ASSOCIATION FOR RESEARCH IN OTOLARYNGOLOGY

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2023 ARO MIDWINTER MEETING

Abstract Book

Saturday, February 11, 2023

Presidential Symposium
8:00 a.m. - 12:00 p.m.
Ocean's Ballroom 5-12

Presidential Symposium:
Chair: Elizabeth Olson, *Columbia University*

This symposium is meant to convey the basic wonder of the ear and of how the ear and brain together provide our sense of hearing. Understanding the operation of the healthy ear and auditory brain is key to understanding how sound sensing can fail.

The session begins with a talk on the historical development of ideas about cochlear sensitivity by physicist Christopher Shera. Karl Grosh, an expert in the dynamic processing of the cochlea, will then review cochlear mechanics. Laurel Carney will discuss how cochlear dynamics shape neural responses to sound, including speech. Raymond Goldsworthy will discuss how the history of cochlear implants has led to the modern device. Richard Einhorn, a composer with hearing loss, will discuss his experience with hearing loss, and share his knowledge of modern hearing aids and personal sound amplification systems. Finally, Debara Tucci will describe the global impact of hearing loss and efforts to improve accessibility.

Hearing is fundamental to communication. The impact of hearing loss, and therefore the impact of hearing remediation, is profound. This symposium is a whirlwind tour of that story -- from the history, through the basics, to what can and should and be done to address hearing health.

The Ear, the Eye, and the ARO: Cochlear Function Through the Ages
Christopher Shera, *University of Southern California*

The cochlea of the inner ear transforms air-borne pressure waves into neural impulses that are interpreted by the brain as sound and speech. The cochlea is a snail-shaped electro-hydromechanical signal amplifier, frequency analyzer, and transducer with an astounding constellation of performance characteristics, including sensitivity to sub-atomic displacements with microsecond mechanical response times; wideband operation spanning three orders-of-magnitude in frequency; and an input dynamic range of 120 dB, corresponding to a million-million-fold change in signal energy. All of this is achieved not with the latest silicon technology, but by self-maintaining biological tissue, most of which is salty water. How does the ear do it? This presentation will review some of what we think we know about how the cochlea works and how these ideas, and the ARO, have changed down through the ages.

Review of Cochlear Mechanics
Karl Grosh, *University of Michigan*

The mammalian cochlea performs a real-time, time-frequency analysis of incoming acoustic signals, and transmits this information to the brain for processing. Normal hearing relies on a carefully orchestrated, tripartite response of the mechanical, electrical, and acoustical (fluidic) domains of this organ. The outer hair cell (OHC) of the mammalian cochlea is at the nexus of the active processes giving rise to the nonlinear, biologically vulnerable, cochlear response that allows for the sensation of sound and system survival over a million-fold change in excitation level. The biological, electromechanical feedback control algorithm that achieves this result is, however, still incompletely understood. In this talk, the fundamental structure-function relations of the cochlea are reviewed along with processes to translate these essential building blocks (such as OHC electromotility and OHC hair bundle mechanoelectric transduction) into a physiological-motivated, complete mathematical model. Fundamental challenges to modeling, including efficient time domain simulation of three-dimensional linear and nonlinear models, are presented. We discuss and exemplify (though numerical experiment) the manner in which biophysically-relevant model elements can be varied and studied to address central questions in cochlear biophysics, such as the role of somatic motility in cochlear amplification, possible fluid paths in acoustic emissions, and essential nonlinearities in the cochlea. The ultimate objective of these experiments is to identify models that are

useful in making predictions, and that test the hypotheses underlying any cochlear mechanics model via comparison to controlled experiments.

From the Ear to the Brain: A Neural Code That Leverages Inner-Ear Nonlinearities
Laurel H. Carney, *Departments of Biomedical Engineering, Neuroscience, and Electrical and Computer Engineering, University of Rochester, Rochester, NY*

This symposium begins with a review of fundamental mechanisms in the inner ear, and works its way towards perception and the problems associated with hearing impairment. In that context, this talk will provide a bridge between response properties of the inner ear and the neural signals that carry information from the ear to the brain. In particular, the nonlinear transduction of mechanical signals into electrical signals by inner hair cells contributes to a robust neural code for sound spectra, carried by the relatively slow fluctuations in neural responses. Illustrations of this neural fluctuation code in the responses of auditory-nerve fibers to speech sounds will be reviewed. An important aspect of this code is its conversion to an average rate profile by midbrain neurons, which are sensitive to fluctuations of their inputs. Implications of this coding strategy for understanding hearing loss will be reviewed. Lastly, recent predictions of speech intelligibility in listeners with and without hearing loss, based on computational models for neural fluctuations in auditory-nerve responses and their representation in the midbrain, will be presented.

Back to the Future: How the History of Cochlear Implants Illuminates Future Directions
Raymond Goldsworthy, *USC*

The first procedure using an auditory implant to restore hearing to a totally deaf person was performed in 1957. In the time since, there have been vigorous debates concerning cochlear implants. The early debates centered on whether they would work, which the years have decisively shown that they do. Today, close to a million implants have been provided to people with hearing loss around the world. Outcomes are impressive with most recipients achieving high levels of speech comprehension in quiet and moderate amounts of background noise. While impressive, many recipients complain about the sound of music. Consequently, many of the most entertaining debates of today center on improving music appreciation for recipients. In this presentation, we will reflect on how the history of cochlear implants has led to a modern design that emphasizes speech, sometimes at the expense of music. Attention will be given to the role of precise timing in the auditory system, and how strategies of today and tomorrow strive to make better use of the exceptional temporal precision that cochlear implants provide.

Current and Near-Future Trends in Hearing Technologies
Richard Einhorn, *Hearing Technology Consultant*

Hearing aids and cochlear implants have evolved considerably in the past 15 years. However, they still have limitations which can often be addressed by modern consumer and professional audio technology. Many of these solutions are little known or understood by both patients and hearing healthcare providers. This talk will introduce basic concepts for effective assistive listening, highlight currently available devices and apps, and discuss some unique solutions for especially difficult hearing situations. Additionally, the talk will discuss Bluetooth LE Audio, the new consumer standard in wireless audio transmission which has significant implications for improving the efficacy and scope of all hearing health technology.

Global Impact of Hearing Loss, and Efforts to Improve Accessibility Worldwide
Debara Tucci, *National Institute on Deafness and Other Communication Disorders/NIH*

Hearing loss is a significant global problem. More than 20% of the world's population has mild to complete loss in the better hearing ear, and more than 5% have moderate to complete loss. While the greatest burden imposed by hearing loss in low- and middle-income countries (LMICs) is in the pediatric age groups, the opposite is true in high income countries, where the burden is reflected predominately in aging populations. Overall, hearing loss is the fourth leading cause of years lived with disability worldwide. The financial burden of unaddressed hearing loss is immense, and it is estimated by the World Health Organization (WHO) to exceed $750 billion annually, including health care and societal costs.

Although proven preventative measures and treatments exist, they are not routinely implemented in low- and middle-income settings. Even in high income countries such as the United States and Europe, the recognition of problems associated with hearing loss in older adults – including increased incidence of hospitalization, falls, social isolation and depression, and apparent association with increased risk of dementia – has not been sufficient to drive public policy and health care strategies toward prevention, identification, and treatment of hearing loss.
Several efforts have been initiated in the past few years to change the trajectory of this ever-increasing burden. One such effort is the WHO World Report on Hearing, which details the problem and proposes strategies for mitigation worldwide. Another, the Lancet Commission on Hearing Loss (LCHL), will build on the findings and recommendations put forth by the World Report on Hearing. The LCHL began in fall 2019, and is expected to submit recommendations late this year, with publication in 2023.

Within this Commission, over 100 commissioners and consultants with wide-ranging subject matter expertise, from all over the world, have developed strategies for addressing these challenges from the perspectives of prevention, mitigation, and treatment. Economic, cost-benefit analyses are expected to inform recommendations to policy makers in a variety of regional and economic settings, who may then apply solutions to their individual circumstances and health care priorities. For example, recognition that otitis media is a major cause of pediatric hearing loss and childhood illness in LMICs may inform approaches and prioritization of hearing health care in these settings. The importance of integrating hearing health care into country level health care systems is of paramount importance, and it will ensure continued improvements in and access to care.

The work to address this global problem will not end with the LCHL, but the commission will serve as a beginning, providing a roadmap for future, prioritized, sustained, and collaborative work among all stakeholders.

spARO Mentoring Session: Navigating the Grant Landscape

12:45 p.m. - 1:45 p.m.
Canaveral 1-2

This session will feature short presentations from a panel of leading scientists to introduce various grants and fellowships for graduate students and postdoctoral fellows in the U.S. and Europe. The session will focus on federal and private research funding sources, eligibility considerations for selected grants, the application process, and the peer review process. This session will be an interactive forum with questions and discussion.

Sarah Verhulst*, Hearing Technology @ WAVES, Dept. of Information Technology, Ghent University*
Peter Steyger*, Creighton University*

Podium #1 - Gene Therapy

2:00 p.m. - 4:00 p.m.
Ocean's Ballroom 5-12

Moderators: Ronna Hertzano and Jennifer Lentz

Delivery of Gene Therapy Through a Cerebrospinal Fluid Conduit to Rescue Hearing

Barbara Canlon*[1], Barbara Mathiesen[2], Leo Miyakoshi[2], Christopher Cederroth[1], Evangelia Tserga[1], Corstiaen Versteegh[1], Peter Bork[2], Natalie Hauglund[2], Ryszard Gomolka[2], Yuki Mori[2], Niklas Edvall[1], Stephanie Rouse[3], Kjeld Møllgård[2], Jeffrey Holt[3], Maiken Nedergaard[4]
[1]Karolinska Institute, [2]University of Copenhagen, [3]Boston Children's Hospital and Harvard Medical School, [4]University of Copenhagen and University of Rochester Medical Center
Background: In mice, inner ear gene therapy has recently proven effective in restoring hearing in neonatal animals but is complicated in adulthood by the structural inaccessibility of cochlea, which is embedded within the temporal bone. New delivery routes may not only serve the advancement of auditory research, but also prove useful when translated to humans with progressive genetic-mediated hearing loss. Cerebrospinal fluid flow via the glymphatic system is emerging as a new approach for brain-wide drug delivery in rodents

as well as humans. The cerebrospinal fluid and the fluid of the inner ear are connected via the cochlear aqueduct. To date, no previous studies have explored the possibility of delivering gene therapy via the cerebrospinal fluid and restore hearing in adult mice.
Methods: Tracers or AAVs into injected into the cisterna magna (CM) were performed in anesthetized mice. A skin incision was made over the occipital crest and the neck muscles were separated to reveal the atlanto-occipital membrane. A 30 G needle attached to a PE 10 tube filled with aCSF was inserted into cisterna magna. Live imaging was performed using a preclinical scanner (BioSpec 94/30 USR). Computer tomography scans were performed using a Vector4uCT system (MILabs, Utrecht, Netherlands). AAV9-PHP.B vectors carrying the coding sequences of eGFP or mouse VGLUT3 were generated by the Viral Core at Boston Children's Hospital. AM/CBA-VGLUT3-WPRE-BGH plasmids containing mouse VGLUT3 cDNA under control of chicken β-actin (CBA) promoter and cytomegalovirus (CMV) enhancer was kindly provided by Omar Akil. AAVs were injected in the cisterna magna at a rate of 1 ul/min for 10 min. Histology and immunohistochemical procedures were performed on fixed cochlea and were either cryosectioned or prepared for surface preparations. Cochleae were stained for the quantification of the pre- and post-synaptic elements, anti-myosin VIIA and anti-vesicular glutamate transporter 3 for observation of the restoration of the hair cells. Auditory brainstem responses were recorded for determining thresholds and signal generation and acquisition was done using Tucker-Davis Technologies System III hardware and software.
Results: Here we show that a single intracisternal injection of adeno-associated virus encoding vesicular glutamate transporter-3 (VGLUT3) rescues hearing in adult deaf Slc17A8 -/- mice by restoring VGLUT3 expression in inner hair cells, with limited ectopic expression in the brain and none in the liver. The cochlear aqueduct was found to exhibit lymphatic-like characteristics. In-vivo time-lapse magnetic resonance imaging, computed tomography and optical fluorescence microscopy show that large-particle tracers injected in cerebrospinal fluid reach the inner ear by dispersive transport via the cochlear aqueduct in adult mice.
Conclusions: Our findings demonstrate that cerebrospinal fluid transport comprises a novel and relatively non-invasive route for effective gene delivery to the adult inner ear and represents a crucial step towards using gene therapy to restore hearing in humans.

Gene Replacement Rescues Transduction and Synaptic Functions of the Clarin-2 Deafness Gene

Clara Mendia*[1], Thibault Peineau[2], Nawal S Yahiaoui[1], Chloé Felgerolle[1], Samantha Papal[1], Maureen Wentling[1], Pranav Patni[1], Audrey Maudoux[1], Carlos Aguilar[3], Sylvie Nouaille[1], Michael R Bowl[3], Sedigheh Delmaghani[1], Didier Dulon[2], Sandrine Vitry[1], Aziz El Amraoui[1]
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Background: Hearing impairment is the most common sensory disorder in humans. While prosthetic devices, such as cochlear implants, help alleviate the burden of hearing loss in a subpopulation of deaf patients, effective biological treatments for cochlear functions are still missing. Promising successes using adeno-associated virus (AAV)-mediated therapeutics have been obtained recently, often with only partial recovery of all or certain sound frequencies. Several challenges such as efficiency (across frequencies), long term stability, and therapeutic window need to be addressed. To tackle these issues, we used a model of postnatal severe-to-profound hearing loss in human and mice. Defects in CLRN2 were recently found to cause hearing deficits in humans, mice, and zebrafish, supporting evolutionary conservation of the key role of the clarin tetraspan-like proteins in the inner ear.
Methods: We characterized auditory function by ABR and DPOAE recordings. Hair bundle structural abnormalities were explored by confocal and scanning electron microscopy, while FM1-43 assays addressed mechano-electrical transduction. Capacitance recordings were performed to assess synaptic function of auditory inner hair cells.
Adeno-associated Viral delivery was used for gene supplementation, and qPCR and RNAscope imaging were used to confirm gene ectopic expression in treated ears.
Results: Our in-depth characterization of the Clrn2−/− defective mouse revealed novel synaptic auditory abnormalities, in addition to priorly established hair bundle structure and function defects. Our new data in Clrn2−/− mice clearly show that gene supplementation using either the murine (Clrn2) or human (CLRN2) clarin-2 gene preserves normal hearing.

Ectopic expression of Clrn2 successfully prevents the loss of mechano-electrical transducing stereocilia (i), restores normal mechano-electrical transduction in auditory hair cells (ii) and IHC synaptic function (iii), and preserves, over time, near normal ABR and DPOAE hearing thresholds (iv), confirming durable recovery of function in both the inner and outer hair cells. Our findings also confirm the extreme pathogenicity of the CLRN2 mutated allele, c.494C>A, recently reported to cause hearing loss in humans. Indeed, in contrast to the expression of intact human CLRN2, leading to full recovery of hearing, neither CLRN2T165K nor CLRN2G146fs26Stop (2 variants from CLRN2 deaf patients) prevent the severe-to-profound hearing loss in Clrn2−/− mice. Finally, we explored the therapeutic time window, showing that the maximal therapeutic beneficial outcomes are achieved by inoculation of the early neonatal mouse cochlea; and that the diminishing improvement in sensory function upon injections at P5, P10 and P14 is linked to decreased transduction efficiency in the auditory hair cells.
Conclusions: In summary, our findings demonstrate the feasibility of effective and durable restoration of hearing using viral-mediated gene therapy in the inner ear.

Adult Gene Therapy Restores Auditory and Vestibular Function in P2rx2 V60L Mouse Model for DFNA41

Wei Wei*[1], Wenliang Zhu[1], Stewart Silver[1], Ariel Armstrong[1], Arun Prabhu Rameshbabu[1], Fletcher Robbins[1], Katherina Walz[2], Xuezhong Liu[2], Zheng-Yi Chen[1]
[1]Mass. Eye and Ear, [2]University of Miami Miller School of Medicine
Background: Gene editing and gene therapy are rapidly developed to treat diverse forms of genetic hearing loss. Most current editing and gene therapies are focused on the neonatal age and minimal studies show efficacious outcome with intervention in adult. P2rx2 is an ATP-gated iron channel protein essential for auditory and vestibular function. A pathogenic mutation in P2rx2, c.178G>T (p.V60L), causes autosomal dominant deafness 41 (DFNA41), a late-onset progressive hearing loss. We created a knock-in mouse model of human DFNA41, which mirrors deafness in humans. We explore the gene editing therapy to abolish the mutation at adult age to rescue hearing and vestibular function in P2rx2 knock-in (KI) mice.
Methods: We designed sgRNAs to target the P2rx2 mutation and constructed plasmids containing saCas9/sgRNA to transfect the primary skin fibroblast cells of P2rx2 KI mice in vitro. Editing efficiency and off-target were determined by next-generation sequencing (NGS). The sgRNA and SaCas9 were packaged into a single AAV2 known to transduce the auditory hair cells efficiently. AAV2-Cas9-sgRNA-P2rx2 was microinjected into the fully mature mouse inner ear at 4 weeks of age. Auditory function and sensory cell morphology were assessed by ABR, DPOAE, immunofluorescence, and Scanning Electron Microscopy. Vestibular functions were tested by Rotarod Test and Open Field Test.
Results: To knock out P2rx2 KI mutant gene expression, we screened 5 sgRNAs for both spCas9 and saCas9, and identified a saCas9/sgRNA-1-P2rx2 with robust editing efficiency and high specificity targeting the P2rx2 V60L allele. We produced a single AAV product with SaCas9-sgRNA-1 and injected AAV2-Cas9-sgRNA-1-P2rx2 into 4-week-old P2rx2 KI inner ear. We detected hearing rescue by significant reductions in ABR thresholds in low and middle frequency starting 12 weeks post injection. By 24 weeks hearing rescue became more profound as the result of continuous hearing deterioration in uninjected control ears whereas hearing was maintained in the injected and edited ears. Concomitantly, lower DPOAE thresholds, higher wave 1 amplitudes, increased hair cell survival, and better hair cell bundle morphology were observed in the injected ear compared to the un-injected ear. By the open field test, injected mice explored the border of the field and displayed fewer full-body rotations compared to un-injected mice.
Conclusions: Our study demonstrates that editing therapy can be successfully applied to mature inner ear to rescue auditory and vestibular function effectively long term and safely in the P2rx2 KI mouse model. The study determined a time window for editing therapy intervention in mice which is relevant for patients with late-onset progressive deafness caused by P2rx2 mutations. Successful editing and gene therapies in mature inner ears open an avenue for the application in humans whose inner ears are fully mature from the birth.

AAV Delivery of Tmc1 Rescues Vestibular Function in Tmc1 KO Mice

Evan Ratzan*[1], John Lee[2], Gwenaelle Geleoc[3], Jeffrey Holt[1]
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Background: Transmembrane channel-like 1 (Tmc1) encodes a protein required for mechanosensory transduction in auditory and vestibular hair cells. TMC1 and its counterpart TMC2 function together as pore

forming subunits of hair cell transduction channels and are necessary for auditory and vestibular function. Mice lacking both Tmc1 and Tmc2 (Tmc1/2 DKO) exhibit severe circling behavior, head bobbing, and imbalance. Patch-clamp recording from Type II vestibular hair cells shows that DKO hair cells also lack electrical responses to mechanical hair bundle deflections. Likewise, Tmc1/2 DKO mice fail produce waveforms associated with normal responses to head jerk stimuli during vestibular evoked potentials (VsEPs).
Methods: Evaluation of hair cell physiology through FM1-43 labeling of saccular hair cells and VsEP of P60, P120, and P180 WT, Tmc1 KO, Tmc2 KO, and Tmc1/2 DKO mice. Gene expression of Tmc1 and Tmc2 was assessed quantitatively with qPCR and distribution of transcripts through fluorescent in situ hybridization hairpin chain reaction. Phenotypic changes in protein expression was evaluated for calretinin, oncomodulin, Pou4f3, and myosin VIIa through immunhistochemistry.
Results: Interestingly, Tmc2 KO mice show normal VsEP responses, but Tmc1 KO mice fail to produce them. Restoration of Tmc1 expression through neonatal inner ear injection of adeno associated virus (AAV) encoding Tmc1 rescues VsEPs to near WT thresholds. FM1-43 dye uptake in vestibular hair cells indicates the presence of functional transduction channels, but dye uptake is not uniform between different Tmc KO genotypes. FM labeling of striolar hair cells in the saccule is stronger in Tmc1 KO mice, weaker in the Tmc2 KO mice, and completely absent from all hair cells in Tmc1/2 DKO mice. Interestingly, Tmc2 KO and Tmc1/2 DKO mice not only lack striolar FM uptake but also lose expression of calretinin in striolar calyxes of the saccule. However, qPCR data shows much higher levels of Tmc1 than Tmc2 in the saccule.
Conclusions: One possible explanation for such a role for Tmc1 is that Tmc1 and Tmc2 are expressed differentially in striolar versus extrastriolar hair cells resulting in differential compensation for VsEP signal. An alternative explanation is that Tmc1 is differently expressed between Type I versus II vestibular hair cells and thus KO mice respond to head jerk stimuli differently. Finally, it is possible that Tmc1 and Tmc2 are expressed in all vestibular hair cells, but not at equal levels which results in Tmc1 being able to compensate for loss of Tmc2, but not vice versa. Taken together, these data suggest that Tmc1 is essential for encoding head jerk stimuli and can compensate in the absence of Tmc2 to provide normal VsEP thresholds.

Congenital Deafness, Vestibular Dysfunction, and Progressive Visual Impairment in a Rhesus Macaque Model of Usher Syndrome Type 1B

John Brigande*[1], Junghyun Ryu[2], Jon D. Hennebold[2], Fernanda C. Burch[2], J. Beth Kempton[1], Edward V. Porsov[1], Lauren Renner[3], Benjamin J. Burwitz[4], Carol B. Hanna[2], Martha Neuringer[3]
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Background: Our long-term goal is to define genetic mutations in the rhesus macaque that recapitulate disease phenotypes in the human inner ear and eye and then identify gene therapies that durably restore sensory function. We predict that therapeutics effective in treating hearing and vision loss in nonhuman primate disease models will also meaningfully improve sensory perception in human patients. Inner ear and eye anatomy, physiology, and development in the rhesus macaque closely models that in humans. Importantly, sensitive low-frequency hearing is present at birth in rhesus macaques and overlaps with the human speech frequencies needed for effective oral communication. The goal of this study is to create a null mutation in the rhesus macaque Myosin 7A (MYO7A) gene using a CRISPR/Cas9 genome editing strategy and to characterize the neonatal sensory phenotypes. MYO7A is an unconventional myosin expressed in auditory and vestibular hair cells, and in the retinal pigment epithelium and photoreceptors of the retina. Mutations in MYO7A are responsible for ~50% of all Usher syndrome cases and affected patients experience severe hearing loss and imbalance at birth with progressive blindness.
Methods: A compound heterozygous infant harboring a 1-base pair and a 63-base pair deletion in MYO7A was born in November 2021. DNA from neonatal cheek, skin, and peripheral blood monocytes was sequenced to confirm genotype. Tympanometry was performed to interrogate the middle ear function. Auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) were conducted to assess auditory function. Retinal multimodal imaging and electroretinograms (ERG) were deployed to assess retinal structure and function.
Results: Tympanometry showed A-wave responses indicating patency of the middle ear conduction pathways bilaterally. Neither ABR nor DPOAE responses were detected through 6 months indicating

congenital deafness. The infant displayed postural instability at birth and stumbling, a broadened gait, and loss of balance while engaging an inclined plane. The infant hangs upside down a disproportionate amount of time. Multimodal imaging at 4 months revealed patchy hyperfluorescence temporal to the optic nerve with disruption of the photoreceptor layers and thinning of the outer nuclear layer. Full field ERGs showed an ~50% reduction in response to scotopic, photopic, and flicker stimuli.
Conclusions: We conclude that the auditory, vestibular, and vision phenotypes in the CRISPR/Cas9-derived MYO7A compound heterozygote faithfully model the pathogenesis of Usher syndrome type 1B (USH1B). The rhesus macaque model of USH1B may be useful for testing novel therapeutics.

Rescue of Autosomal Dominant Hearing Loss by in Vivo Delivery of Mini dCas13X-Derived RNA Base Editor

Yilai Shu*[1], Qingquan Xiao[2], Zhijiao Xu[1], Yuanyuan Xue[1], Chunlong Xu[3], Geng-Lin Li[1], Hui Yang[2], Huawei Li[1]
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Background: Programmable RNA editing tools enable the reversible correction of mutant transcripts, reducing the potential risk associated with permanent genetic changes irreversibly installed by DNA editing tools. However, validity of these RNA tools to treat disease remains unknown. Here, we explored the potential of RNA correction therapy with Cas13-based RNA base editors in the Myo6C442Y/+ mouse model that recapitulated the phenotypes of human dominant-inherited deafness.
Methods: In vitro, we screened and compared the Myo6C442Y correction efficiency and the off-target effects among different RNA base editors and gRNAs targeting C442Y mutation. In vivo, single adeno-associated virus (AAV)-mediated delivery of mxABE system was injected into the mouse cochlea, and the auditory functions were assessed by auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE). Immunostaining, scanning electron microscope were preformed to observe the hair cell survival and hair bundle morphology.
Results: The mxABE exhibited both high efficiency of A > G conversion and low off-target edits, and showed remarkable correction of Myo6C442Y to Myo6WT allele in homozygous Myo6C442Y/C442Y mice and induced significant increase of Myo6WT allele in the injected cochlea of Myo6C442Y/+ mice compared with the uninjected ones. Rescue of auditory function was observed up to 3 months post AAV-mxABE-Myo6 injection in Myo6C442Y/+ mice. We also observed increased survival rate of hair cells and decreased degeneration of hair bundle morphology in the treated ears compared to untreated ears.
Conclusions: In summary, our results provide a proof-of-concept study for RNA editing tools as a therapeutic treatment for various semi-dominant forms of hearing loss and other diseases.

In Vivo Outer Hair Cell Gene Editing With Cas9 Nuclease Partially Improves Progressive Hearing Loss in Dominant-Negative Kcnq4 Mouse Model

Byunghwa Noh*[1], John Hoon Rim[2], Ramu Gopalappa[2], Haiyue Lin[3], Kyu Min kim[3], Min Jin Kang[3], Heon Yung Gee[2], Jae Young Choi[3], Hyongbum Henry Kim[2], Jinsei Jung[3]
[1]Department of Otorhinolaryngology, Graduate School of Medical Science, Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, [2]Department of Pharmacology Yonsei University College of Medicine, [3]Department of Otorhinolaryngology Yonsei University College of Medicine
Background: Hearing loss is a common sensory disorder with an incidence of 2.5 in 1,000 births, but there are no effective drugs to treat hearing loss. KCNQ4 W276S variant that is one of the various pathogenic variants in KCNQ4, voltage-gated potassium channel protein subfamily Q member 4 and expressing in Outer hair cells (OHCs) and spiral ganglion neurons (SGNs), and is the only mutation found in common among several DFNA2 families causes non-syndromic autosomal dominant progressive hearing loss (DFNA2) by strong dominant-negative effect. Recently, the potential of gene editing technology using the CRISPR/Cas9 system in the inner ear to treat hearing loss caused by various gene dysfunction has been demonstrated. In this study, we found that OHCs gene editing with Cas9 nuclease in the Kcnq4W276S/+ murine model partially rescued progressive hearing loss by improving the OHC membrane potential. This result will provide a basis for the clinical application of the CRISPR/Cas9 system to treat deafness.

Methods: sgRNAs were generated to target c.830G> C, a missense mutation region in exon5 of the Kcnq4 gene. Two anc80L65 vectors were packaged with N-term of SpCas9, sgRNA sequence, and C-term of SpCas9, respectively. Post-auricular incision was performed in mice between postnatal day one (P1) and P3 for inner ear injection through round window membrane. At 7 weeks, auditory brainstem response (ABR) measurements were performed to determine whether hearing was improved. In vivo Indel rate was measured through the deep sequencing method. Immunohistochemistry was performed to compare the number of live hair cells in the injected and noninjected sides. We developed a new technique using live-cell imaging, Tl+ flux assay, to measure the membrane potential of the OHCs ex vivo.
Results: In the 7weeks ABR results, the hearing of the AAV-injected ear was improved by 20dB on average compared to the non-injected ear, and the whole cochlea of the injected ear with improved hearing showed 0.6% indels. Also, through live cell imaging, it was confirmed that the OHCs membrane potential of the AAV-injected ear was more hyperpolarized, suggesting KCNQ4 activity was improved, but no difference was confirmed in cochlear histology.
Conclusions: Improvement of progressive hearing loss in the Kcnq4W276S/+ murine model through AAV-SpCas9 injection was successfully performed, but we need to improve the indels efficiency more than in this study's low correction rate.

Precise Targeting of GJB2 Cells Results in Safe and Efficacious Gene Therapy for Hearing Loss Due to GJB2 Deficiency (DFNB1)

Kathryn Ellis*[1], Yoojin Chung[1], Kasey Jackson[1], Theresa Abell[1], Joseph Goodliffe[1], Quynh-Anh Fucci[1], Chris Thompson[1], Madeline Barnes[1], Kevin Lebo[1], Sarah Cancelarich[2], Daniela Di Battista Miani[2], Leah Sabin[2], Meghan Drummond[2], Gabriela Pregernig[1]
[1]Decibel Therapeutics, [2]Regeneron Pharmaceuticals, Inc.
Background: Mutations in Gap junction beta protein 2 (GJB2) are the leading cause of non-syndromic, prelingual deafness (DFNB1) worldwide. One major challenge in developing a gene replacement therapy is the ability to replicate the endogenous expression pattern in the cochlea, as GJB2 is expressed in a variety of cell types at different expression levels. A successful DFNB1 gene therapy must provide benefit by sufficiently targeting the diverse set of GJB2-expressing nonsensory cells but also must avoid toxicity by excluding expression from critical sensory cells. Here we demonstrate that selective targeting of GJB2-expressing cells results in robust and durable hearing restoration in a translationally relevant mouse model of GJB2 deficiency.
Methods: Adult or neonatal mice were injected via the posterior semicircular canal with AAV encoding GJB2 or nuclear GFP driven by a ubiquitous promoter or GJB2 regulatory elements. Hearing function was assessed using ABR or DPOAE. After animal sacrifice, immunohistochemistry was performed to assess cochlear morphology and transgene expression.
Results: We evaluated whether off-target GJB2 expression was detrimental to hearing by delivering AAVs encoding hGJB2 under control of a ubiquitous promoter (AAV-CMV-hGJB2) to the cochlea of adult wildtype mice. Three quarters of injected animals had elevated hearing thresholds. Histological analysis showed accumulation of GJB2 protein in inner hair cells after just 1 week, followed by almost complete loss of these cells by 2 weeks.
We identified a combination of GJB2 proximal and distal regulatory regions which mirrors endogenous GJB2 expression. Integrative bioinformatic analyses of bulk and single cell epigenomic datasets were leveraged to select candidate regulatory regions, and proximal promoter/enhancer designs were screened in neonatal cochlear explants, identifying a series of combinations which successfully drive expression in GJB2-expressing cells while excluding expression from hair cells and neurons. In vivo experiments confirmed that our regulatory elements closely replicated the endogenous GJB2 expression pattern in both mouse and non-human primate, and they eliminated off-target toxicity observed with CMV in adult wildtype mice.
Next, we evaluated the ability of our various regulatory elements to restore hearing in a mouse model of GJB2 deficiency. We achieved robust hearing restoration in ears injected with human GJB2 compared to contralateral ears and naïve controls, achieving wildtype thresholds in some animals. We demonstrate that this hearing recovery is stable and durable for at least six months.
Conclusions: Our results underscore the importance of using the proper regulatory elements to drive GJB2 expression and avoid toxicity in AAV-based gene therapy for the most common form of genetic deafness, and they highlight the power of bioinformatics to achieve a successful design. The robust and durable

recovery observed in mice with our vector represents significant progress towards the development of a gene therapy to restore natural hearing to DFNB1 patients.

Podium #2 - Tinnitus: Experiments and Models

2:00 p.m. - 4:00 p.m.
Oceans Ballroom 1-4

Moderators: Fatima Husain and William Sedley

Gating Mechanism in Tinnitus: Explored in Surgery-Induced Unilateral Deafness in Adult Humans

MinChul Park*[1], Greg O'Beirne[1], Philip Bird[2], Mike Maslin[1]
[1]University of Canterbury, [2]University of Otago

Background: An important question in the relationship between hearing loss and tinnitus is why some people with hearing loss experience tinnitus while others with hearing loss do not. The "TRN noise-cancellation" model refers to a failure of the so-called noise-cancellation circuitry of the auditory brain, leaving it unable to "tune out" the neural noise (tinnitus) signal triggered by hearing loss. There are limited opportunities to test this model and its corresponding predictions in humans due to factors like heterogeneity of hearing loss. However, participants with surgery-induced unilateral deafness (UD) allow an opportunity. In normally hearing listeners, the auditory brainstem response (ABR) exhibits amplitude and latency effects to sounds heard in noise as a function of both signal-to-noise ratio (SNR) and absolute level (effects are additive). On the other hand, long-latency responses like the P1-N1-P2 complex tend to show a subtractive effect. The dual effect of these different AEPs has been associated with the noise cancellation process acting at the level of the thalamus. A comparison of AEPs arising from generators above and below the thalamus level may offer an indirect means to test the noise-cancellation model in humans. If a failure of noise-cancellation circuity is related to the perception of tinnitus, we hypothesise an absence of the subtractive effect in P1-N1-P2 data in individuals with UD and tinnitus compared to UD without tinnitus, and controls, but no such changes in the ABR in all three groups.
Methods: To test this idea, we are gathering data in three sub-groups: i) UD adults with tinnitus ii) UD adults without tinnitus, and iii) age-matched controls without UD or tinnitus. A total of 20 in each subgroup are sought. ABR and P1-N1-P2 data are collected in response to monaural stimulation of the intact ear in quiet and in noise at three SNRs (-10 dB, 0 dB and +10 dB), via a 64-channel BioSemi device. Sensor level analysis will consist of a parametric comparison of amplitudes and latencies, while source analysis will be used to explore altered generators associated with tinnitus and no-tinnitus conditions.
Results: The study has been approved by the University of Canterbury Human Research Ethics Committee (Ref. HEC 2021/68/LR-PS), data collection started in April 2022, and it is anticipated to be completed by September 2023. We are currently processing the data gathered to date for communication of preliminary findings by January 2023. The work is funded by the University of Canterbury Doctoral Scholarship and the Eisdell Moore Centre.
Conclusions: This study will offer an opportunity to test the predictions of the TRN-mediated noise-cancellation model. Findings that support the model will help advance the understanding of tinnitus onset in humans, whereas findings that do not support the model may suggest a need to refine it.

Tinnitus Heterogeneity and Subtyping - Results From the Swedish Tinnitus Outreach Project

Christopher Cederroth*[1], Niklas Edvall[1], Natalia Trpchevska[1], Alvaro Gallego-Martinez[2], Alessandra Lugo[3], Andra Lazar[4], Inger Uhlen[4], Winfried Schlee[5], Barbara Canlon[1], Jan Bulla[6], Silvano Gallus[3], Jose Antonio Lopez-Escamez[2]
[1]Karolinska Institute, Dept of Physiology and Pharmacology, Sweden, [2]Otology and Neurotology Group CTS 495, Department of Genomic Medicine, Pfizer-University of Granada-Junta de Andalucía Centre for Genomics and Oncological Research (GENyO), [3]Department of Environmental Health Sciences, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy, [4]Hörsel och balansmottagningen, Karolinska Universitetssjukhuset, Stockholm, Sweden, [5]University of Regensburg, Germany, [6]University of Bergen, Norway

Background: Despite being a highly prevalent condition, tinnitus remains a scientific and clinical enigma. Not only, is tinnitus heterogeneous in its manifestations, but also it can be caused by numerous audiological related dysfunctions, varies in the psychological reactions, as well as in the response to treatments.
Methods: In 2015, we launched the Swedish Tinnitus Outreach Project (STOP), which was established in order to better understand tinnitus subtyping, identify relevant biomarkers, under a large phenotyping strategy. Through the partnership with local and national prospective cohorts, we recruited 5,671 adult individuals with and without tinnitus, representative of the general Swedish population. Demographic, tinnitus and psychological data was collected between 2015 to 2019. With available plasma and DNA, STOP took advantage from several state-of-the-art OMICs analyses: Plasma screening of 184 biomarkers (O'link) was performed on 1046 individuals, Whole Exome Sequencing (WES) and Whole Genome Sequencing (WES) was performed on 327 and 97 individuals respectively. In-depth auditory phenotyping was performed on 927 individuals, and auditory brainstem responses (ABR) collected on 405 participants.
Results: Initial studies on the Swedish cohorts have revealed that more frequent occasional tinnitus increases the odds of transitioning to constant tinnitus and that constant tinnitus increases the odds that tinnitus will persist. Using the ABR data collected in STOP, we could show that constant tinnitus can be distinguished from occasional tinnitus or non-tinnitus groups by means of electrophysiology (increased latency of the ABR Wave V) but not plasma biomarkers. From a genetic perspective, we showed in twins a higher heritability of bilateral tinnitus vs unilateral tinnitus, and using national registry data in adoptees, we show that the transmission of tinnitus within the family is influenced by genetic factors. Using STOP, we performed a familial aggregation study on 2305 individuals to show that among unilateral, bilateral, constant or severe tinnitus, the later showed the greatest sibling recurrence risk with women with severe tinnitus having 10 times greater risk of having a sibling with tinnitus. In contrast, individuals with severe tinnitus display a burden of rare variants in ANK2, AKAP9 and TSC2 genes suggesting the role of connectivity genes being involved in the pathogenicity of severe tinnitus.
Conclusions: Tinnitus appears to be progressively transitioning from occasional to constant states, when alterations in neuronal function may be associated with the persistence of tinnitus. On the other hand, severity appears genetically driven. Based on our findings, we propose that new tinnitus definitions consider all new dimensions in perception (unilateral/bilateral), frequency and chronicity (occasional/constant), and severity as subtypes. We hope this standardization will help clinical data globally and facilitate future meta-analyses.

Sound Elicits Rapid and Involuntary Fluctuations in the Eyes, Skin, and Face that Provide a Sensitive Biomarker for Tinnitus and Hyperacusis-Related Burden

Samuel Smith*[1], Kelly Jahn[2], Jenna Sugai[1], Kenneth Hancock[1], Daniel Polley[3]
[1]Eaton-Peabody Laboratories, Massachusetts Eye and Ear, [2]Department of Speech, Language, and Hearing, University of Texas at Dallas, [3]Eaton-Peabody Laboratories, Massachusetts Eye and Ear, Harvard Medical School
Background: For some, the experience of sound is a burden. Moderate intensity sound can feel distressing; phantom ringing is invasive and uncontrollable; noisy auditory scenes present as overwhelming. Such complaints go beyond acoustic sound qualities, but instead focus on affective, "post-sensory", qualities. Presently, the psychologic burden of these disorders can only be assessed with questionnaires, emphasizing the need for objective, rapid and low-cost physiological measures to index the distress associated with hyperacusis, tinnitus, and aging.
Methods: To quantify affective sound responses, we simultaneously recorded pupil diameter, skin conductance, and high-speed videography of the face while subjects listened to 60 tokens of environmental sounds drawn from the International Affective Digitized Sounds database. Participants rated each token for the subjective valence, arousal, and loudness at the conclusion of each trial. Measurements were made in a cohort of 53 young (<41 yrs) or older (>41yrs) normal hearing adults, classified as neurotypical, tinnitus, and hyperacusis. Participants completed a set of self-reported questionnaires designed, in part, to quantify self-perceived tinnitus presence and severity (TRQ) and/or hyperacusis handicap (HHQ). Linear mixed-effects models with random effects for participant were used for group-level analyses, and multivariate linear regression was used to predict within-group hearing burden.
Results: We found that affective sound tokens elicited rapid and involuntary fluctuations in pupil diameter, skin conductance, and facial movements that clearly differed as a function of perceived sound valence (i.e., positive, neutral, or negative; marginal ANOVA, $p<0.01$ for pupil, skin, and face). Response amplitudes for

these three autonomic markers distinguished young neurotypical participants from older neurotypical participants or younger participants with tinnitus or hyperacusis at a group level. For example, large sound-evoked pupil dilations discerned the young hyperacusis group from the others. By contrast, in subjects with chronic tinnitus, pupil and skin response amplitude across valence categories were the primary distinguishing feature. Most intriguingly, the combination of all involuntary behavioral signatures were strongly predictive of tinnitus and hyperacusis burden, as assessed with questionnaires (TRQ, adjusted R-squared=0.83, p=0.02) or (HHQ, adjusted R-squared=0.95, p<0.01).
Conclusions: Diagnosing and treating suprathreshold hearing disorders such as tinnitus, hyperacusis, and hidden hearing loss is hampered by a reliance on subjective self-report questionnaires. Here, we provide new evidence that rapid facial movements can index affective sound processing. When combined with measures of pupil dilation and skin conductance, these autonomic markers can be used to accurately predict the tinnitus and hyperacusis-related burden reported by individual participants. This approach identifies a new avenue towards low-cost, non-invasive, and objective measurements for the severity of suprathreshold hearing disorders.

Psychophysics of Patient Decisions Applied to the Tinnitus Patient Journey

Alexander Hoetink*[1], Sarah Kaldenbach[2], Arno Lieftink[1], Huib Versnel[1], Robert J. Stokroos[1], Karin H. Wiefferink[2]
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Background: Chronic tinnitus management requires a multidisciplinary approach with attention for medical, audiological, psychological and social-emotional issues. Most therapies are based on psychological intervention (e.g. cognitive behavioral therapy), audiological intervention (sound therapy), or a combination. Several Cochrane reviews indicate that psychological intervention is the most effective intervention, yet many patients seem to prefer other options. The aim of this study is to develop a new method that combines Signal Detection Theory and Health Belief Model and apply it to map patient decisions during the tinnitus patient journey.
Methods: We used the clinical data of 148 tinnitus patients who visited an Audiological Center in The Netherlands during a two-year period. Inclusion criteria were age (older than 15 years) and referral for tinnitus care. Before and after audiological assessment and tinnitus intervention patients were asked to fill in the Tinnitus Handicap Index (THI) and Glasgow Health Status Index (GHSI) questionnaires. All patients received standard of care tinnitus psychoeducation. As follow-up, they were offered to start a trial period with a sound therapy device (hearing aid, ear worn tinnitus masker or combination device), and they were offered psychosocial counseling based on elements of cognitive behavioral therapy and mindfulness. After the trial period with the sound therapy device, patients were free to purchase the device or not. The proportion of patients that accepted the different interventions were analyzed as a function of hearing level, preintervention THI-score and preintervention GHSI-score. Using delta_THI > 7 as clinically relevant change in THI-score to distinguish between successful decisions (true positives) and unsuccessful decisions (false positives), we constructed Receiver-Operator-Curves to map the decision criteria that patients adopted when accepting psychological intervention, starting a trial period with a sound therapy device and finally purchasing the sound therapy device.
Results: The results show that patients adopt a strict decision criterion (accepting few false positives at the expense of few true positives) when deciding about taking up psychological intervention. For sound therapy, on the other hand, a lax decision criterion is adopted (resulting in more true positives at the expense of an increased rate of false positives). A more unbiased decision criterion is adopted when deciding about purchasing the sound therapy device after the trial period. We found that decision criteria also depended on patient characteristics such as hearing level, pre THI-score, age and laterality of tinnitus.
Conclusions: This study shows that combining Signal Detection Theory and Health Belief Model allows mapping of patient decisions during the tinnitus patient journey. The results can be used to create patient profiles that allow for more individualized counseling in shared decision making. This individualized counseling may lead to adoption of more unbiased decision criteria by patients with a profile associated with a strong bias.

Developing a Generalized Risk Model for Tinnitus

Valerie Ingalls*[1], Ishan Bhatt[1]

[1]*University of Iowa*
Background: Tinnitus, or the phantom perception of sound, is a common otological condition that affects nearly 15% of the general population. Tinnitus varies in its severity, but millions of people in the United States experience debilitating effects from it. Past epidemiological studies have identified aging, noise exposure, smoking, reoccurring ear infections, and health conditions (e.g., hypertension, diabetes) as risk factors for tinnitus. However, the predictive utility of the associated risk factors in determining an individual's risk for tinnitus remains elusive. In this study, we sought to examine the predictive utility of the risk factors to ascertain an individual's risk of tinnitus.
Methods: We developed risk models for tinnitus using known risk factors as input and the presence of tinnitus as the models' target. Data for both the training and testing of these models were taken from the National Health and Nutrition Examination Survey (NHANES). Four cycles, 2005-2006 through 2011-2012, were combined to form a training data set (N = 40,790, ages <1-84) while an additional two cycles were combined to form the testing set (N = 19,225, ages 1-80). Model construction utilized the individuals in these data sets with a known presence or lack of tinnitus (training N = 11,530, ages 12-84; testing N = 6,403, ages 16-80). Our input variables were age, sex, ethnicity, education level, both recreational and work-related noise exposure, reoccurring ear infections, firearm usage, hearing protection usage, BMI, veteran status, smoking, hypertension, dyslipidemia, diabetes, and PTA in the worse ear at high frequencies (3kHz – 8kHz).
Results: Decision tree modeling produced the strongest results, with promising accuracy (>80%) for both the training and testing data sets. Worse-ear high-frequency PTA emerged as the most important predictive factor. For individuals with normal high-frequency hearing thresholds, noise exposure unrelated to work was the best secondary predictor. For those with mild high-frequency hearing loss, work-related noise exposure was the most important secondary predictor, followed by gender in those who did not have exposure. For those with more moderate high-frequency hearing loss, work-related noise exposure was also the best secondary predictor, followed by firearm usage and age in those who did have exposure. For those with the highest high-frequency thresholds, sex was the best secondary predictor followed by repeated ear infections in males and hearing protection usage in females.
Conclusions: Our model confirms the significance of many previously identified risk factors for tinnitus and provides insight into their relative importance as predictors. The emergence of worse-ear high-frequency PTA as the most influential factor aligns with previous evidence that even chronic tinnitus sufferers with normal hearing at mid-low frequencies often experience high-frequency hearing loss. The decision tree structure suggests that there are notable interactions between predictors.

A Population-Based Study of Plasma Metabolomic Profiles and Risk of Developing Persistent Tinnitus

Oana Zeleznik*[1], Raji Balasubramanian[2], D. Bradley Welling[3], Konstantina Stankovic[4], Gary Curhan[1], Sharon Curhan[1]
[1]Brigham and Women's Hospital, Harvard Medical School, [2]University of Massachusetts, [3]Harvard/Massachusetts Eye and Ear, [4]Department of Otolaryngology Head and Neck Surgery, Stanford School of Medicine
Background: Tinnitus is a heterogeneous disabling disorder that affects >740 million adults globally, and is severe in >120 million, yet treatments are limited and there is no cure. There is a critical need to improve the understanding of the diverse etiologies underlying tinnitus onset and persistence and to identify potentially modifiable risk factors that could inform targeted treatment and prevention strategies. Likely, complex interactions between individual-level and environmental factors influence tinnitus development. Accumulating evidence suggests involvement of specific metabolites and metabolic pathways in processes underlying several auditory and neurodegenerative disorders; metabolic dysregulation may also play a role in tinnitus etiology. Metabolomic assays are powerful tools to identify disease biomarkers and pathoetiologic processes. Metabolite levels are 'downstream' of transcriptional and translational processes and reflect direct input from the diet, environment and microbiome, thus the metabolomic assays are powerful tools to uncover valuable insights into how metabolic factors influence the onset of this complex condition.
Methods: We prospectively investigated the association of plasma metabolite profiles and risk of developing incident persistent tinnitus (defined as daily tinnitus lasting ≥5 minutes) among 9490 women (mean age at blood collection: 54 years) in Nurses' Health Study (NHS) and Nurses' Health Study II (NHSII). Information on tinnitus, demographic, medical, diet and lifestyle factors was collected on biennial

questionnaires. Liquid-chromatography mass spectrometry was used to measure 463 metabolites. We used logistic regression and nested models adjusted for potential confounders to estimate odds ratios [OR (95% CI) per 1 standard deviation increase in metabolite levels] and identify individual metabolites associated with risk of developing persistent tinnitus among 1603 cases and 7887 controls (no tinnitus). We used Metabolite Set Enrichment Analysis (MSEA) to identify metabolite classes associated with risk of developing persistent tinnitus. We used the number of effective tests (NEF, logistic regressions) and the false discovery rate (FDR, MSEA) to account for testing multiple correlated hypotheses.
Results: In the fully adjusted models, we identified several plasma metabolite classes that were significantly associated with risk of developing persistent tinnitus. Higher circulating levels of triglycerides and fatty acyls were statistically significantly (FDR<0.05) associated with an increased risk while higher levels of phosphatidylethanolamine plasmalogens, and steroid and steroid derivates were associated (FDR<0.05) with a lower risk of developing persistent tinnitus. Several individual metabolites (triglycerides, fatty acyls, phosphatidylethanolamine plasmalogens) were associated with risk of developing persistent tinnitus at a nominal significance level ($p<0.05$) but none remained statistically significantly after multiple testing correction (NEF-corrected $p<0.05$).
Conclusions: This first large population-based metabolomics study of persistent tinnitus identified metabolite classes that were significantly associated with risk of developing persistent tinnitus. These findings may provide insight into metabolic pathways involved in tinnitus etiology and contribute to the discovery of novel disease biomarkers and therapeutic targets for effective treatment.

Visual Evoked Potentials in Cochlear Implant Users With and Without Tinnitus Suppresion.

Mina Stojanovic[1], Joseph Chen[1], Trung Le[2], Vincent Lin[2], Andrew Dimitrijevic*[3]
[1]Department of Otolaryngology - Head and Neck Surgery, Sunnybrook Health Sciences Centre, [2]Otolaryngology—Head and Neck Surgery, Sunnybrook Health Sciences Centre, [3]Sunnybrook Hospital ENT University of Toronto
Background: Numerous studies have shown that many patients with hearing loss that have a cochlear implant (CI) experience a suppression of tinnitus when their CI is turned on. However, the neural correlates of this suppression are currently unknown. Additionally, CI patients exhibit higher degrees of cross-modal plasticity than normal-hearing (NH) individuals. Cross-modal plasticity can be quantified as the degree of auditory cortex activation to a visual stimulus. This study compared the brain activity using high density EEG (electroencephalography) elicited by visual stimuli in conditions where CI users with single-sided deafness (SSD) were experiencing tinnitus versus no tinnitus. We hypothesized that CI users with SSD that experience greater degrees of self-reported tinnitus suppression will also have greater degrees of cross-modal plasticity than SSD CI patients with less or no suppression of tinnitus.
Methods: Before the collection of EEG data, an online questionnaire was sent to existing SSD users. The survey quantified the subjective tinnitus loudness and annoyance when the CI is turned on versus off using a Likert scale from 1 to 10. Patients who completed the questionnaire completed a face versus house discrimination task with their CI on and off while recording 64-channel EEG. The task consisted of ten blocks of 40 stimuli displayed in a random order, resulting in a total of 400 stimuli. There were four different stimulus conditions: upright face, upside down face, upright house, and upside down house. Participants were required to indicate via keyboard press whether a house or face was perceived. Evoked potentials and subsequent source analysis was performed. Left and right auditory cortex regions of interest were examined for activity related to cross-modal plasticity.
Results: Of the 34 CI users with SSD that completed the survey, 21 reported a reduction in perceived tinnitus loudness ranging from complete suppression to very little suppression. A subset of the participants completed the EEG portion of the study (n=11). These initial data showed that participants with tinnitus suppression (from the CI being turned on) had elevated left and right auditory cortex activation compared to those who did not report tinnitus suppression.
Conclusions: These pilot data confirm previous studies showing that a CI can help suppress tinnitus to varying degrees. Moreover, these data also suggest that tinnitus in SSD CI users may arise from "maladaptive" cross modal activation that is reduced once the CI is switched on

A Combined Image- And Coordinate-Based Meta-Analysis of Whole-Brain Voxel-Based Morphometry Studies Investigating Subjective Tinnitus

Punitkumar Makani*[1], Marc Thioux[1], Sonja Pyott[1], Pim van Dijk[1]
[1]*Department of Otorhinolaryngology, Head and Neck Surgery, University Medical Center Groningen, Groningen, the Netherlands*
Background: Previous voxel-based morphometry (VBM) studies investigating subjective tinnitus (here referred to as simply tinnitus) have reported structural differences in a variety of spatially distinct cortical regions (Adjamian et al. 2014; Elgoyhen et al. 2015). However, results have been highly inconsistent and sometimes contradictory. In the current study, we conducted a combined image- and coordinate-based meta-analysis of whole-brain VBM studies to identify robust gray matter (GM) differences associated with tinnitus, as well as examine the possible effects of hearing loss on the outcome of the meta-analysis.
Methods: The PubMed and Web of Science databases were searched for studies published up to August 2021. Additional manual searches were conducted using BrainMap, Neurosynth, and NeuroVault websites for studies published up to December 2021. The meta-analysis was conducted using Seed-Based d Mapping with Permutation of Subject Images, which allows the combination of statistical maps from the original study results and tables of coordinates reporting significant group differences. The results of the whole-brain meta-analyses were corrected for multiple comparisons using threshold-free cluster enhancement ($P_{FWE} \leq 0.05$).
Results: Of the 153 identified studies, a total of 15 studies met the inclusion criteria, resulting in the inclusion of a total of 423 individuals with tinnitus and either normal hearing or hearing loss (mean age 51 years; 173 female) and 508 individuals without tinnitus and either normal hearing or matched hearing (mean age 52 years; 234 female). Unthresholded statistical images were obtained for 5 studies. We found a small but significant gray matter reduction in the left inferior/middle temporal gyrus for groups of normal hearing individuals with tinnitus compared to hearing-matched individuals without tinnitus. In sharp contrast, in groups with hearing loss, tinnitus was associated with larger gray matter volumes in the lingual gyrus and precuneus bilaterally. Those results appear heavily dependent upon matching the hearing levels between the groups with or without tinnitus.
Conclusions: The results of this meta-analysis suggest that the absence or presence of hearing loss is the driving force of changes in GM across individuals with and without tinnitus. Future studies should carefully account for confounders, such as hearing loss, hyperacusis, anxiety, and depression, to identify GM changes specifically related to tinnitus. Ultimately, the aggregation of standardized individual datasets with both anatomical and useful phenotypical information will permit a better understanding of tinnitus-related gray matter differences, the effects of potential comorbidities, and their interactions with tinnitus.

Symposium #3 - Brain Plasticity in Deafness

2:00 p.m. - 4:00 p.m.
Crystal Ballroom D-E

Brain Plasticity in Deafness

Chair: Pascal Barone, *CNRS UMR5549*

The symposium is aimed on the mechanisms of brain plasticity after hearing loss and their primordial role in the success of rehabilitation by neuroprosthesis. The symposium will address the mechanisms of intra- and crossmodal brain plasticity in a multidimensional approach, from animal models to the patient with a life-time perspective. The investigation will offer a multi-scale standpoint from single cell recording to functional connectivity with a specific aim to link adaptive behavior to brain reorganization.
Intramodal plasticity will be discussed in an animal model of congenital deafness (A. Kral) which shows a predominance of inhibitory interactions arising from the affected ear. This alteration in binaural integration is directly related to the observations of a loss of spatial abilities observed in UHL adults (P. Barone) whose magnitude of deficits is associated to the loss of cortical lateralization. All together these studies accounts for the constraints of delays in sequential cochlear implantation in congenitally deaf children (K. Gordon) in the restoration of spatial and binaural abilities. Similarly, in humans we will show how visual crossmodal influences largely depend on the type of single or bilateral cochlear implantation (P. Sandmann). Thus, our symposium at the crossroads of basic and clinical research proposes complementary studies as a continuum in the understanding of the neural mechanisms involved in deafness rehabilitation during development and adulthood. It is targeting a wide audience of researchers in auditory neurosciences and psychophysics, but also it is directly addressed to clinicians and professionals in hearing rehabilitation.

Deaf Ear Inhibits Hearing Ear Following Single-Sided Congenital Deafness
Andrej Kral, *Dept. of Experimental Otology, Medical University Hannover*

Performance of sequential cochlear implantations in prelingually deaf children shows that the second implanted ear underperforms in speech comprehension (review in Gordon and Kral, 2019, Hear Res). An inhibition between the ears in such condition has been suggested (Burdo et al., 2016, Eur Ann ORL), but the physiological mechanism remained elusive. In cats with single-sided congenital deafness (SSD) the aural preference shifted towards the hearing ear on both hemispheres (Kral et al., 2013, Brain). Binaural integration was also compromised (Tillein et al., 2016 Cereb Cortex). The present study investigated the nature of binaural interactions with focus on excitation and inhibition.
In the present study, 9 adult hearing controls (HCs), 9 adult bilaterally congenitally deaf cats (CDC) and 2 adult SSD cats were used. All animals were acutely electrically stimulated by cochlear implants (CI). Cortical responses were evoked by a train of 3 biphasic electric pulses (200 µs/phase, 500 Hz). Intensities up to 12 dB above auditory brainstem response threshold were used, whereas the contralateral ear was kept constant at 6 dB above threshold, and the current level of the ipsilateral ear was varied from -2 to 12 dB above threshold. Multiunit activity was recorded using 16-channels arrays covering all layers of the primary auditory cortex. Responses were classified to excitation or inhibition depending on whether the stimulation at the ipsilateral ear significantly increased or reduced the firing rate with increasing level.
In HCs, the ipsilateral ear induced inhibition of the responses to the contralateral ear in ~40% of recording sites, whereas in CDCs this proportion was smaller (~30%). In SSD animals, the deaf ear consistently induced suppression of the responses to the hearing ear in ~60% of units, whereas vice versa the hearing ear caused excitation and inhibition was exceptionally rare (< 2%). These data document the extraordinary extend of the reorganization of binaural interactions and demonstrate that the previously deaf ear causes inhibition of the responses to the hearing ear in abnormally high proportion of units. That explains why after long periods of unilateral early deafness learning speech comprehension through the previously deaf ear is difficult and does not profit from what was learned through the other ear. Early binaural hearing is necessary in preventing these adverse consequences.
Supported by Deutsche Forschungsgemeinschaft (Exc 2177) and National Science Foundation in collaboration with BMBF (DLR # 01GQ1703), and MedEl Comp., Germany.

Unilateral Deprivation in Children is Not a "Minimal" Hearing Loss: Evidence From Measures of Brain Plasticity
Karen Gordon, *The Hospital for Sick Children*

Cochlear implants are the standard of care for children with bilateral deafness but are less readily adopted for children who have one deprived ear including those with single sided deafness (SSD). This discrepancy has several potential origins including a clinical notion that audibility in one ear is "good enough" and persists despite consistent reports of developmental delays in children with unilateral hearing loss. In this symposium talk, evidence of cortical reorganization measured by EEG in children who have unilateral deafness and/or asymmetric hearing will be presented. Effects of age as well as duration of unilateral deafness and/or asymmetric hearing on responses from auditory cortices and cortical connectivity will be discussed in context with implications for promoting binaural/spatial hearing through cochlear implantation.

Restoring Cortical Processing for Spatial Hearing Following Cochlear Implantation for Asymmetric Hearing Loss
Pascal Barone, *CNRS UMR5549*

A cortical lateralization is a functional organization observed in most sensory modalities and in the auditory domain, the functional lateralization is devolved to spatial hearing, with each hemisphere primarily involved in processing the localization of sound in contralateral space. The representation of the contralateral auditory field is underpinned by contralateral aural dominance which results from complex neural interactions between inputs from each ear. We showed (Vannson et al 2020) that unilateral hearing loss (UHL) induces deficits in binaural integration (spatial localization and speech understanding in noise) but also reverses contralateral aural dominance in favor of the preserved ipsilateral ear. The extent of this brain reorganization

is directly correlated to the extent of spatial behavioral deficit. A second study (Karoui et al 2022) in a group of UHL patients treated with a cochlear implant demonstrated that restoration of auditory inputs to the deaf ear through electrical stimulation restored contralateral hemispheric dominance of both the better and impaired ear. Finally, mirroring what was observed in UHL patients, the extent of restoration of contralateral dominance was directly correlated with the ability to localize sounds after implantation. Lastly in a 3rd group of UHL patients we observed clearly a subgroup of subjects with near normal sound localization performances and a near normal spatial Mismatch Negativity (MMN) a neural marker of spatial abilities. This suggest the existence of behavioral adaptive linked to adaptive brain compensation.
Altogether, we clearly demonstrated a link between brain reorganization and spatial auditory performance in deafness. Our results are crucial for further progress in the rehabilitation of unilaterally deaf patients and show that the success of rehabilitation depends mainly on brain plasticity mechanisms, and that the restoration of contralateral dominance is essential for an optimal functional recovery.

Enhanced Audio-Visual Interactions in Cochlear-Implant Users
Pascale Sandmann, *University of Cologne*

Cochlear implantation has been a well-established procedure to treat patients with severe to profound sensorineural hearing loss. However, a cochlear implant (CI) provides only limited spectro-temporal information, and after implantation, the central nervous system needs to learn to recognise the new, artificial input as meaningful sounds. Interestingly, these cortical changes – also referred to as neuronal plasticity – are not restricted to the auditory system. Rather, CI users show altered cortical processing of both auditory and visual stimuli (Stropahl et al., Hearing research, 2017). In addition, the CI users reveal an increased interaction between the auditory and visual system, which allows improved recognition of speech and environmental sounds when CI users have simultaneous access to auditory and visual information (Radecke et al., NeuroImage Clinical, 2022). This remarkable (behavioural) audio-visual gain in CI users is supported by electroencephalography (EEG) results, which suggest that in the CI users, visual stimuli have a stronger influence on auditory processing when compared to the normal-hearing listeners (Layer et al., NeuroImage Clinical, 2022). Preliminary results suggest that this visual modulation effect is more pronounced in CI users with bilateral hearing loss compared to CI users with unilateral deafness. Nevertheless, both groups of CI users show improved lip-reading ability and increased recruitment of the visual cortex during the processing of auditory and audio-visual speech stimuli when compared to normal-hearing listeners. Overall, these results indicate that CI users develop altered multisensory processing and visual enhancements to compensate for the limited auditory signal provided by the CI.

gEAR Workshop - Explore and Analyze Basics: Creating Gene Carts and Dataset Comparisons

4:00 p.m. - 5:15 p.m.
Biscayne 1

This will be a walk-through of the basic features of the portal, giving the background needed for the more tool-specific sessions. Topics include account creation, basic gene expression searches, data sharing, site navigation and overall feature discussion. We will also overview some of the new features of gEAR and point users to online webinars where they can learn more about them.

Explore and Analyze Basics: Creating gene carts and dataset comparisons (45 min)
Learn how to create collections of genes (carts) which can be used with many tools on the portal. Then, see how the dataset comparison tool is used to visualize differences in expression between two conditions of the same dataset, with statistical tests. This workshop includes a hands-on component, bring your laptop along.

Ronna Hertzano, *University of Maryland School of Medicine*
Joshua Orvis, *Institute for Genome Sciences*

Charles Steele Symposium

4:15 p.m. - 6:15 p.m.

Crystal D-E

Honoring Charles Steele

Chair: Sunil Puria, *Harvard Medical School, Mass. Eye and Ear Infirmary*
Co-Chair: Elizabeth Olson, *Columbia University*

In this special symposium, we plan to honor the lifetime contributions by Prof. Charles Steele to the field of Cochlear and Middle Ear mechanics.

Charles R. “Chuck” Steele, professor emeritus of mechanical engineering and of aeronautics and astronautics at Stanford University, contributed significantly to cochlear and middle ear mechanics, died Dec. 9, 2021 in Redwood City, CA. He was 88. Steele’s first paper on the cochlea was published in 1974 and many of his subsequent works on the topic are still cited today. In total, it is estimated Steele published some 100 journal articles, five review articles and numerous abstracts in conference proceedings, while delivering guest lectures around the world. As an editor, he was perhaps even more influential, becoming editor-in-chief of the International Journal of Solids and Structures. As teacher, Steele was similarly admired. He taught plates and shell structures, differential geometry and the mechanics of hearing to graduate students. He mentored more than 70 doctoral students through their dissertations and maintained contact with many of them throughout their careers. Professional recognition followed these accomplishments. He was elected a fellow of American Society of Mechanical Engineers in 1980 and the American Academy of Mechanics in 1985. In 1995, he became a member of the National Academy of Engineering. In 1988, the National Institutes of Health bestowed the Claude Pepper Award upon Steele for his work in hearing. In 2014, he was given the lifetime achievement award at the Mechanics of Hearing meeting in Greece.

Charles Steele – The “Mad” Scientist
Kelly Steele, *Consultant*

George Springer, longtime colleague of Charles and former chair of Stanford Aeronautics and Aerospace Department says Charles is a “mad” scientist– which best describes him in many ways. Charles is not a “normal” scientist. He is a musician who plays French Horn professionally. He is an avid opera fan who would tell you everything about an opera with sparking eyes. He is a multilingual who had worked on Russian, German, Chinese, Spanish, Swedish, Portuguese and delivered speeches with native language in different counties. He is a birder who would walk into woods, hear bird calling and tell you what kind of bird it is. He kept a schedule of going to gym every morning at 5:30 and going to work at his office till before the Pandemic closedown at Stanford. He practically worked till the last moments of his life by advising students, writing papers and collaborating with his colleagues and partners worldwide. I’d love to share more personal aspects of Charles life with everyone at the Symposium.

Charles Steele’s Entrance Into Cochlear Mechanics: JASA Papers From the 1970s Through 1980
Elizabeth Olson, *Columbia University*

My presentation will review the Steele and Steele + Taber papers published in the Journal of the Acoustical Society of America during the 1970s and through 1980. These papers described the basic physics of the cochlea, showing how the cochlear traveling wave and passive tuning emerged from known physical properties. These papers treated the fluid component of cochlear mechanics with quantitative care, producing the "Effective fluid mass" curves that are fundamental to understanding the dispersive properties of the cochlear traveling wave. Charles Steele's pre-cochlear-amplifier work remains central to our current understanding of cochlear mechanics. After the discovery of cochlear amplification, Steele and colleagues developed an active cochlear model that employs the longitudinal coupling of the organ of Corti to produce active tuning. My talk will focus on Steele's early work from the 1970s-1980, in which he laid down the passive substrate for active mechanics.

From Rockets to Plant Growth, Charles Steele Leaves His Positive Impact
Karl Grosh, *University of Michigan*

I first came to know Charles in his role as a member of the Applied Mechanics faculty while I was a student at Stanford, and have been lucky to have him as a colleague in cochlear mechanics for many years. Charles taught a canonical sequence of classes on the mechanics of plates and shells. I will recount a delightful spring term I spent learning shell theory from Charles, who always brought a human view of the material he covered. The Applied Mechanics community was tight-knit back in the 1990's, and I enjoyed interacting with Charles's students and learning of the diversity of topics his group covered. Charles made an impact in four different areas: the analysis of shells as structural elements, bone remodeling and density estimation, cochlear mechanics, and plant morphogenesis, an amazing breadth of study. Charles's work has been recognized in many ways; one was the 1999 ASME Koiter medal. In his acceptance presentation for that award, he lucidly described some of his work on the influence of shell buckling in plant morphogenesis, and I will endeavor to summarize his work with P. B. Green, J. Dumais, and other colleagues in this area. Finally, I wanted to recognize Charles's incredible service to the community as editor of the influential International Journal of Solids and Structures (Editor in Chief from 1985-2005!). With his passing, we have lost a giant in the field and a role model for how engineers can make an impact in biology.

How the Analytical WKB Approach to 3d Cochlear Models by Charles Steele is Still the Basis for Our Understanding of Cochlear Mechanics

Renata Sisto, *INAIL Research*

Although numerical models may be more efficient for including realistic details of the Organ of Corti in cochlea models, the analytical approach to cochlear mechanics still prevails whenever a deeper intuitive understanding of the most relevant phenomena is required.
Charles Steele used his uncommon mathematical skill to elaborate cochlear model in terms of the most relevant physical phenomena and their equations. His 3d WKB models are probably still the best way to fully describe the cochlear phenomenology in terms of fluid field dynamics and fluid-structure interaction. In this presentation we will show how the most relevant aspects of the cochlear hydrodynamics had already been taken into account by Steele's WKB solutions, developed in the 70's, and propose our interpretation. The complete approach to the 3d hydrodynamics of the cochlea permits the correct understanding of an important short-wave effect, which we name the "fluid focusing effect". This effect is related to the flux conservation of an incompressible fluid, and becomes relevant in the "peak region", where the traveling wave (TW) wavelength is shorter than the cochlear duct height, H. In this region, only a layer of thickness comparable to wavelength of fluid is involved in the TW dynamics and in the energy exchange between structure and fluid. Charles took this phenomenon into account by defining an effective mass of the system reduced by a factor of order of the ratio between H and the wavelength of the traveling wave in the short-wave region. Following Shera et al. (2005), we describe this effect as a boost to the differential pressure, driving the BM motion, produced by a geometrical shape factor.
In the 3d fluid field equations developed by Charles, the effect of viscosity in the peak region is also analytically taken into account. Without the damping effect of viscosity, which in the peak region is also proportional to the local wavenumber, it is quite difficult to get the stabilization of the response that permits to get both a high peak gain and a rapid decay of the response immediately after the peak. Recently, we showed how the pressure focusing and viscous damping effects can be included in a correction to the 1d transmission line admittance. Charles had understood the importance of geometrical and viscous effects from the beginning of his activity. Based on this ansatz, he was also able to give accurate estimates of the power balance in the traveling wave propagation.

Charles Steele's Contributions to Numerical Solutions for Shells of Revolution

Sunil Puria, *Harvard Medical School, Mass. Eye and Ear Infirmary*

Charles Steele was a gentle soul and I miss his calm demeanor. We started working together when I moved to the Bay Area in 1997. Charles contributed to many fields but in our field, he was known for cochlear mechanics. We co-advised many trainees together and shared a flourishing lab. As he learned more about the middle ear, he said 'who knew that the middle ear was so interesting.' He was driven by curiosity. When I get stuck on a problem, I often think about how Charles would address this. But fortunately for us, he left us with many breadcrumbs to follow in the scientific literature. In addition to the 3D WKB approach, one of the computational approaches he developed is the FAST4 method and its application to the organ of Corti. This approach used axisymmetric shells of revolution and allowed calculations at multiple length scales

from the nano-meter dimension tip links to the mm scale fluid spaces. This was an alternative finite-element modeling approach but one that was far more efficient. He would boast that he could compute organ of Corti responses in a matter of seconds on his laptop while standard finite-element approaches took days for the same problem. I will attempt to summarize a few of his papers in this area (Steele and Shad, 1995; Steele et al. 2009). He was my primary collaborator at Stanford, but he mentored all of us in the Mechanical Engineering Department. Just a few weeks before he passed away, he provided constructive feedback on a paper we co-authored. How amazing! He gave us hope that as we ourselves get older, we can do good science late into life.

Emeritus Professor Charles Steele
Anthony Ricci, *Stanford University*

I knew of Charles's work since I was graduate student, but I did not get to know Charles until 2006 when I moved to Stanford. At that time, Charles had emeritus status, something I never realized until years later because his work ethic, his enthusiasm for science and for mentoring and his productivity were unmatched. During the past several years, Charles was a regular member of our lab meetings and journal clubs and regardless of the topic had information and insight and a unique perspective. You always felt good after talking with Charles. I will present on several recent projects with Charles that highlight his creativity, his ability to simplify difficult questions and his diversity. Examples include calculating diffusion coefficients for dyes within the lipid bilayer, estimating pressure drops across cochleostomies, and estimating fluid flow around sensory hair bundles.

Mentoring the Love of Life Beyond WKB and Finite Element Modeling of the Cochlea
Yanli Wang, *Harvard Medical School, Massachusetts General Hospital*

Charles not only made cochlear mechanics fascinating and beautiful for me, but also filled my world with wonder and warmth. I was Charles' last PhD student working with him from 2013 to 2018. Our first work investigates the energy flow in the cochlea based on a 3D WKB model of the mouse cochlea. Charles always had the magic to make the equations and symbols dance in front of my eyes. Charles' curious and patient eyes can inspire the dullest soul. My second project was an experimental project investigating the in situ motion of individual inner hair cell stereocilia. For an engineering student, learning wet lab experiments from ground zero wasn't easy. Charles' encouragement was paramount, not to mention the support of quick hands-on finite element models here and there in parallel with the experiments, for example, the investigation on the effect of the holes on the pressure drop in the cochlea. Long after I graduated, it was still fun to pick up the phone and talk about my newest model with Charles, not to mention the road trip he and his wife took me on to visit relatives and friends, and the cultural gems in Germany. A true mentor like Charles doesn't just teach engineering and science with clarity, passion, and beauty, but also embodies the groundedness and spaciousness in front of difficulties, the wisdom of staying true and humble, and a deep love to the world, to the people, and to the earth. There is nothing more humbling than Charles' humbleness and loving presence. The impact of a PhD advisor on their students can be a lifelong fortune, and I definitely consider myself one of the most fortunate to be under Charles' wings of wisdom and love. This heart is forever changed to be warmer, more patient, and more loving. Thank you Charles.

ARO Diversity and Minority Affairs Workshop: Microaggressions, Allyship and Equitable Mentoring

4:15 p.m. - 6:15 p.m.
Crystal C

Microaggressions, Allyship and Equitable Mentoring

Chair: Tejbeer Kaur, *Creighton University*
Co-Chair: Radha Kalluri, *University of Southern California*
Co-Chair: Melanie Barzik, *National Institutes of Health*
Presenters:
Melanie Barzik, *National Institutes of Health*
Matheus Macedo-Lima, *University of Maryland - College Park*

Diana Peterson, *Philadelphia College of Osteopathic Medicine*
Radha Kalluri, *University of Southern California*
Tejbeer Kaur, *Creighton University*

The Association for Research in Otolaryngology (ARO) is committed to increasing diversity, equity, and inclusion in science by fostering a welcoming community that removes barriers and embraces diverse people, approaches, and ideas. Despite ongoing efforts, the ARO community is still largely non-diverse and overrepresented by Caucasian/white and male-identifying members. Thus, the first objective of this workshop is to provide participants with an overview of the demographics of the ARO community with the goal of identifying areas that could be targeted for action towards increasing diversity. Moreover, the lack of diversity in the ARO community could be representative of a known larger issue in higher education – the "leaky pipeline" problem, i.e., the progressive evasion of women and people from minority groups at each stage of the educational system. With this phenomenon in mind, the second objective of this workshop is to provide strategies to improve the experience of and support for underrepresented populations in our communities, such as how to recognize and avoid microaggressions, equitable mentoring, anti-racism and effective allyship.

This 2 hour workshop will commence with a ~15-minute platform presentation by a Diversity and Minority Affairs Committee member on ARO demographics, followed by a ~30-minute platform presentation Dr. Sharon Milgram from the Office of Intramural Training and Education at the National Institutes of Health to introduce the topics. In the next 45 minutes, the presenters will facilitate discussions and guide group activities with 4 - 6 participants per group, making use of pedagogic approaches such as case studies and role-play. In summary, this workshop aims to raise awareness about the demographic imbalances in the ARO community, and to provide ARO members with tools for supporting and promoting diversity in their own work environments and organizations. In addition, the small group discussions aim to assist workshop attendants with tools for identifying and avoiding microaggressions, practicing powerful allyship, and becoming a more inclusive and equitable mentor.

NIDCD SBIR/STTR Grants for Product Development Workshop

6:00 p.m. - 7:30 p.m.
Labrid A

Roger Miller*, NIDCD/NIH*
Shiguang Yang*, NIDCD/NIH*

NIDCD Early Stage and New Investigators Workshop: Navigating the NIH

6:00 p.m. - 7:30 p.m.
Labrid B

Janet Cyr*, NIH/NIDCD*
Andrea Kelly*, NIDCD/NIH*

NIDCD Research Training and Career Development Workshop

6:00 p.m. - 7:30 p.m.
Mako

Alberto Rivera-Rentas*, NIDCD/NIH*
Katherine Shim*, NIDCD/NIH*

Sunday, February 12, 2023

Podium #4 - Hearing Loss: Consequences and Adaptation

8:00 a.m. - 10:00 a.m.
Ocean's Ballroom 5-12

Moderators: Fan-Gang Zeng and Carolyn McClaskey

Frequency-Following Responses in Subjects With Sensorineural Hearing Loss: A Systematic Review

Laura Jacxsens*[1], Lana Biot[1], Annick Gilles[1], Vincent Van Rompaey[1], Carles Escera[2], Willem De Hertogh[3], Marc J.W. Lammers[1]
[1]Antwerp University Hospital, [2]University of Barcelona, [3]University of Antwerp

Background: Scalp-recorded frequency-following responses (FFRs) are sustained neuro-electrical potentials representing the periodicity of acoustic stimuli and can be used to reveal the integrity of sound processing. They are believed to be generated predominantly in the auditory midbrain. Both the envelope (F0) and temporal fine structure (TFS) component of sound are represented in FFRs and are, therefore, appropriate for studying the mechanisms underlying sound processing and speech recognition. The present systematic review aims to assess whether hearing loss has an impact on different FFR parameters.
Methods: A systematic review was performed and reported according to PRISMA guidelines. The online databases PubMed, Web of Science, and Scopus were searched up to September 2022. Studies evaluating FFRs in patients with hearing loss and normal-hearing controls were included. No restriction on age or severity of hearing loss was implemented. Two independent reviewers performed study selection, data extraction, and risk of bias assessment.
Results: Thirteen case-control studies met our inclusion criteria. Acquisition parameters differed widely across studies. Nine of the studies used the speech stimulus /da/, however, with varying stimulus duration, intensity, and presentation rate. The most frequently analyzed FFR parameter was the F0. Five studies reported a statistically significant smaller envelope in patients with hearing loss. Conversely, three studies reported a significantly larger envelope and two studies did not find a significant difference between both groups. TFS was studied in six studies, three of which found no significant difference between patients with hearing loss and controls. In the time domain, all four studies that investigated latencies reported prolonged latencies in patients with hearing loss. The specific waves that were prolonged, differed across studies. There was no consensus across studies regarding the wave amplitude in the time domain.
Conclusions: Patients with hearing loss may require a longer time for processing (speech) stimuli, reflected in the prolonged latencies. It is however not clear if this delay occurs in the onset, transition, steady-state, or offset of the response. No definite conclusions could be drawn on the influence of hearing loss on wave amplitude in the time domain, the envelope component, and the TFS. Subject characteristics, acquisition parameters, and FFR outcome parameters differed greatly across studies. The instability in results can partially be explained by the fact that several studies obtained FFRs with a short stimulus of about 40 ms, which is too short to cause a proper neural phase locking. It is also notable that most studies used the same stimulus intensity for hearing loss subjects as for controls. Moreover, ages of the subject groups significantly differed in several studies, which possibly also affected results of individual studies. Future studies should be performed in larger subject groups, with longer stimuli presented at maximum loudness level in subjects with hearing loss.

Relationships Between Cognitive Abilities and Benefit From Visual and Contextual Cues in a Speech-In-Noise Recognition Task in Individuals With Normal Hearing and Hearing Loss

Andreea Micula*[1], Emil Holmer[1], Ruijing Ning[1], Henrik Danielsson[1]
[1]Linköping University

Background: Visual and contextual cues facilitate speech recognition in sub-optimal listening conditions (e.g. background noise, hearing loss). Moreover, successful speech recognition in challenging listening conditions is linked to cognitive abilities such as working memory capacity and fluid intelligence. However, it is unclear which cognitive abilities facilitate the effective use of visual and contextual cues in individuals with normal hearing and hearing loss. The first aim was to investigate whether individuals with hearing loss rely on visual and contextual cues to a higher degree than individuals with normal hearing in a speech-in-noise recognition task. The second aim was to investigate whether working memory capacity and fluid intelligence are associated with the effective use of visual and contextual cues in these groups.
Methods: Groups of participants with normal hearing (NH) and hearing aid users (HA) were included (n = 169 per group). The Samuelsson and Rönnberg task was administered to measure speech recognition in speech-shaped noise. The task consists of an equal number of sentences administered in the auditory and

audiovisual modalities, as well as without and with contextual cues (visually presented word preceding the sentence, e.g.: “Restaurant”, “Train”). The signal-to-noise ratio was individually set to 1 dB below the level obtained for 50% correct speech recognition in the Hearing-In-Noise test. All participants received linear amplification, the HA group based on individual audiometric thresholds and the NH group 20 dB flat gain. The Reading Span test was used to measure working memory capacity and the Raven test was used to measure fluid intelligence. The data were analyzed using linear mixed effects modelling.
Results: Both groups exhibited significantly higher speech recognition performance when visual and contextual cues were available. A two-way interaction showed that the HA group performed significantly worse compared to the NH group in the auditory modality but performed on par with the NH group in the audiovisual modality. A three-way interaction suggested that the group difference in the auditory modality was moderated by the Raven test score. Additionally, a significant positive relationship was found between the Raven test score and speech recognition performance only for the HA group in the audiovisual modality. There was no significant relationship between the Reading Span test score and performance.
Conclusions: Both NH and HA participants benefited from contextual cues, regardless of cognitive abilities. The HA group relied on visual cues to a higher degree than the NH group, reaching a similar speech-in-noise recognition performance level as the NH group in the audiovisual modality despite a worse performance in the auditory modality. Importantly, the effective use of visual cues was associated with higher fluid intelligence in the HA group.

Societal Costs and Long-Term Outcomes of Severe to Profound Hearing Loss

Ivette Cejas*[1], David Barker[2], Esteban Petruzello[1], Christina Sarangoulis[1], Alexandra Quittner[3]
[1]University of Miami Miller School of Medicine, [2]The Alpert Medical School of Brown University, [3]Behavioral Health Systems Research

Background: According to the Project HOPE study, cost estimates for individuals with severe to profound hearing loss is $297,000 over a person’s lifetime (Mohr et al., 2000). As the landscape of hearing healthcare continues to change with hearing technology and expanded criteria for cochlear implantation, more research is needed on the societal costs of hearing loss and long-term outcomes post-implantation. The aims of the current study are 1) to estimate the lifetime costs of severe to profound hearing loss (SPHL) and 2) evaluate academic achievement and quality of life outcomes in adolescent children using CIs.
Methods: To estimate current lifetime costs and burden of SPHL, we used the 2021 Medicare rates for CPT codes, IDEA estimate of twice the cost-per-pupil of public education supplemented by a written report for the California state legislature on these costs (Taylor, 2016), and the 2018 National Health Interview Survey (NHIS).
The current study used several national available data sources, including the Childhood Development after Cochlear Implantation (CDaCI), National Longitudinal Transition Study – 2 (NLTS-2), and published samples of non-implanted children (Meyer et al., 2013, Rachakonda et al., 2014, and Umansky et al., 2011) to assess the long-term educational and quality of life outcomes for children with SPHL with and without CIs. Measures included: Woodcock-Johnson III (academic achievement), Comprehensive Assessment of Spoken Language (spoken language), Pediatric Quality of Life Inventory (quality of life), and Youth Quality of Life Instrument – Deaf and Hard of Hearing (quality of life).
Results: The overall societal cost of SPHL for the birth cohort 2020 is estimated at $45.58 billion. The breakdown of educational and medical costs will be presented. Additionally, individuals with SPHL earned less income with the largest income gap happening to individuals 30 – 45 years of age (SPHL: $30, 127; No SPHL: $53,319) and lower educational attainment compared to individuals without SPHL (33.42 vs 45.62% completed higher education). More importantly, children with cochlear implants consistently had higher academic achievement scores, particularly in reading comprehension and writing compared to non-implanted samples. Adolescents with CIs also reported better QoL on the generic PedsQL measure across all subscales compared to the non-implanted comparison samples abstracted from the published studies. The differences among the implanted CDaCI cohort and non-implanted samples were larger than the established minimally clinical important difference score of 4.4 for the PedsQL.
Conclusions: Overall, while the medical costs are higher for individuals who pursue cochlear implantation, they are ultimately offset by higher earning potential and higher education achievement compared to those with SPHL who do not pursue cochlear implants. Children with cochlear implants also out perform non-implanted children in educational outcomes and quality of life.

The Role of Central Gain in Auditory Processing Deficits of Older Adults

Kelly Harris*[1], James Dias[1], Carolyn McClaskey[1], Judy Dubno[1]
[1]*Medical University of South Carolina*

Background: Our recent research demonstrated that increased central gain, shown to be related to auditory nerve (AN) deficits and decreased GABA levels, negatively affects speech recognition in noise (SIN). These associations occur independently of the effects of pure-tone thresholds on SIN, suggesting that individual differences in GABA not related to age or hearing loss contribute to SIN. There is accumulating evidence in both animals and humans that decreased cortical inhibition and central gain may negatively affect spectral, spatial, and temporal processing, contributing to deficits in SIN. However, the extent to which central gain and decreased GABA contribute to well-established deficits in temporal processing and signal-in-noise detection in older adults remains largely unknown. Here, temporal processing was assessed by examining changes in cortical P1 response amplitudes as a function of click presentation rate. Signal-in-noise encoding was assessed by examining differences in P1 response amplitudes for conditions in which the click is presented in broadband noise and in which the click is presented in quiet. We predicted that increased central gain in older adults would be associated with larger effects of click rate and background noise on P1 response amplitudes.
Methods: Participants were older (55+ y/o) and younger (18-30 y/o) adults. AN compound action potentials (CAP-N1) and cortical P1-N1 responses were elicited by a 100 dB pSPL click. Associations between CAP-N1 and P1 amplitudes were modeled in younger adults and the resulting coefficients were applied to the CAP-N1 responses of older adults to estimate older adults' predicted cortical P1 response amplitudes. Observed P1 values greater than predicted was used to quantify central gain in older adults. We then determined effects of central gain on two measures of temporal processing as assessed by P1 response amplitudes. Proton Magnetic Resonance Spectroscopy was collected in a subset of participants to estimate cortical levels of GABA.
Results: CAP-N1 responses to clicks were smaller in older adults than in younger adults, but cortical P1 responses to clicks presented at a slow rate in older adults were similar to or larger than those in younger adults. Smaller CAP-N1 response amplitudes were also associated with reduced cortical levels of GABA, which were associated with increased central gain. P1 response amplitudes decreased in broadband noise as compared to quiet and with increasing click presentation rate and both effects were associated with increased central gain.
Conclusions: Greater central gain in older adults was associated with a stronger detrimental effect of background noise and faster stimulus rate on cortical P1 response amplitudes. These results suggest that, although central gain may help maintain cortical responses in quiet, these same mechanisms may be maladaptive for listening in challenging conditions.

Association of Hearing Aids and Cochlear Implants With Cognitive Decline and Dementia: A Meta-Analysis of Longitudinal Studies

Brian Sheng Yep Yeo*[1], Harris Jun Jie Muhammed Danial Song*[1], Emma Min Shuen Toh[1], Li Shia Ng[2], Cyrus Su Hui Ho[3], Roger Ho[4], Reshma Aziz Merchant[5], Benjamin Kye Jyn Tan[1], Woei Shyang Loh[2]
[1]*Yong Loo Lin School of Medicine, National University of Singapore, Singapore,* [2]*Department of Otorhinolaryngology-Head and Neck Surgery, National University Hospital, Singapore,* [3]*Department of Psychological Medicine, Yong Loo Lin School of Medicine, National University of Singapore,* [4]*Department of Psychological Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; Institute of Health Innovation and Technology (iHealthtech), National University of Singapore, Singapore.,* [5]*Geriatric Medicine, Department of Medicine, National University Hospital, Singapore, Singapore*

Background: Hearing loss is associated with cognitive decline. However, it is unclear if hearing restorative devices may have a beneficial effect on cognition. We sought to evaluate the associations of hearing aids and cochlear implants with cognitive decline and dementia.
Methods: The review was conducted in accordance to the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) Guidelines. Two authors independently searched PubMed, Embase, and Cochrane databases from inception to 23 July 2021 for randomised clinical trials or observational studies published as full-length articles in peer-reviewed journals relating to the effect of hearing interventions on cognitive function, cognitive decline, cognitive impairment and dementia in patients with hearing loss. Maximally adjusted hazard ratios were used for dichotomous outcomes and ratio of means for continuous

outcomes. We also investigated sources of heterogeneity using sensitivity and subgroup analyses, and assessed publication bias using visual inspection, Egger's test and trim-and-fill.
Results: We screened 3243 studies and included 31 studies (25 observational studies, 6 trials) with 137,484 participants, of which 19 (15 observational studies, 4 trials) were included in our quantitative analyses. Meta-analysis of eight studies with 126,809 participants studying long-term associations between hearing aid use and cognitive decline showed significantly lower hazards of any cognitive decline among hearing aid users compared to participants with uncorrected hearing loss (HR=0.81, 95% CI=0.76–0.87, I2=0%). Additionally, meta-analysis of 11 studies with 568 participants studying the association between hearing restoration and short-term cognitive test score changes revealed a 3% improvement in short-term cognitive test scores after the use of hearing aids (RoM: 1.03, 95% CI: 1.02-1.04, I2=0%).
Conclusions: Usage of hearing restorative devices by participants with hearing loss is associated with a 19% decrease in hazards of long-term cognitive decline. Furthermore, usage of these devices is significantly associated with a 3% improvement in cognitive test scores that assessed general cognition in the short-term. A cognitive benefit of hearing restorative devices should be further investigated in randomised trials.

Challenges of Auditory Model-Based Diagnostics

Mathias Dietz*[1], Anna Dietze[1], Jörg Encke[1], Jonas Klug[1], Julia Zimmer[1], Dirk Oetting[2]
[1]University of Oldenburg, [2]Hörzentrum Oldenburg
Background: Auditory models can be employed to fit almost any specific data set a modeler would want to fit. Models have become more comprehensive and even models of perception are built on an increasingly solid physiological foundation. From here, attempts have been made to simulate various hearing impairments, often with some form of diagnostic goal. For model-based diagnostics, models have to be capable of actual predictions rather than postdictions. Furthermore, models face new challenges in the inherently individual nature of diagnosis. Future models which are capable of comprehensively simulating an impaired individual should also be able to predict the aided performance of this individual and thus help to optimize hearing aid fitting.
Methods: This overview talk first summarizes the prerequisites for model-based diagnostics and reviews past studies. We then report examples from binaural hearing that have imposed a significant challenge to our model-based diagnostics approach. We use auditory models as artificial patients and fit model parameters so that its simulated data matches that of the actual patient. The fitted model parameters represent the diagnosis.
Results: One example is a study in 19 patients with asymmetric hearing thresholds. We confirm a long-known issue that equal left-right loudness in sequential stimulation can lead to a strongly lateralized percept, even when stimulating simultaneously with the loudness-balanced levels. Large individual deviations from this trend are observed. Exceptionally large binaural broadband loudness summation is found in many but not all patients.
Conclusions: As soon as hearing impairment is more complex than a symmetric single cause, individuals perceive sound in unique ways that cannot be simulated by present day models. If we do not want to give up on the long-term goal of a comprehensive model-based diagnosis, larger-scale efforts are necessary to develop a range of tools, models with the right number of diagnostically relevant parameters, and openly available experimental data and metadata from both clinical and basic research studies.
Supported by the European Research Council (ERC) under the European Union's Horizon 2020 Research and Innovation Programme grant agreement No. 716800 (ERC Starting Grant to M.D.)

Stress Affects Central Compensation of Neural Responses to Cochlear Synaptopathy in a cGMP-Dependent Way

Morgan Hess*[1], Daria Savitska[1], Dila Calis[1], Philine Marchetta[1], Lukas Rüttiger[1], Marlies Knipper[1], Wibke Singer[1]
[1]Molecular Physiology of Hearing, University of Tuebingen
Background: Increasing evidence supports a link between hearing loss and dementia. In a mouse model, we previously demonstrated that an age-related cochlear synaptopathy (decoupling of inner hair cell synapses from auditory nerve fibers) leads to poorer temporal auditory and memory-related processing. We could show that cochlear synaptopathy can, in some individual cases, be centrally compensated through enhanced input/output function of auditory brainstem responses (neural gain), preventing an age-dependent temporal

discrimination loss. Therefore, mice can be subdivided by their compensation capacity into a group of low compensators and another group of high compensators.
Methods: We utilized a reporter mouse line allowing for the quantification of activity-dependent usage of brain-derived neurotrophic factor (Bdnf) exon-IV and -VI. We measured hearing function in these mice through auditory brainstem response and auditory steady state response, then injected them for 10 days with the "memory-enhancing" phosphodiesterase 9A inhibitor or a placebo. After this time, we again measured hearing function, then performed long-term potentiation or histology in brain slices.
Results: Low compensators displayed an associated decrease in temporal sound coding, memory-linked processes, and recruitment of activity-dependent Bdnf transcripts in hippocampal regions in comparison to high compensators. We further found that successful modulation of this phenotype was dependent upon a normal stress response.
Conclusions: The successful central auditory- and memory-dependent adjustment to cochlear synaptopathy is a cGMP- and glucocorticoid-dependent process.

Neural Entrainment of a Naturalistic Conversation in Varying Working Memory Loads

Priyanka Prince*[1], Joseph Chen[2], Trung Le[2], Vincent Lin[2], Andrew Dimitrijevic[3]
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Background: In a noisy environment with auditory and visual distractions, selective attention to target stimuli can be cognitively demanding especially in individuals with a hearing impairment or using a hearing protheses such as a cochlear implant (CI). CI users have been shown to rely more on visual input for the understanding of speech stimuli; this can result in an increased listening effort and therefore, more resources utilized from a limited cognitive load. The neural basis of this relationship between cognition and speech perception and understanding is not fully understood.
Methods: In this study, using a high-density electroencephalogram (EEG) in normal hearing adults, we investigated the neural correlates of speech entrainment to two people having a conversation with background multitalker noise whilst visual digits appeared on the screen around them. The participant task was to answer conversation content questions and recall the digits that were presented. Three memory loads were assessed, no digits, three digits and seven digits.
Results: Behavioural results showed that as visual load increases, performance on recall for both the conversation and digits decrease. The degree of neural entrainment varied as a function of memory load such that larger memory load resulted in greater neural tracking.
Conclusions: These data suggest that non-specific, cross-modality attention increases auditory-speech encoding. These data provide evidence that natural conversations be used as a stimulus when probing cognitive functions related to speech in noise listening and working memory.

Podium #5 - From Cortical Processing to Perception

8:00 a.m. - 10:00 a.m.
Oceans Ballroom 1-4

Moderators: Bonnie Lau and Andrew Oxenham

Decoding Auditory Working Memory Content From Intracranial Broadband High Frequency Activity

Isil Uluc*[1], Parker Kotlarz[1], Angelique Paulk[2], Alan Bush[2], Valentina Gumenyuk[3], Kaisu Lankinen[1], Teppei Matsubara[1], Nao Matsuda[1], Gavin Belok[2], Alexander Zhang[2], Daniel Soper[2], Jessica Chang[2], Sonika Agarwal[2], Alexandra O'Donnel[2], Dustine Reich[4], Virginia Rosenberg[2], Steven Stufflebeam[1], Mark Richardson[2], Sydney Cash[2], Jyrki Ahveninen[1]
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Background: The neural mechanism underlying the maintenance of relevant auditory information in working memory (WM) remains a significant question. Research on other primates has led to a discussion on whether WM maintenance is based on persistent delay-period firing activity, alternatively, on more dynamic, distributed activation patterns modulated by an underlying synaptic trace. Unfortunately, the prevailing non-invasive neuroimaging methods, such as fMRI or magnetoencephalography and EEG, provide limited means to test these competing hypotheses on humans.
Methods: Here, we investigated auditory WM in 11 patients with medically intractable epilepsy who were implanted with intracranial stereo-EEG (sEEG) depth electrodes in their brains for presurgical monitoring. The neuronal mechanism underlying WM was inferred from broadband high-frequency activity (BHA) patterns, a putative marker of local firing activity. During sEEG recordings, the participants performed a retro cue WM paradigm with parametric auditory ripple sounds as to-be-remembered stimuli. We used multivariate pattern analysis with non-parametric permutation testing to show that content-specific WM information can be decoded from BHA in superior temporal and inferior frontal brain areas during information maintenance.
Results: The areas containing auditory spatiotemporal WM information did not appear to overlap with sustained increases in BHA activity relative to the pre-retro-cue baseline.
Conclusions: Our working hypothesis for further studies is that patterns of BHA in superior temporal auditory cortices carry human auditory WM information.

Subject-Specific Adaptation for a Causal, Real-Time, Auditory-Attention Decoding System

Christine Beauchene[1], Stephanie Haro*[2], Michael Brandstein[1], Thomas Quatieri[1], Christopher Smalt[1]
[1]MIT Lincoln Laboratory, [2]Speech and Hearing Bioscience and Technology Program, Harvard Medical School

Background: Future hearing-aid technology may allow the listener to automatically isolate a single talker of interest from a mixture through the shifting of their attentional focus as measured by Electroencephalography (EEG). A challenging aspect of using EEG for clinical and rehabilitative applications is the variability both across and within patients over time. This often results in algorithms that are based on a group model, or the necessity of a long initial training period to calibrate the system to each user. One method to reduce the training time is transfer learning, which uses labeled data from a separate pool of participants to speed up the process. In this study, we apply transfer learning to an auditory attention decoding (AAD) algorithm that correlates the EEG data and speech envelopes to predict a listeners attentional focus.
Methods: We used two published AAD datasets, containing 18 (Dataset 1) and 22 (Dataset 2) participants, to compare three separate model implementations: individual, group, and augmented. For the individual model, we implemented a sliding window approach to update the model on each trial by using data from the participant's previous two trials. To identify the group model for a single participant, we used all the data from the pool of other participants (i.e., leave-one-out approach). The augmented model was also implemented using a sliding window and a weighted combination of both the group and individual models.
Results: The augmented model developed in this study outperformed the individual and group model at a decision window of 10 seconds. For the Dataset 1, the accuracy of the augmented model (Mean 69.5%, SD ± 7.9%) was higher than both the group model (Mean 65.5%, SD ± 9.5%) and the individual model (Mean 62.2%, SD ± 4.9%). Similarly, for the Dataset 2, the augmented model (Mean 78.4%, SD ± 9.7%) outperformed both the group model (Mean 74.1%, SD ± 10.1%) and the individual model (Mean 67.3%, SD ± 9.5%). Preliminary results of the two separate one-way ANOVAs, one for each dataset, show that significant differences were observed between the models (Dataset 1: $F(2, 198) = 14.8$, $p = 9.6E-7$, Dataset 2: $F(2, 264) = 29.2$, $p = 3.4E-12$). Compared to the individual models, the augmented model significantly improved 10 second decoding accuracy by 7% and 11% for Dataset 1 and 2, respectively.
Conclusions: This study demonstrates that decoding accuracy can be improved by combining transfer learning with conventional AAD algorithms. Moreover, we can help reduce the initial training time requirement for a neurofeedback system by using the group model until the required amount of participant data is collected. Overall, these results are directly applicable to the development of neurofeedback applications that uses the output of an AAD model as a bio-feedback measure.

Laminar Specificity of the Auditory Awareness Negativity Under Multitone Masking: A Biophysical Modeling Study

Carolina Fernandez Pujol*[1], Elizabeth Blundon[2], Andrew Dykstra[3]
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Background: How perception of sensory stimuli emerges from brain activity is a fundamental question of neuroscience. To date, two disparate lines of research have examined this question. On one hand, human neuroimaging studies have helped us understand the large-scale brain dynamics associated with perception. On the other hand, work in animal models (mice, typically) has led to fundamental insight into the micro-scale neural circuits that underlie perception. However, translating such fundamental insight from animal models to human brain circuits has been challenging.
Methods: Here, using biophysical modeling (the Human Neocortical Neurosolver or HNN), we show that the auditory awareness negativity (AAN), an auditory evoked response associated with perception of target sounds in noise, can be accounted for by additional input to the supragranular layers of auditory cortex (AC) that is present when target sounds are heard but absent when they are missed.
Results: This additional input likely arises from corticocortical feedback or non-lemniscal thalamic projections and leads to increased local field potential activity in layer V, in turn resulting in both increased spiking activity in layer-V pyramidal neurons and the AAN.
Conclusions: The results are consistent with current cellular models of conscious processing (such as Apical Amplification and Dendritic Integration theory) and help bridge the gap between the macro and micro levels of perception-related brain activity.

Using Frequency Selectivity to Examine Category-Informative Dimension-Selective Attention

Sahil Luthra[1], Chisom Obasih[1], Adam Tierney[2], Frederic Dick[3], Lori Holt*[1]
[1]Carnegie Mellon University, [2]Birkbeck College, University of London, [3]University College London

Background: In everyday listening, task- and goal-relevant information is not uniformly distributed along and across auditory dimensions. For instance, information that is diagnostic for categorizing particular auditory objects or speech sounds may preferentially lie within a particular set of amplitude modulation rates or frequency bands. A number of theoretical accounts suggest that listeners learn to attend to the category-diagnostic range along that auditory dimension, and potentially to suppress less category-informative information, However, there is limited mechanistic evidence for this account, particularly in more complex auditory learning environments.
Methods: We test this attentional account using novel non-speech auditory categories requiring reliance on information in high or low spectral bands. Prior to fMRI scanning, participants learn four novel nonspeech categories to criterion across five days of training. Exemplars are composed of three concatenated high bandpass-filtered hums and three simultaneous low bandpass-filtered hums; hums are nonspeech pitch contours, derived from multiple-talker Mandarin productions varying in lexical tone. Two categories are defined by category-consistent hums in the high-frequency band and inconsistent (between-category) hums in the low band. The other two categories have category-consistent hums in the low band and category-inconsistent hums in the high band. Thus, category learning requires reliance on – and perhaps selective attention to – category-diagnostic patterns within high or low frequency bands. Control trials involve categorization across an orthogonal dimension, stimulus amplitude. In a post-training fMRI session, listeners categorize sounds differentiated by information in either high or low spectral bands, or on relative amplitude. We combine this with tonotopic mapping across auditory cortex and "attention-o-tonotopic" mapping driven by overt endogenous attention to high and low frequency bands. We examine how dimension-selective attention driven by the demands of categorization may impact cortical activation within auditory cortical regions with frequency preferences corresponding to category-diagnostic versus non-diagnostic acoustic patterns and how selective attention effects may differ when demands are driven implicitly by task relevance (categorization) versus by directed attention ('listen high').
Results: While challenging, many listeners are able to use spectrally-delimited information to identify novel auditory categories. Initial results suggest that there is modulation of frequency-selective cortical regions corresponding to which band is category-informative.
Conclusions: This work will help to illuminate the cortical mechanisms supporting dimension-based auditory selective attention; through a comparison with our control condition (stimulus amplitude

judgments), it also allows us to assay a putative role for suppression when listeners deploy selective attention for categorization. We provide a theoretical bridge from effects of explicitly directed attention (i.e., "listen high") to effects of selective attention that may emerge over the course of auditory category learning, as listeners learn which dimensions are informative for categorization. Finally, the study links human studies of auditory attention to speech and non-human animal studies of frequency-selective auditory attention with non-speech stimuli.

Functional Geometry of Human Auditory Cortical Resting State Networks Derived from Intracranial Electrophysiology

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[1]University of Wisconsin, [2]University of Iowa, [3]Albert Einstein College of Medicine, [4]The University of Iowa

Background: Unresolved questions about the organization of human auditory cortex (AC) hinder understanding of speech and language processing. These questions include the relationship of canonical AC to higher-order auditory-responsive areas, the definition of auditory information processing streams, and the degree of hemispheric asymmetry in these organizational features. Here, we address these questions using resting state functional connectivity (RSFC) derived from human intracranial electroencephalography (iEEG). We elucidated underlying network structure by applying diffusion map embedding (DME) to RSFC matrices. DME maps recording sites to a space where proximity represents functional similarity.
Methods: Resting state iEEG data were obtained from 6741 recording sites in 49 epilepsy patients (22 female) undergoing chronic monitoring to identify seizure foci prior to resection surgery. Research protocols were approved by the University of Iowa Institutional Review Board, and written informed consent was obtained from all participants. Research participation did not interfere with clinical care, and participants could rescind consent for research without interrupting their clinical management. Implants were in the language dominant hemisphere in 22 of 43 participants evaluated. RSFC (band power envelope correlations) was averaged within cortical regions of interest (ROI) and across subjects, thresholded, normalized, and analyzed using DME, which maps data into a Euclidean space using eigenvector expansion of the normalized RSFC matrix. Auditory responsiveness was quantified as percent of recording sites in each ROI with significant responses to monosyllabic words.
Results: DME revealed a hierarchical organization of ROIs that aligned with auditory responsiveness. Cluster analysis revealed a tight group of canonical AC ROIs that excluded planum polare but included the upper bank of the superior temporal sulcus. This auditory cluster segregated maximally in embedding space from multiple prefrontal clusters. In addition to clusters representing ventral and dorsal auditory processing streams, a limbic cluster was identified whose proximity to AC suggested a distinct stream subserving memory- and emotion-related auditory processing. Positions of ROIs in embedding space distinguished network hubs and spokes. Lower order sensory and motor regions were identified as spokes. Analysis of RSFC in the gamma band (30-70 Hz) identified anterior temporal lobe ROIs as hubs, consistent with their role in semantic processing. In the theta band (4-8 Hz), anterior and posterior cingulate cortex ROIs were identified as hubs, suggesting a temporal scale-dependent rearrangement of network organization. Positions in embedding space did not differ for language-dominant versus non-dominant hemispheres, and while inter-ROI distances for all ROIs did differ between hemispheres, distances between language-specific ROIs behaved similarly to non-language-specific ROIs. Thus, no evidence was observed for specific lateralization of speech and language networks.
Conclusions: Results provide new insights into the organization of human AC and related ROIs. This approach will facilitate identifying network changes during active speech and language processing and elucidating mechanisms underlying disorders of auditory processing.

Enhanced Interaural Level Cues Affect Hemodynamic Responses in Superior Temporal Gyrus

Benjamin Richardson*[1], Jana Kainerstorfer[1], Barbara Shinn-Cunningham[1], Christopher Brown[2]
[1]Carnegie Mellon University, [2]University of Pittsburgh

Background: Spatial cues help listeners both segregate the sources in an auditory scene and focus attention on whichever source they wish to analyze (Shinn-Cunningham and Best, 2008). HI and CI listeners hear degraded spatial cues (e.g., Dai et. al., 2018; Jones et. al., 2014; Williges et. al. 2019), which may contribute to their difficulties in completing this task. I interaural level difference (ILD) cues alone produce less spatial

release from masking (SRM) as interaural time difference (ITD) cues (Ihlefeld and Litovsky, 2012; Glyde et. al., 2013). Yet, ILDs improve speech reception when there are competing talkers, especially when they are larger than what occurs naturally (Brown, 2014). The current study used a vocoded speech intelligibility paradigm (Zhang et al, 2021) while recording functional Near-Infrared Spectroscopy (fNIRS) to better understand the relationship between magnified ILD cues and neural activity in superior temporal gyrus (STG).

Methods: 19 native English speakers with no reported hearing deficits participated (13 females, mean age 38 years). Subjects, seated in a sound-treated booth, heard target (spatialized left) and masker (spatialized right) sequences of object and color words. They pressed a button when they detected a color word. Target and masker were vocoded into 16 log-spaced frequency bands, nine of which were randomly selected to represent the target and the remaining seven, the masker. Seven spatial conditions were tested. Two replicated Zhang et al., using whole-waveform ITDs with either speech or noise maskers (ITDSpeech and ITDNoise, respectively). The remaining five used speech maskers and whole-waveform ILDs of different magnitudes (ILD0, ILD10, ILD20, ILD30, ILDinf). fNIRS recorded hemodynamic activation over STG and lateral frontal cortex.

Results: The ITD conditions replicate Zhang et. al. (2021): hemodynamic activation in STG is greater for a speech masker than a noise masker. In the main experiment in which broadband ILDs were manipulated, we found that increasing the magnitude of ILD in the ILD10, ILD20, and ILD30 conditions led to increased activation in STG. Activation was low, however, in the cases where spatial cues were unavailable (ILD0) and for dichotic presentations (ILDinf). Behavioral sensitivity did not vary across conditions.

Conclusions: The replication of the results from Zhang et. al. 2021 suggests that hemodynamic activation in STG may be a proxy for release from masking from a competing talker, confusable with the target. Results from magnified ILD conditions allow us to assess whether these cues facilitate spatial release from masking. The pattern of results observed suggests that broadband ILDs elicit a hemodynamic response as large as that of ITD conditions. We show that enhanced ILDs increase activation of auditory-related areas associated with spatial release from masking.

Emergence of the Cortical Encoding of Phonetic Features in the First Year of Life

Giovanni Di Liberto*[1], Adam Attaheri[2], Giorgia Cantisani[3], Richard Reilly[1], Áine Ní Choisdealbha[2], Sinead Rocha[2], Perrine Brusini[4], Usha Goswami[2]

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Background: Even prior to producing their first words, infants are developing a sophisticated speech processing system, with robust word recognition present by 4-6 months of age. These emergent linguistic skills, observed with behavioural investigations, are likely to rely on increasingly sophisticated neural underpinnings. The infant brain is known to robustly track the speech envelope, however to date no cortical tracking study could investigate the emergence of phonetic feature encoding.

Methods: Here we utilise temporal response functions computed from electrophysiological responses to nursery rhymes to investigate the cortical encoding of phonetic features in a longitudinal cohort of infants when aged 4, 7 and 11 months, as well as adults.

Results: The analysis indicate EEG tracking of both acoustic and phonetic features in infants and adults. Crucially, TRF models show an increasingly detailed and acoustically-invariant phonetic encoding over the first year of life, providing the first direct evidence that the pre-verbal human cortex learns phonetic categories. By 11 months of age, however, infants still did not exhibit adult-like encoding.

Conclusions: This study demonstrated the emergence of phonetic encoding from 7 months of age using direct neural measurements during natural speech listening. The data provide clear-cut evidence of the emergence of phonetic categories that contributes to the current debate regarding their role in the development of speech processing. Our demonstration that phonetic encoding can be assessed with nursery rhyme stimuli in ecologically-valid conditions opens the door to cross-language work using TRFs that investigates the interaction between characteristics of natural language such as phonological complexity and the development of phonetic encoding. It also provides opportunities for novel mechanistic investigations of the development of bi-lingual and multi-lingual lexicons during language acquisition.

Podium #6 - Inner Ear Therapeutics

8:00 a.m. - 10:00 a.m.
Crystal Ballroom D-E

Moderators: Arnaud Giese and Jonathan Bird

Development of a Dual Porous Nanocomposite Coating as an Implant-Associated Local Drug Delivery System for Cochlear Implants

Mosaieb Habib*[1], Timo Herrmann[1], Karen Hindricks[1], Jennifer Harre[2], Thomas Lenarz[2], Athanasia Warnecke[2], Peter Behrens[1]
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Background: Research on the release of drugs from neural electrodes has been ongoing for some time. So far, no sustainable and universal concept has been found. Currently, platinum is used as an electrode material in neural electrodes, such as in the cochlear implant (CI). By provision of neuroprotective substances, neuronal growth factors, anti-inflammatory drugs or antibiotics, it is possible to restore the balance in the inner ear and thus stabilize the remaining spiral ganglion neurons (SGNs). Furthermore, the outgrowth of neurites from these cells can be induced to improve the electrode-nerve contact and increase signal resolution for an improved hearing perception. Although platinum can also be produced in porous form, controlled release from its pore system is not possible due to its chemical inertness and consequently the difficulty of surface modifications for interactions with drug molecules.

To nevertheless achieve local implant-associated drug delivery, we devised a novel nanocomposite material of nanoporous silica nanoparticles (NPSNPs) embedded in the pores of nanoporous platinum (NPPt) which can be employed as a coating on the platinum surfaces of the CI electrode. NPPt exhibits high conductivity and favorable electrochemical properties. NPSNPs provide large surface area, permanent porosity and high versatility in terms of easily tunable surface properties to achieve high drug loading.
Methods: To fabricate the NPSNPs@NPPt material on the surface of platinum substrates, unmodified and sulfonic acid modified NPSNPs were first encapsulated in a polystyrene shell. After the 100 nm sized silica-polystyrene core-shell nanoparticles were applied on the surface of the substrates, platinum was electrolytically deposited between the particles. In the final step, the polystyrene was removed by extraction to obtain the dual porous nanocomposite coating on platinum.
To test the potential as a drug delivery system, the material was incubated in a methylene blue solution followed by release experiments. In addition, in vitro tests with NIH/3T3 fibroblasts and SGNs of neonatal Sparague-Dawley rats were performed to verify cytocompatibility.
Results: Scanning electron microscopy images showed the successful incorporation of the modified and unmodified NPSNPs into the NPPt. Impedance and cyclic voltammetric measurements demonstrated improved electrochemical properties compared to dense platinum due to the enhanced surface of the NPPt. The release experiments indicate that the sulfonic acid modified particles in the composite coating lead to higher loading and release of methylene blue compared to the unmodified particles. Cell culture studies with fibroblasts and SGNs showed good cytocompatibility.
Conclusions: The novel nanocomposite material combines the favorable properties of both nanoporous platinum and nanoporous silica nanoparticles, specifically the excellent electrochemical behavior of NPPt and the high specific surface area, large pore volume and amenability to surface modifications of the NPSNPs. Overall, the material shows great potential for use as an implant-associated local drug delivery system for cochlear implants.

Drugs Delivered by Semicircular Canal Injection Enter the Perilymphatic Space

Jinkyung Kim*[1], Dorothy Pan[2], John Oghalai[2], Anthony Ricci[1]
[1]Stanford University, [2]University of Southern California

Background: The safe delivery of genes or pharmacological agents into the cochlea will be a requirement for future inner ear therapy. Similarly, the delivery of sensors is also required for functional imaging of sound-evoked responses in the auditory periphery. Posterior semicircular canal injection has been shown to be a safe and effective approach for the delivery of therapeutics to the inner ear in neonatal and adult mice; however, we do not yet understand which cochlear compartment the injection is delivered into.
Methods: Either artificial perilymph, pharmacological agents, or gold nanoparticle was injected via posterior semicircular canal in 4-6 week old C57BL/6 mice. After the artificial perilymph injection, the hearing function was verified by auditory brainstem response (ABR) recordings and vibrometry

measurement. Vestibular function was also verified by head-bobbing, trunk-curl, swimming tests, and vestibular sensory-evoked potential (VsEP) recording. To investigate which cochlear compartment the injection enters, ABRs were measured an hour after the injection of 1 mM curare, 100 µM CNQX, or 1 mM lidocaine. Lastly, in vivo monitoring of gold nanoparticles delivered by the injection was performed using optical coherence tomography imaging.
Results: No changes in ABR, vibrometry, VsEP, or any behavioral tests were observed after the artificial perilymph injection, indicating that posterior semicircular canal injection induces no defects in hearing and vestibular functions at the injection volume and rates selected. Curare, a MET channel blocker, expected to block MET function when it is in the endolymphatic space, did not change ABR threshold an hour after the injection. However, CNQX (AMPA blocker) and lidocaine (Na+ channel blocker), which block synaptic activity and action potential generation when in the perilymphatic space, greatly elevated the ABR threshold. The results from the pharmacological manipulation indicate the posterior semicircular canal injection targets the perilymphatic, not the endolymphatic space. This conclusion was confirmed in vivo by real-time monitoring of gold nanoparticle distribution within the cochlea when injected via the posterior semicircular canal.
Conclusions: Posterior semicircular canal injection is a safe method to deliver therapeutics into the cochlea. It targets only perilymphatic, not endolymphatic space. Our finding will therefore allow for precise delivery of drugs/ sensors within the cochlea for therapeutic and research purposes.

Investigation of Inner Ear Drug Delivery With a Cochlear Catheter in Piglets as a Representative Model for Human Cochlear Pharmacokinetics

Erdem Yildiz*[1], Anselm Joseph Gadenstaetter[1], Matthias Gerlitz[1], Lukas Landegger[1], Rudolfs Liepins[1], Michael Nieratschker[1], Rudolf Glueckert[2], Hinrich Staecker[3], Clemens Honeder[1], Christoph Arnoldner[1]
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Background: Intricate pharmacokinetic examinations to better understand drug distribution within the inner ear could facilitate the development of novel therapeutics. For such translational research projects, animal models are indispensable, but differences in inner ear dimensions and other anatomical features complicate the transfer of experimental results to the clinic. Results obtained in rodents often poorly correlate with their human counterparts. The translational gap between these species may be bridged using larger animal models such as non-human primates. However, their use is challenging and impeded by administrative, regulatory, and financial hurdles. Therefore, we conducted an in-depth evaluation of the inner ear anatomy and pharmacokinetics in piglets to assess their potential use in inner ear research.
Methods: Prior to in vivo experiments, anatomical landmarks were identified, and a step-by-step surgical approach for inner ear drug delivery and perilymph (PL) sampling was established in the cadaver model. Controlled intracochlear delivery of fluorescein isothiocyanate-dextran (FITC-d) was carried out after the insertion of a novel, clinically applicable CE-marked cochlear catheter through the round window membrane in piglets. Sequential apical PL sampling was performed two, six, and twenty-four hours after a single injection of FITC-d and concentration levels were determined with fluorometric measurements. PL levels two hours after injection were compared to the FITC-d content in control groups, which either had been injected with a simple needle puncture through the round window membrane or the above-mentioned cochlear catheter in combination with a stapes vent hole. Additionally, intraoperative acoustic compound action potentials and cochlear microphonics were measured to determine cochlear function before and after compound administration. Extracted inner ears were scanned via micro-CT to determine cochlear dimensions.
Results: We found significantly increased apical FITC-d concentrations when using the cochlear catheter and higher total concentrations in all PL samples compared to the round window membrane injection. Additionally, the concentrations decreased after six and twenty-four hours, and the distribution was more homogenous compared to shorter observation times. The stapes-venting group also showed a more uniform distribution with a ten times lower maximum FITC-d concentration when compared to the non-venting group. Additionally, segmentations of micro-CT scans comprised a porcine inner ear volume of 84.55 µl. Objective acoustic measurements after cochlear catheter insertion revealed no difference in threshold shifts compared to control groups.

Conclusions: Local inner ear drug delivery with a novel CE-marked cochlear catheter is feasible in piglets. The obtained pharmacokinetic insights represent essential findings regarding the compound distribution within human-like cochleae, as the porcine inner ear dimensions largely match the human anatomy. Due to the widespread availability, fewer ethical concerns, and easy handling, these animals should be assessed in various translational hearing research settings to confirm their potential as an alternative to non-human primates.

Evaluating Protective Effects of Non-Invasive Hypothermia and a Pan-Caspase Inhibitor as Combination Therapy in an NIHL Rodent Model

Maria Fernanda Yepes*[1], Kayla Minesinger[2], Federica M. Raciti[1], Samantha Rincon Sabatino[3], Rachele Sangaletti[4], Suhrud Rajguru[5]
[1]University of Miami, [2]University of Miami, Biomedical Engineering Department and Otolaryngology, [3]University of Miami, Department of Biomedical Engineering, [4]Department of Otolaryngology, University of Miami, [5]Department of Otolaryngology and Biomedical Engineering, University of Miami,Department of Veterans Affairs, Bruce W. Carter Department of Veterans Affairs Medical Center

Background: Noise-induced hearing loss involves cochlear stereociliary bundle and hair cell damage, as well as synaptic and neural degeneration. Underlying mechanisms involve elevation of reactive oxygen species, excitotoxicity, and apoptosis. Prior studies from our group have proven Mild Therapeutic Hypothermia (MTH) to be neuroprotective against acoustic trauma. Z-VAD-FMK, a pan-caspase inhibitor, has been demonstrated to mitigate apoptotic processes and thus protect against hair cell loss and auditory threshold shifts induced by ototoxic agents. Beneficial effects of Z-VAD in combination with MTH after oxidative stress have been reported in in-vitro studies. In the present study, we investigated the protective effects of MTH in addition to a pan-caspase inhibitor in a rodent model exposed to acoustic trauma.
Methods: Brown Norway rats were exposed to noise (4-8 kHz at 110 dB) for 1 hour under normal awake and behavioral conditions and subsequently divided into three different groups: (1) Noise exposure + MTH + Z-VAD combination (n=11), (2) Noise + ZVAD (n=12), (3) Noise alone (n=12). Local and non-invasive hypothermia was applied 20 minutes after noise exposure. z-VAD-FMK was delivered intraperitoneally at a dosage of 1.5mg/kg following hypothermia. Cochlear (ABR) and vestibular (cVEMP) functions were assessed prior of trauma and post-interventions serially. Immunohistology studies were carried out to characterize hair cell loss.
Results: Post-noise exposure, the auditory thresholds across the tonotopic map increased by 26 dB on average when compared to baseline levels. The auditory thresholds recovered across all groups by day 28 (average shift at day 28 was ~9 dB in group 1, ~14 dB in group 2 and ~14 dB in group 3). The combination therapy of MTH + ZVAD resulted in quicker recovery with reduced auditory threshold shifts when compared to ZVAD alone and noise alone groups. The protection was observed across all time points. The most significant changes between groups were seen at 4kHz and 8khz frequencies.
Conclusions: Our findings overall suggest that MTH and ZVAD could potentially have synergistic beneficial effects against cochlear dysfunction caused by acoustic overexposure. Based on our results, systemic drug administration of Z-VAD, in addition to non-invasive localized cooling techniques, could potentially be explored as viable therapies in clinic to treat NIHL.

Demonstration of Durable Anti-Vegf Protein Expression and Otic Tolerability following Intracochlear Delivery of AK-AntiVEGF (AAVAnc80-AntiVEGF Vector) Across Multiple Doses in Non-Human Primates

Shimon Francis*[1], Yuan Gao[1], Michael J. McKenna[1], Jean Phillips[1], Kathleen Lennon[1], Brian Lin[1], John Connelly[1], Pascal Schamber[1], Junaid Syed[1], Jenna Soper[1], Ann E. Hickox[1], Emmanuel J. Simons[1], Eva Andres-Mateos[1], Jennifer A. Wellman[1], Michelle D. Valero[1]
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Background: AK-antiVEGF (AAVAnc80-antiVEGF), a gene therapy candidate in preclinical development, encodes a vascular endothelial growth factor (VEGF) inhibitor (anti-VEGF protein) under the control of a ubiquitous promoter. AK-antiVEGF is designed for the potential treatment of patients with vestibular schwannoma (VS), a benign tumor that originates from the cells surrounding the vestibulocochlear nerve within the internal auditory canal. Common symptoms associated with VS include hearing loss, tinnitus, and dizziness; as tumors continue to grow, they can compress the brainstem, representing a concern for more serious morbidity and, in very rare cases, mortality. Current interventional options are invasive approaches,

such as surgical resection and/or radiation therapy, which can cause significant morbidity (e.g., facial paralysis and hearing loss). An alternative treatment approach using systemic Avastin® (bevacizumab) has been shown to decrease VS tumor size and improve hearing in neurofibromatosis type 2 (NF2) patients; however, long-term systemic administration of VEGF inhibitors can be associated with significant safety concerns. A one-time, local delivery of AK-antiVEGF to the inner ear is intended to transduce cochlear and vestibular cells, resulting in secreted anti-VEGF protein into perilymph, an inner ear fluid that is in diffusional continuity with the interstitial and perineural spaces of the vestibulocochlear nerve where VS tumors are located. The limited systemic exposure to VEGF inhibitor in this more targeted approach has the potential to minimize adverse effects. In previous nonclinical studies, non-human primates (NHPs) that underwent simultaneous bilateral intracochlear administration of AK-antiVEGF (with doses spanning > a one-log range) demonstrated tolerability and biologically active levels of anti-VEGF protein in perilymph.
Methods: Here, we performed histologic evaluations of tolerability and durability of anti-VEGF protein expression in NHP cochleae at 1-, 3-, or 6-months following simultaneous bilateral intracochlear administration of AK-antiVEGF at one of three doses. There were three animals per AK-antiVEGF dose level, as well as two vehicle control animals, per survival duration. Left cochleae were microdissected and immunostained for anti-VEGF protein detection and hair cell survival.
Results: Overall, hair cell survival was robust and anti-VEGF protein was observed in a broad range of cochlear cell types, consistent with the tropism of AAVAnc80 in previous studies using reporter transgenes expressed via a ubiquitous promoter. Using fluorescence intensity in the confocal micrographs as a metric for protein expression, anti-VEGF was detected at similar levels across each of the dose levels of AK-antiVEGF and across each of the survival durations.
Conclusions: These data demonstrate that a one-time intracochlear administration of AK-antiVEGF results in durable expression of anti-VEGF protein that is well tolerated in the NHP cochlea. In addition to confirming durable expression of transgenes delivered to the cells of the cochlea using an AAVAnc80 vector and ubiquitous promoter, these data support the planned clinical development of AK-antiVEGF for the treatment of VS.

Individual Healing Attempt to Treat Idiopathic Sudden Sensorineural Hearing Loss Using an Additively Manufactured, Individualized, Dexamethasone Releasing Round Window Niche Implant

Verena Scheper*[1], Chunjiang Wei[2], Ziwen Gao[2], Jana Schwieger[2], Martin Ulbricht[3], Stefan Senekowitsch[3], Werner Weitschies[3], Anne Seidlitz[4], Felix Repp[5], Samuel John[6], Thomas Lenarz[2], Farnaz Matin[2]
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Background: Idiopathic sudden sensorineural hearing loss (ISSHL) affects millions of people worldwide. The standard therapy is systemic glucocorticoid application. A therapeutic option for secondary treatment, i.e. after failure of hearing improvement with systemic therapy, are intratympanically injected glucocorticoids. The outcome varies substantially and some patients don't benefit from the suggested treatment options. We hypothesize that a sustained local drug delivery to the inner ear has the potential to improve hearing outcomes. Individualized, drug-releasing, round window niche implants (RNIs) may be a suitable approach for applying drugs locally to the cochlea as non-invasively as possible. We developed a RNI delivering dexamethasone to individually treat ISSHL patients where guideline-following therapy was not successful.
Methods: Five patients with unilateral ISSHL were treated. A cone beam computed tomography imaging of the temporal bone was performed preoperatively. Round window niches were segmented semi-automatically using a newly developed software-tool. The reconstructed niches were processed for the desired implant design and subsequently the RNIs were additively manufactured using silicone that has been tested for compliance with USP Class VI, ISO 10993 (parts 6, 10 and 11) and FDA extractables, containing dexamethasone. The UV-irradiated RNIs were implanted under general or local anesthesia in an approximately 20-minute procedure via a transmeatal approach. Follow up visits were arranged on a weekly basis and pure tone audiometry was controlled after 29 days.
Results: No otogenic complications such as vertigo were observed within 4 weeks postoperatively. One patient developed a benign paroxysmal positional vertigo 4 weeks after RNI insertion, which resolved 3 months postoperatively. Four patients did not request an explantation of the RNI and the RNI was explanted

from one patient on day 29. The hearing thresholds of 2 patients were stable 29 days after RNI insertion, and an improvement of more than 10 dB in ≥ 3 frequencies was observed in 3 patients.
Conclusions: Using additive manufacturing, we developed an individualized drug releasing RNI. The implant was safely inserted into the round window niche and no adverse events were observed. The individual healing attempt resulted in an improvement of the hearing thresholds in 3 out of 5 cases, which showed no improvement after first line therapy with systemic glucocorticoids.

Preclinical Development of a Genetic Medicine for Otoferlin Gene-Mediated Hearing Loss: AK-OTOF

Yuan Gao*[1], Shimon Francis[1], Robert Ng[1], Yukako Asai[1], Yuanzhao Darcy[1], Danielle R. Lenz[1], Hao Chiang[1], Samantha Davis[1], Ye-Hyun Kim[1], Michael J. McKenna[1], Brian Lin[1], Jean Phillips[1], Kathy Lennon[1], Chris Tarapata[1], Christian Supina[1], Aaron Graham[1], Ann E. Hickox[1], Emmanuel J. Simons[1], Eva Andres-Mateos[1], Jennifer A. Wellman[1], Michelle D. Valero[1]
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Background: Millions of people worldwide have disabling hearing loss due to mutations in one of their genes that result in loss of expression or generation of an incorrect version of a protein required for hearing. The otoferlin gene (OTOF) encodes otoferlin, a protein critical for afferent signaling at the inner hair cell (IHC) synapse; individuals with biallelic mutations in OTOF typically present with congenital, Severe to Profound sensorineural hearing loss. Recent advances in both gene therapy and delivery to the intracochlear space support the potential to restore hearing in individuals with OTOF-mediated hearing loss by enabling IHCs to produce otoferlin using a one-time, local administration of AK-OTOF (AAVAnc80-hOTOF). Here, we describe the preclinical development strategy and key nonclinical studies that support the translation of AK-OTOF and an investigational delivery device designed for intracochlear administration to clinical development.
Methods: The approach to deliver genetic medicines to the inner ear, including the design of a dual adeno-associated viral (AAV) vector, the Akouos delivery device, and an intracochlear administration procedure, was evaluated in in vitro studies, as well as in in vivo studies in otoferlin knock-out (Otof -/-) mice and non-human primates (NHPs). Nonclinical studies were conducted to inform the design of clinical investigation of AK-OTOF, including demonstration of biological plausibility by the intended clinical route of administration, evaluation of intervention window (with respect to otoacoustic emission [OAE] status), identification of biologically active dose levels, assessment of onset and durability of functional recovery, and evaluation of safety.
Results: A one-time intracochlear administration of a dual AAVAnc80 vector encoding human otoferlin (hOTOF), under control of a ubiquitous promoter, to mice and NHPs results in robust expression of full-length hOTOF in the target IHCs (and not in other cell types). The Otof -/- mouse phenotype supports its utility for preclinical development, including determination of biologically active dose levels of AK-OTOF that restored auditory function by 2 weeks post-administration, and through at least 6 months post-administration (the longest survival duration evaluated), and determination of potential for restoration of auditory function based on status of cochlear integrity (as evaluated by OAEs). Evaluation of safety and otic tolerability of AK OTOF was conducted in both NHPs and mice; no adverse effects were observed in clinical pathology, otic pathology, systemic histopathology, and/or cochlear / auditory function, demonstrating that AK-OTOF was systemically and locally well tolerated.
Conclusions: The strategy employed for preclinical development of AK-OTOF, leading to FDA clearance for a clinical trial in pediatric individuals with OTOF-mediated hearing loss, can serve as an exemplary path to achieving the broader goal of developing precision genetic medicines with the potential to restore, improve, and preserve high-acuity physiologic hearing for individuals worldwide who live with disabling hearing loss.

Restoration of Vestibular Function by Regeneration of Type I and Type II Hair Cells in Mature Mouse Utricle

Hanae Lahlou*[1], Hong Zhu[2], Wu Zhou[2], Albert S. B. Edge[1]
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Background: The vestibular sensory organs of the inner ear contain highly specialized mechanoreceptive hair cells essential for balance. Vestibular hair cells regenerate spontaneously in response to damage;

however the extent of regeneration declines after the first postnatal week. In this study, we sought to develop a pharmacological treatment to enhance vestibular hair cell regeneration in adult utricle.
Methods: We used adult mice that express the diphtheria toxin receptor in hair cells (Pou4f3-DTR) for targeted ablation of hair cells. Mice received a unilateral injection of small molecules, a combination of a glycogen synthase kinase inhibitor (CHIR99021) and histone deacetylase inhibitor (valproic acid), after systemic administration of diphtheria toxin. This combination of drugs, previously shown to stimulate supporting cell proliferation and hair cell differentiation in cochlear organoids, was delivered locally via the semi-circular canal, and the extent of spontaneous vs drug-induced regeneration was compared between the treated and untreated (contralateral) ear.
Results: We observed a significant increase in cells expressing hair cell marker, MYO7A, in drug-treated ears relative to ears without treatment where hair cells regenerated spontaneously. Drug treatment resulted in regeneration of 58% of the normal number of hair cells after 8 weeks as compared to 32% in the DT-treated ear without drug treatment. Lineage tracing of supporting cells showed newly regenerated type I and type II vestibular hair cells identified by immunoreactivity to MYO7A and SOX2 antibodies, with MYO7A+SOX2- being type I hair cells while MYO7A+SOX2+ co-labeled cells specific to type II hair cells. Changes in hair cell number were associated with a significant functional improvement as assessed by the vestibuloocular reflex and single fiber recordings from vestibular neurons.
Conclusions: This work provides further knowledge of the molecular mechanisms required for vestibular hair cell differentiation and suggests that the drug combination may be a candidate for clinical application for balance disorders related to loss of hair cells.

Podium #7 - Development and Patterning of Sensory Structures

10:30 a.m. - 12:30 p.m.
Ocean's Ballroom 5-12

Moderators: Melissa McGovern and Brent Wilkerson

The Transcription Factor Myt1, an Atoh1 target, is Necessary for Patterning the Organ of Corti

Elizabeth Driver*[1], Rebecca Hipp[1], Abaigeal Donaldson[1], Likhitha Kolla[1], Matthew Kelley[1]
[1]NIDCD/NIH
Background: During cochlear development, the prosensory domain, which will give rise to the organ of Corti, is patterned into a highly ordered array of inner and outer sensory hair cells surrounded by at least six types of non-sensory supporting cells. To achieve this cellular diversity, the sensory progenitor cells must undergo a series of cell fate decisions governed by transcription factors, the most well-known of which is Atoh1. Atoh1 is known to be necessary and sufficient for hair cell differentiation, and null mutations in this gene result in the complete loss of hair cells.
Methods: To identify novel genes involved in hair cell development and function, we performed RNA-seq on cochlear epithelia from embryonic day 15 Atoh1+/+ and Atoh1-/- mouse embryos. From this, we identified several genes significantly downregulated in the Atoh1-/- cochlea whose function has not been reported in the inner ear. We determined the wild-type expression patterns of several genes of interest and confirmed their downregulation in the Atoh1-/- cochlea through in situ hybridization and/or immunohistochemistry.
Results: We characterized the expression of several previously unreported genes found in hair cells, supporting cells, or both during cochlear development. Of particular interest was the transcription factor Myt1, which is known to be important for neural fate selection in other systems. In the cochlea, Myt1 is specifically expressed in hair cells during differentiation, from embryonic day 14 to at least post-natal day 1. Using a conditional mutant mouse line for Myt1, we deleted Myt1 either throughout the cochlear epithelium or specifically in hair cells. At birth, Myt1 mutant cochleae demonstrated disrupted cellular patterning within the organ of Corti and a significant increase in the number of inner and outer hair cells. In addition, both hair cells and supporting cells appeared immature when compared to cells in control littermate cochleae. Future experiments will determine the effects of over-expression of Myt1 using an adenovirus vector in cochlear explants.
Conclusions: Our data suggest that expression of the Atoh1 target Myt1 during hair cell differentiation is important for patterning the organ of Corti and for hair cell maturation. Future directions of this project will

investigate the mechanisms through which this occurs. Myt1 has been demonstrated to suppress Notch downstream targets and to upregulate proneural genes in other neural cell types, and we hypothesize that loss of Myt1 disrupts Notch-mediated lateral inhibition during prosensory cell differentiation, leading to the conversion of supporting cells to hair cells. If this is the case, then overexpression of Myt1 in prosensory cells may promote a hair cell fate. These studies should enhance our understanding of the factors needed for proper hair cell differentiation and patterning of the organ of Corti.

Genetic Lineage Tracing Reveals the Shared Origins of the Line of Polarity Reversal in Mouse Vestibular Maculae

Ellison Goodrich[1], Michael Deans*[1]
[1]University of Utah

Background: The planar polarized organization of vestibular hair cells in the utricle and saccule is unique because these sensory epithelia contain two groups of hair cells with oppositely oriented stereociliary bundles that meet at the Line of Polarity Reversal (LPR). This organization allows the maculae to detect motion in opposite directions, and is coordinated with parallel afferent projections to the hindbrain and cerebellum. Emx2 is a transcription factor expressed in hair cells located on one side of the LPR that reverses the orientation of their bundles and thereby establishes the position of the LPR. Since Emx2 expression begins before hair cell specification, we tested the hypothesis that the position of the mature LPR is pre-determined at earlier stages of inner ear development.
Methods: To determine when Emx2 might establish an antecedent LPR we generated Emx2 P2A-CreERt2 transgenic mice for Cre-mediated lineage tracing. The gene targeting strategy preserved endogenous regulatory sequences by inserting the P2A-CreERt2 coding sequence at the Emx2 stop codon and generating a bicistronic mRNA without impacting Emx2 expression. No mutant phenotypes were detected when Emx2 P2A-CreERt2 mice are homozygosed. Lineage tracing was initiated by activating CreERt2 with a single dose of Tamoxifen delivered on a single developmental day between E10.5 through E14.5 to mice with the Cre-dependent tdTomato reporter (Ai9). Lineage tracing experiments were repeated using Dreher mutant mice in which the prosensory domain fails to segregate into a distinct utricle, saccule and cochlea.
Results: Tamoxifen delivered after the LPR has formed (E14.5) results in tdTomato expression in the lateral region of the utricle and the inner region of the saccule when evaluated at P0, similar to Emx2 mRNA distribution seen by in-situ hybridization. Tamoxifen delivery at E11.5 results in a similar pattern of labeled cells when viewed at P0. Both hair cells and supporting cells were labeled in either condition. In contrast, lineage tracing at E10.5 failed to label any cells within vestibular sensory epithelia. In Dreher mutants, lineage tracing at E11.5 or E14.5 resulted in a continuous field of labeled cells along one edge of the combined vestibulo-acoustic sensory epithelia.
Conclusions: We demonstrate that the developing pro-sensory domain in the mouse otocyst is patterned at E11.5 into regions destined to generate hair cells with oppositely oriented stereociliary bundles. Moreover, this occurs prior to the separation of the utricle and saccule. Although the evidence for this patterning is Emx2 expression, we cannot determine whether Emx2 function is also required at this stage. Nonetheless, the lineage tracing profile and the continuous field of labeled cells in Dreher mutants suggests that factors regulating the spatial distribution of Emx2 in the developing otocyst ultimately determine the position of the LPR in the mature maculae.

TRIM71 Regulates Auditory Progenitor Cell Behavior and is Essential for Hearing

Angelika Doetzlhofer*[1], Xiaojun Li[1], Charles Morgan[1], Duy Pham[2], Lale Evsen[1], Waldemar Kolanus[3], Kristopher Kahle[4], Sandrine Marlin[5]
[1]Johns Hopkins University School of Medicine, [2]Yale University School of Medicine, [3]University of Bonn, [4]Harvard Medical School, [5]Hôpital Necker; AP-HP, Université Paris Cité

Background: This study investigates the role of progenitor-gene Trim71 in cochlear development and hearing. TRIM71 protein functions as an ubiquitin ligase and RNA-binding protein and is an essential regulator of stemness, pluripotency and vertebrate development. Point mutation in TRIM71's RNA-binding domain have been recently identified in patients with congenital hydrocephaly and hearing loss. How Trim71 deficiency impacts cochlear development and its sound processing function is unknown.
Methods: To address Trim71 function, we stage specifically deleted the Trim71 gene using Trim71 floxed (f) mice that also carried R26rtTA*M2 and TetO-Cre transgenes for doxycline-mediated Cre induction (Trim71Δ/Δ). Littermates that lacked the TetO-Cre transgene were used as control (Trim71f/f). In a subset

of experiments, the floxed alleles was replaced with an allele that harbors a point mutation in TRIM71's RNA-binding domain found in patients with congenital hydrocephaly and hearing loss (Trim71Δ/R595H). Bulk RNA-sequencing was used to analyze transcriptional changes in response to Trim71 deletion. Auditory brainstem response (ABR) measurements in one-month old mice were used to analyze hearing acuity.
Results: We found that Trim71 is highly expressed in the developing otocyst (E9.5-E11.5) and that its expression persists in cochlear hair cell and supporting cell progenitors (pro-sensory cells), but rapidly declines upon their differentiation at around E13.5. Knockout of Trim71 during early otic development (E8.5-E10.5) results in premature cell cycle exit and premature differentiation of cochlear pro-sensory cells. RNA-sequencing of E13.5 control and Trim71 homozygous mutant cochlear epithelia revealed that TRIM71 negatively regulates the expression of pro-differentiation genes, including Inhba (Activin A) and Tgfbr2, suggesting that TRIM71 maintains pro-sensory cells in a proliferative and undifferentiated state by antagonizing TGF-β type signaling. Consistent with such role, we found that inner ear-specific deletion of Inhba and Tgfbr1 delayed pro-sensory cell cycle withdrawal and differentiation. Trim71 homozygous mutant (Trim71Δ/Δ) mice had hearing deficits, and qualitative similar hearing defects were observed in Trim71 mutant mice, which harbor a point mutation in TRIM71's RNA-binding domain found in patients with congenital hydrocephaly and hearing loss (Trim71Δ/R595H). RNA-sequencing of P30 control and Trim71 homozygous mutant cochleae suggest that early embryonic loss of Trim71 de-regulates synaptic and hair cell bundle –specific gene expression. Indeed, phenotypic analysis revealed that Trim71 deficient inner hair cells have mild hair cell bundle defects and form fewer synapses.
Conclusions: Trim71 is highly expressed in otic and cochlear progenitor cells, its maintains pro-sensory cells in an undifferentiated and proliferative state and its function is essential for hearing in mice and humans.

Tbx2 is a Master Regulator of Inner Vs Outer Hair Cell Differentiation

Jaime Garcia-Anoveros*[1], John C. Clancy[2], Chuan Zhi Foo[2], Ignacio García-Gómez[2], Yingjie Zhou[2], Kazuaki Homma[2], Mary Ann Cheatham[2], Anne Duggan[2]
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Background: The cochlea utilizes two types of mechanosensory cells to detect sounds. A single row of inner hair cells (IHCs) synapse onto neurons to transmit sensory information to the brain, whereas three rows of outer hair cells (OHCs) selectively amplify auditory inputs. To date, two transcription factors have been implicated in the specific differentiation of OHCs, and none in that of IHCs. One of these, INSM1, acts during an embryonic critical period to consolidate the OHC fate, preventing OHCs from transdifferentiating into IHCs. In the absence of INSM1, embryonic OHCs misexpress a core set of IHC-specific genes, which we postulated as likely involved in IHC differentiation. Here we find that one of these genes, Tbx2, is a master regulator of IHC vs OHC differentiation.
Methods: Ablation of Tbx2 in embryonic IHCs (Atoh1-Cre;Tbx2-F/F and Gfi1-Cre;Tbx2-F/F mice) results in their developing as OHCs, expressing early OHC markers like Insm1 and not early IHC markers like Fgf8) and eventually becoming completely mature OHCs in the position of IHCs. These inner compartment outer hair cells (ic-OHCs) are molecularly, anatomically and physiologically like OHCs, and display electromotility. Hence, Tbx2 is necessary for IHCs to differentiate distinctly from OHCs.
Because in the absence of INSM1 many embryonic OHCs express TBX2 and differentiate as IHCs, we tested both regulators for epistasis by generating double conditional KOs (Atoh1-Cre;Tbx2-F/F;Insm1-F/F) and found that Tbx2 is epistatic to Insm1. In the absence of both genes, OHCs do not become IHCs whereas IHCs become OHCs. Hence, TBX2 is necessary for the abnormal transdifferentiation of INSM1-deficient OHCs into IHCs, as well as for normal IHC differentiation.
Results: Because IHCs express TBX2 throughout development, we wondered whether it would play a role past the initial specification of IHCs. Ablation of Tbx2 in postnatal, largely differentiated IHCs (Fgf8-CreER;Tbx2-F/F with Tamoxifen administration at postnatal day 9), makes them transdifferentiate directly into OHCs, replacing IHC features with those of mature, but not embryonic, OHCs.
Finally, ectopic expression of Tbx2 (delivered by Anc 80 serotype AAVs in cochlear explant cultures) in OHCs results in their transdifferentiation into IHCs. Hence, Tbx2 is both necessary and sufficient to make, and maintain throughout development, IHCs distinct from OHCs.
Conclusions: From an evolutionary perspective OHCs, appear as the novel cell type, as they are unique to mammals and differ from other hair cells by displaying electromotility instead of robust presynaptic transmission. However, while evolutionarily the features of OHCs may be more recent than those of IHCs,

the developmental program that distinguishes the two cells from each other operates by TBX2 preventing IHCs from acquiring and maintaining a default, OHC fate. We propose a model in which a failure to differentiate of some ancestral IHC-like cell contributed to generating a new cell type, the OHC.

The Role of Tcf/Lef Transcription Factors in the Formation of Inner Ear Sensory Organs

Magdalena Zak*[1], Javier de Andres[1], Stephen Terry[1], Nicolas Daudet[1]
[1]*UCL Ear Institute, London, England*

Background: The inner ear is composed of several sensory organs responsible for the detection of sound, head position and acceleration. During embryonic development, these organs originate from neurosensory-competent domains within the otocyst, but the molecular signals controlling their formation remain unclear. The transcription factor Sox2 is required for neurosensory specification since its absence abolishes the differentiation of sensory organs and their associated neurons. Sox2 is initially present throughout the otocyst, then it becomes restricted to its ventro-medial aspect. Our recent work suggests that this restriction is regulated by a dorso-ventral gradient of (high to low) canonical Wnt activity. To find out which particular effectors of the Wnt signalling pathway are implicated in this process, we analysed the expression and function of the four members of the Tcf/Lef family of transcription factors in the chicken otocyst.
Methods: To assess the role of each specific Tcf/Lef transcription factor in the sensory organ formation, we combined gene overexpression, Wnt reporter and CRISPR/Cas9 mediated knock-out in the embryonic chicken inner ear.
Results: We found that Lef1, Tcf7, Tcf7l1 and Tcf7l2 exhibit distinct expression patterns in the chicken otocyst. Lef1 and Tcf7 expression was found in the dorsal regions specific also for Wnt reporter activity, while Tcf7l1 and Tcf7l2 were detected in the Sox2-positive neurosensory domain localised in the ventral otocyts. Our functional experiments showed that overexpression of Lef1 and Tcf7l1 induced ectopic Sox2 expression in the otocyts.
Conclusions: The distinct expression patterns of Tcf/Lef genes suggests that they could participate to different functions of Wnt signalling during otic development. Our initial results point at Lef1 and Tcf7l1 as the most promising candidate regulators of neurosensory specification. Further studies will evaluate their importance for early morphogenesis of the otocyst and the differentiation of sensory organs

Prdm1 Drives a Sensory Hair Cell Fate Switch in Zebrafish

Jeremy Sandler*[1], Nhung T.T. Tran[1], Shiyuan Chen[1], Malcolm Cook[1], Tatjana Piotrowski[1]
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Background: A major cause of hearing loss in mammals is the lack of regeneration in the cochlea following damage to mechanosensory hair cells. Regenerating hair cells is a central strategy for restoring hearing, but triggering proliferative regeneration and maturation in mammals remains elusive. The zebrafish Danio rerio has hair cells in an array of mechanosensory organs along the trunk along the trunk, called the lateral line, and in the inner ear. While sharing genetic, functional, and structural similarity with mammalian inner ear hair cells, zebrafish hair cells readily and rapidly regenerate following death to restore full function. The transcription factor prdm1a is expressed in hair cells of the zebrafish lateral line, but not in hair cells of zebrafish or mammalian inner ears. Mammalian Prdm1 has been shown to control a fate switch in various cell types. The combination of its hair cell-specific expression and its involvement in cell fate decisions led us to investigate prdm1a for its role in hair cell development and regeneration.
Methods: We mutated prdm1a in zebrafish by introducing a point mutation that truncates the protein and eliminates zinc finger domains. Using this mutant, we assayed hair cell development and regeneration, including the number of developing or regenerated hair cells, and support cell and hair cell proliferation. We performed scRNA-seq on sorted lateral line cells from prdm1a mutants and siblings, and used the data to cluster hair cells and identify changes in gene expression in the mutant. Finally, we combined our scRNA-seq with ATAC-seq and ChIP-seq generated from lateral line cells to identify enhancers and promoters of genes targeted by Prdm1a, and investigate their ability to drive expression of a GFP reporter.
Results: In prdm1a mutants, we found a drastically reduced number of hair cells and cell proliferation in the lateral line during development and regeneration. scRNA-seq revealed a cell type fate switch between lateral line and inner ear hair cells, with a multitude of inner ear hair cell-specific genes ectopically expressed in mutant lateral line hair cells. Motif searches revealed Prdm1 binding sites highly enriched in the promoters and enhancers of ectopically expressed genes. Using these enhancers to drive GFP expression in zebrafish

confirmed their ectopic expression in prdm1a mutant hair cells, but not in siblings. Finally, expressing prdm1a in the inner ear hair cells, where it is not normally expressed, was sufficient to repress inner ear hair cell genes.
Conclusions: These findings show that prdm1a plays a crucial role in repressing an inner ear hair cell fate in lateral line organs and highlight prdm1 as a potential driver of hair cell type specification and regeneration in other vertebrates. Combined, our data show that prdm1 is an essential gene to consider in future regeneration attempts in the mammalian cochlea.

CHD7 and SOX2 Cooperate to Regulate Development of the Inner Ear

Jingxia Gao*[1], Jennifer Skidmore[2], Jelka Cimerman[2], Elaine Ritter[2], Yehoash Raphael[2], Donna Martin[3]
[1]Department of Pediatrics, University of Michigan,2[3]Kresge Hearing Research Institute, Department of Otolaryngology - Head and Neck Surgery, Michigan Medicine, [3]Department of Pediatrics and Genetics, University of Michigan
Background: Hereditary hearing loss and balance disorders remain important clinical problems that have become easier to diagnose but have not greatly benefitted from advances in molecular therapies. An important step in designing therapies for hereditary hearing loss and balance disorders is understanding their underlying mechanisms. In this study, we explored genetic interactions between Chd7 and Sox2, two genes that are both highly expressed in and critical for early inner ear development. Chd7 encodes the ATP-dependent chromatin remodeler CHD7, mutated in CHARGE syndrome. Sox2 encodes Sex determining Region Y-box 2 family transcription factor SOX2. In humans, single copy loss of either CHD7 or SOX2 disrupts development of multiple organs and tissues, including the brain, eye, ear, and heart. SOX2 and CHD7 have been shown to physically interact in neural stem cells, but the extent of their interaction in the developing ear has not been explored.
Methods: Chd7 germline and conditional mutant mice (Chd7Gt/+; Chd7flox/flox), Sox2 inducible Cre knockin and conditional mutant mice (Sox2CreER/+; Sox2flox/flox), and Lhx1 germline heterozygous knockin mice (Lhx1TLZ/+) were used to explore temporal and spatial interactions of these genes in the developing inner ear. Expression of Sox2 and Chd7 in the ear was examined by immunohistochemistry. The inner ear membranous labyrinth was evaluated using paint-fill. The cochleae of postnatal day 1 (P1) pups were dissected, measured, and stained in whole-mount assays for confocal imaging. Microdissected E10.5 otocysts were processed for RNA sequencing.
Results: We found that Chd7 and Sox2 are dynamically co-expressed in the developing otocyst and cochleovestibular ganglion. Chd7 is required for normal Sox2 expression in the E10.5 otocyst, while Chd7 expression is Sox2-independent. Double heterozygous (Chd7Gt/+;Sox2CreER/+) mice exhibit early postnatal death and severely malformed inner ear vestibular and auditory structures, including defects of the semicircular canals and shortened cochlea. Through inducible deletions, we identified a critical window (~E9.5) for Chd7-Sox2 genetic interactions in the developing semicircular canals. Supernumerary outer hair cells were present in Chd7Gt/+;Sox2CreER/+ cochleae while innervation and stereociliary orientation of hair cells were normal. Differential gene expression analysis revealed 75 down-regulated genes and 56 up-regulated genes in E10.5 Chd7Gt/+;Sox2CreER/+ developing otocysts compared to wild type. The most highly upregulated gene was the LIM homeodomain transcription factor gene Lhx1, confirmed by immunostaining to be ectopically expressed in the Chd7Gt/+;Sox2CreER/+ otocyst. Interestingly, mice with single copy loss of both Chd7 and Lhx1 exhibited worsened cochlear hypoplasia compared to Chd7Gt/+, suggesting complex genetic interactions in the developing ear.
Conclusions: These results demonstrate tightly regulated expression of Chd7 and Sox2 during inner ear development, and perturbation of the balance between these two genes leads to complex inner ear malformations. These results could inform the design of novel targets for molecular therapies to treat hearing and balance disorders.

Conditional Expression of Constitutively Active Bmp Signaling in the Developing Cochlea Causes Duplication of the Organ of Corti

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Background: Early in cochlear development, around embryonic day 10 (E10), a gradient of BMP signaling is established along the cochlear epithelium's radial axis, which defines it into three domains. Low, moderate, and high levels of BMP respectively promote specification of the neural, prosensory, and abneural

domains, suggesting that BMP acts in a cell-autonomous manner. Notably, the prosensory domain fails to form with the loss of BMP signaling in BMPR1A; BMPR1B receptor compound mutants and the cochlear epithelium instead adopts the low BMP signal fate of the neural domain. In this study, we are investigating how developing cochlear epithelial cells differentially interpret the BMP gradient signals to acquire their unique cell fates, specifically testing if these fates change with the perturbance of the BMP signaling gradient.
Methods: We designed two knock-in mouse lines using the human BMPR1A (hBMPR1A) gene, one with a wild-type allele, and another with constitutively active kinase activity containing a point mutation (Q233D) in the kinase domain. These alleles were inserted at the ROSA26 locus downstream of a floxed STOP cassette. Expression of the hBMPR1AQD and hBMPR1AWT transgenes was conditionally activated using Sox2CreER driver mice at E9.5, just as the BMP gradient becomes defined. We subsequently analyzed the developmental phenotype of the cochlear ducts at early (E13.5), mid (E15.5), and late (E18.5) stages by performing immunofluorescence assays of tissue sections and the whole mount cochleae with antibodies specific to distinct cell types of the cochlear epithelium. We also used light sheet microscopy (LSM) at each time point to determine whether overall cochlear morphology was altered by BMP disruption.
Results: Activation of the constitutively active hBMPR1AQD transgene in Sox2 expressing progenitors at the onset of cochlear extension resulted in a duplicated mirror image of the organ of Corti on the medial side of the “regular” organ of Corti at E18.5. LSM images of these cochleae, immunostained with an epithelial cell marker, showed a significantly shorter length along the apical-basal axis, compared to the controls. Furthermore, we also see an increased number of rows of inner and outer hair cells in both the regular and the duplicated organ of Corti. We do not observe any aberrant phenotype following activation of the hBMPR1AWT allele in the cochlea. In light of these intriguing findings, we are further characterizing BMPR1AQD mice with markers specific to the cells of the neural and abneural domains to better understand the patterning defects caused by this perturbation.
Conclusions: In summary, using loss of function studies, our lab has previously shown that BMP signaling is necessary for cochlear patterning. Our current, gain-of-function studies support and extend this discovery by showing that conditional expression of constitutively active BMP signaling is sufficient for the formation of a duplicate organ of Corti.

Podium #8 - Peripheral Auditory Mechanisms

10:30 a.m. - 12:30 p.m.
Oceans Ballroom 1-4

Moderators: Rick Nelson and Rick A. Friedman

Atomistically Detailed Simulations of the Voltage-Response of Prestin

Oliver Beckstein*[1], Edis Jakupovic[1], Dhasakumar Navaratnam[2], Joseph Santos-Sacchi[3]
[1]Arizona State University, [2]Yale University School of Medicine, [3]Yale University School of Medicine, Surgery, Neuroscience, Cellular and Molecular Physiology
Background: Prestin is an integral membrane protein in outer hair cells (OHC) in the inner ear that is ultimately responsible for sound amplification by a change in cross sectional area in response to transmembrane voltage changes. Prestin is a member of the SLC26 family of transporters that normally transport anions across the cell membrane but prestin does not exhibit sizable transport activity. Biochemical evidence and recent cryo-electron microscopy structures strongly suggest that prestin undergoes at least a partial "elevator"-type movement, reminiscent of other secondary active transporters. Unlike most other transporters, the conformational change is strongly voltage dependent (reminiscent of voltage sensors in voltage-gated potassium channels). Chloride anions bind to prestin but it is not clear if they are actually transported and they only modulate prestin function but are not absolutely required. Structures of the 0 mV prestin dimer are compact and "contracted" and it is believed that the conformation at the normal resting potential of -70 mV is expanded. Similar to depolarization, measurements of non-linear capacitance showed that an increase in temperature can also shift the conformational equilibrium of prestin. We hypothesized that we could directly observe the effect of the membrane potential and temperature on chloride binding and the conformational equilibrium of prestin.
Methods: We performed all-atom molecular dynamics (MD) simulations of prestin from the "compact" gerbil (PDB ID 7SUN) in a mixed POPC/cholesterol membrane environment with a 150 mM NaCl

concentration and explicit water under a range of applied membrane voltages, ranging from -150 mV to +150 mV.
Results: The gerbil cryo-EM structure is of a good quality, as evidenced by stable MD simulations over more than 5 μs in total. Chloride ions bind spontaneously to the chloride binding site in a voltage-dependent manner with the hyperpolarized state disfavoring Cl− as expected from experiments. The reduction in binding in the expanded state has been hypothesized to be due to a widening of the binding site compared to the compact state. Starting from the compact conformation, the system samples a range of conformations towards a more expanded state, including widening of the binding site and downward shift of the core domain. These fluctuations are more pronounced at elevated temperature.
Conclusions: MD simulations capture the voltage-dependent binding of Cl- to prestin and suggest that the equilibrium between compact and expanded conformations of prestin is shifted to the expanded state at elevated temperatures.

Highly Efficient Transduction of the Human Cochlea and Vestibule From the Cerebrospinal Fluid Space Using MRI Gadolinium Contrast

Douglas Totten*[1], Kevin Booth[2], Kristine Mosier[1], Nicholas Koontz[1], Rick Nelson[1]
[1]Indiana University School of Medicine, [2]Harvard Medical School
Background: Delivery of therapeutic drugs and viral-mediated gene augmentation have been widely studied from the transtympanic approach. Marker or drug distribution into the cochlea from the cerebrospinal fluid (CSF) space intracranially in human patients is not well characterized.
Methods: MRI imaging was performed on adults using intrathecal MRI contrast gadolinium to evaluate for CSF leak of the cranial base. Quantification of contrast in the cochlea, vestibule and semicircular canals at 2 hours and on delayed imaging.
Results: 16 patients (10 females) at an average (standard deviation) age of 46 (19) years underwent MRI imaging which revealed 5 spontaneous CSF leaks, 2 post-surgical CSF leaks, and 1 traumatic CSF leak, while 8 patients did not have a CSF leak. All patients demonstrated uptake of contrast in bilateral cochleae. Peak contrast density was 53 (23)% (range: 14 – 97%) of the CSF density. Delayed imaging at an average 4.5 hours after contrast administration demonstrated increased contrast density within the cochlea to 65.5 (21.5)% (range: 31-100%). Contrast within the vestibule was seen in 7 patients on initial imaging and all patients on delayed imaging.
Conclusions: Transduction of bilateral intact cochleae from the CSF space is highly efficient and increases over several hours. This has implications for future therapeutic delivery to the inner ear and raises the possibility of contralateral transduction of transtympanically delivered harmful drugs, such as gentamicin.

Development of Individualized, Additively Manufactured Drug Releasing External Ear Canal Implants for Prevention of Postoperative External Ear Canal Restenosis

Farnaz Matin-Mann*[1], Ziwen Gao[1], Chunjiang Wei[1], Jana Schwieger[1], Martin Ulbricht[2], Vanessa Domsta[2], Stefan Senekowitsch[2], Werner Weitschies[2], Anne Seidlitz[3], Katharina Doll[4], Thomas Lenarz[1], Verena Scheper[1]
[1]Department of Otorhinolaryngology, Head and Neck Surgery, Hannover Medical School/ Lower Saxony Center for Biomedical Engineering, Implant Research and Development (NIFE), Cluster of Excellence Hearing4all, [2]Department of Biopharmacy and Pharmaceutical Technology, Institute of Pharmacy, University of Greifswald, Center of Drug Absorption and Transport, Greifswald, Germany, [3]Institute of Pharmaceutics and Biopharmaceutics, University of Duesseldorf, Germany, [4]Department of Dental Prosthetics and Biomedical Materials Science, Hannover Medical School, Hannover, Germany
Background: Reducing the rate of postoperative external ear canal (EEC) stenosis is essential following canaloplasties. To prevent the need for revision surgeries, our aim was to explore the feasibility of using three dimensionally (3D) printed, patient individualized, drug releasing (dexamethasone (DEX) and ciprofloxacin (cipro)) external ear canal implants (EECI) as postoperative stents.
Methods: The EECI models were manually segmented from temporal bone cone beam computed tomography images and subsequently 3D printed using medical-grade UV silicone (60A MG, BIO-83-6001, EnvisionTEC, silicone elastomer curing at 365 nm, USP Class VI)). The implants were 3D printed using a 3D-Bioplotter® Manufacturers Series (EnvisionTEC GmbH, Gladbeck, Germany) and the silicone was loaded with DEX and cipro. Test specimen of the drug loaded silicone were pre-clinically tested for drug

release (high-performance liquid chromatography), biocompatibility (MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay with fibroblasts), bio-efficacy (TNFα (tumor necrosis factor-alpha)-reduction test (DEX) with dendritic cells, inhibition zone test against the bacteria Staphylococcus aureus and Pseudomonas aeruginosa (cipro)), and microbial contamination (formation of turbidity or sediments in culture media). Following, individualized EECI were implanted in three patients with a history of either congenital EEC atresia, postoperatively acquired EEC stenosis or EEC restenosis after several canaloplasties.
Results: The preclinical tests of the EECI made of UV silicone containing 1% DEX and 0.3% cipro proved the release of the drugs and revealed no cytotoxic effect of the used materials. A general anti-inflammatory and an antibacterial effect was verified and the tested UV irradiated EECI showed no microbiological contamination. The EECI were implantable into the EEC with ease and explanted three months postoperatively. None of the patients showed otogenic symptoms or infections during the postoperative follow up visits. Even 12 months postoperatively the EECs showed a good epithelialization and patency.
Conclusions: We report different clinical applications of individualized, mechanically flexible, drug-releasing EECI and suggest that our novel approach represents a safe and effective method for stenting the reconstructed EEC postoperatively.

Simulating Tympanometry With a Nonlinear Viscoelastic Finite Element Model of the Human Middle Ear

Ignacio Kahnlein*[1], Hamid Motallebzadeh[2], Andrew Tubelli[3], Hong Zhu[4], Wu Zhou[4], Sunil Puria[5], Richard Rabbitt[1]
[1]University of Utah, [2]Harvard Medical School, Mass. Eye and Ear, [3]Mass. Eye and Ear Infirmary, [4]Department of Otolaryngology-Head and Neck Surgery, University of Mississippi Medical Center, [5]Harvard Medical School, Mass. Eye and Ear Infirmary
Background: The biomechanics of tympanometry are inherently nonlinear, and clinical results cannot be accurately interpreted in terms of linear models of the middle ear. In the present work we developed a fully nonlinear finite-element model of the human middle ear with the goal of improving interpretation of traditional single-frequency tympanograms and improving our understanding of wideband reflectance measured in the presence of high ear canal static pressure loads. The model includes kinematic nonlinearities (e.g., the Helmholtz tympanic membrane lever) as well as strain stiffening in the tympanic membrane that occurs as stress increases in the radial and circumferential fibers. The model is suitable for comparing the relative contributions of kinematic vs. constitutive nonlinearities.
Methods: A 3D geometrical representation of the middle ear of an adult male was constructed based on μCT imaging data. The three-dimensional segmented morphology was meshed into 89,636 HEX8 finite elements for numerical simulations of nonlinear mechanics. The model includes the ear canal, tympanic membrane, tympanic cavity, ossicles, middle-ear muscles, ligaments, a termination impedance at the oval window, and was implemented in the software FEBio Studio (https://febio.org). The tympanic membrane was modeled using a visco-hyperelastic material, air was modeled as an inviscid fluid, bone was modeled using linear elasticity, and ligaments were modeled as either visco-hyperelastic or hyperelastic models. The tympanogram stimulus was approximated as a sweeping quasi-static pressure at the ear canal probe entrance plus a sinusoidal perturbation of 226 Hz, repeated over multiple static pressures to generate the simulated tympanogram. Simulations were done in the time domain using an implicit solver with kinematic nonlinearities fully included. The spatially averaged pressure, volume velocity, and entrance area at the probe entrance were extracted from the three-dimensional finite-element results and used to compute the admittance from the Fourier components of the stimulus perturbation frequency.
Results: Preliminary results capture tympanogram trends present in a normal ear, including peak admittance at zero trans tympanic membrane pressure and decreased admittance at both positive and negative pressures. Results also show an asymmetry that putatively arises from the classical Helmholtz tympanic membrane effect. Parameter optimization and subject-specific validation has not yet been completed.
Conclusions: Although computationally expensive, results demonstrate potential for subject-specific analysis of tympanograms. The approach also has potential for analysis of the middle ear under extreme loading conditions such as blast exposure or head impact. [This work was supported in part by NIDCD grant R01 DC018919 (HZ and WZ)]

Effects of Unilateral Eye Closure on Middle Ear Muscle Contractions

Heath Jones*[1], Stephen Tasko[2], Kristy Deiters[2], Gregory Flamme[2], Madeline Smith[2], William Murphy[2], Nathaniel Greene[3], William Ahroon[1]
[1]U.S. Army Aeromedical Research Laboratory, [2]Stephenson and Stephenson Research and Consulting, [3]University of Colorado School of Medicine
Background: Middle ear muscle contractions (MEMCs) are most considered a response to high-level acoustic stimuli. However, MEMCs have also been observed in the absence of sound, either as a response to somatosensory stimulation or in concert with other motor activity. The relationship between MEMCs and non-acoustic sources is unclear.
Methods: This study examined associations between measures of voluntary unilateral eye closure and impedance-based measures indicative of middle ear muscle activity while controlling for demographic and clinical factors in a large group of participants (N = 190) with present clinical acoustic reflexes and no evidence of auditory dysfunction. Participants were instructed to voluntarily close the eye ipsilateral to the ear canal containing a detection probe at three levels of effort. Orbicularis oculi muscle activity was measured using surface electromyography. Middle ear muscle activity was inferred from changes in total energy reflected in the ear canal using a filtered (0.2 to 8 kHz) click train.
Results: Results revealed that middle ear muscle activity was positively associated with eye muscle activity. MEMC occurrence rates for eye closure observed in this study were generally higher than previously published rates for high-level brief acoustic stimuli in the same participant pool suggesting that motor activity may be a more reliable elicitor of MEMCs than acoustic stimuli.
Conclusions: These results suggest motor activity can serve as a confounding factor for auditory exposure studies as well as complicate the interpretation of any impulsive noise damage risk criteria that assume MEMCs serve as a consistent, uniform protective factor. The mechanism linking eye and middle ear muscle activity is not understood and is an avenue for future research.

Single-Cell RNA-Seq Defines the Cell Types of the Normal Human Middle Ear Mucosa

Allen F. Ryan*[1], Rick Friedman[1], Art Nasamran[1], Arwa Kurabi[1]
[1]University of California, San Diego
Background: The middle ear (ME) plays a critical conductive role in hearing, but is a frequent site of infection. Many cell types make up the lining mucosa of the ME, but their function, molecular characteristics and response to pathogens are not well understood. We recently evaluated the transcriptomes of normal ME mucosal cells in the mouse. In this study we similarly assessed the various cells of the normal human ME and compare them to murine ME cells.
Methods: Samples of the normal human ME mucosa were obtained as wastage from trans-labyrinthine surgeries. Each sample was individually prepared for single-cell RNA-Seq on a 10X Chromium Controller according to 10X protocols. Single-cell libraries were sequenced on an Illumina HiSeq 2500. Transcriptome data were analyzed using Cellranger 2.0.2 and mkfastq in conjunction with bcl2fastq 2.17.1.14 and aligned to a human reference genome. Human ME sample data were assessed individually and collectively. Cell clusters in cLoupe and UMAP were identified using recognized marker genes. The expression of murine homologues of human genes was then compared to human genes for various cell types.
Results: The majority of cell types observed in the human samples were also present in the murine ME. However, melanocytes which form a cluster in some murine samples were not observed in the human ME. Schwann cells formed a distinct cluster in the human ME, but only a few scattered cells were seen in the mouse. The proportions of some cell types were distinct. Many more ciliated and basal epithelial cells were present in the human than the mouse ME. Many homologous genes showed comparable expression levels within a given cell type. For example, the expression of innate immune receptor mRNAs was highly similar across human and mouse ME cell types. An exception was the gene encoding NLRP1, a receptor component of inflammasomes that activate the pro forms of IL1b and IL18. Human ciliated epithelial cells expressed significant levels of this gene, with lower expression by endothelial and stroma cells, but all mouse ME cells were negative. In contrast to most innate immune receptors, many cytokine genes displayed different expression patterns: e.g. the CXCL2 gene was strongly expressed only in mouse ME monocytes, but in the human only in mature, non-ciliated epithelial cells. Aquaporin gene mRNAs were distributed across different epithelial cell types in the two species.
Conclusions: Overall, our results suggest that the cells of the normal human and mouse MEs have similar transcriptomes. However some differences with relevance to innate immunity, fluid management and blood

vessel homeostasis were noted. These would likely influence the responses of the human ME to infection. Our cross-species transcriptome compendium can serve as a resource for future translational studies.

The Evolving Description and Scientific Understanding of an Otological Disease Entity: Otitis Media

Robert Ruben*[1]
[1]*Albert Einstein College of Medicine*

Background: The human ear is a constant structure and the disease entities that have affected the ear for the most part have also been constant. The description and scientific understanding of these entities through the accumulation of knowledge, however, has evolved through time. This study documents a paradigmatic example: the evolution of the identification and scientific understanding of otitis media.

Methods: The descriptions and scientific understanding concerning the disease entities that currently fall within the rubric of otitis media are documented through an examination of the primary sources from the 5th millennium BCE to the present.

Results: Prehistorical pathological evidence of infected mastoid has been documented by the examination of paleolithic temporal bones.

The earliest descriptions of what could be considered as otitis media are found in medical treatises of the 5th millennium BCE from Mesopotamia and in Egyptian papyri of the 16th century BCE. From 5th century BCE through the 3rd century CE, conditions which can be interpreted in terms of otitis media are noted in the Hippocratic corpus. Aristotle in the 4th century BCE comments on barotrauma to the middle ear in divers. Roman medicine, based on work in the 2nd and 3rd centuries and continuing through the 9th century and beyond, describes inflammation of the tympanic cavity; it is noted that long-lasting suppuration tends to involve bone. Arabic medicine of the 9th through the 17th centuries describes ear infections although it does not differentiate between otitis externa and otitis media. From the end of the 17th century to the middle of the 18th century, otitis media is recognized through pathological examination, but the observations are not applied clinically. From the end of the 18th century to the beginning of the 20th century, three forms of otitis media are recognized. In the first two-thirds of the 20th century, more than twenty-five different forms of otitis media are recognized. Countering expectations of evolving toward increasing complexity, during the latter part of the 20th century, for all intents and purposes, just three forms of otitis media are recognized. In the 21st century, multiple forms of otitis media are being identified through the use of genetic analysis.

Conclusions: The development of the description and scientific understanding of otitis media came about through the increased knowledge of pathological anatomy, histopathology, diagnostic tools, e.g., the otoscopic, measurement of hearing ability, and the observation of the morbidity of hearing loss.

The development of scientific knowledge has enabled advances in description and understanding of otitis media through the centuries; it should continue to do so.

Symposium #9 - Auditory Expectations, Learning and Plasticity

10:30 a.m. - 12:30 p.m.
Crystal Ballroom D-E

Auditory Expectations, Learning and Plasticity

Chair: Christopher Petkov, *University of Iowa and Newcastle University*
Co-Chair: Maria Geffen, *University of Pennsylvania*
Co-Chair: Kerry Walker, *University of Oxford*

The auditory system constantly generates predictions about the sensory world based on prior experience, which creates expectations about upcoming future events. Auditory expectations powerfully shape sensation, perception and cognition, and a theoretical ‘predictive coding’ framework has advanced in large part from studies in the auditory domain. The expectancy process is thought to be mediated by neural interactions between feedforward and feedback processes all along the auditory pathway, using corticofugal projections from auditory cortex to the periphery, and back. However, until recently it had been unclear how different types of auditory expectations are detected and the role of different stages of the auditory pathway.

This symposium will provide a timely update to address:

- How do auditory expectations form, as an important basis for learning?

- What happens when expectations are not met and the system needs to adapt?
- In disorders that affect the auditory system how are expectancy signals affected?
- Is there plasticity that compensates for impact on the auditory system, and can auditory system impact be remediated?

The symposium will feature presentations from prominent early career and senior scientists, experts in the field of auditory expectancy, learning and plasticity. The presentations will cover a broad range of animal species, including murine models, nonhuman primates and humans. The speakers will showcase the latest neurophysiological approaches possible with each species, ranging from insights at the single neuron levels to mesoscopic and macroscopic studies of the auditory system in humans, including patients with auditory and communication impairments. The presentations will highlight cutting-edge approaches, including circuit-level optogenetic and pharmacological manipulations to identify causal elements involved in auditory expectancy or processes studied with human intracranial recordings and neuroimaging. The speakers will also provide insights on how the auditory field is the leading modality helping to test and advance computational theories on predictive coding.

Corticofugal Regulation of Predictive Coding
Maria Geffen, *University of Pennsylvania*

Sensory systems must account for both contextual factors and prior experience to adaptively engage with the dynamic external environment. In the central auditory system, neurons modulate their responses to sounds based on statistical context. These response modulations can be understood through a hierarchical predictive coding lens: responses to repeated stimuli are progressively decreased, in a process known as repetition suppression, whereas unexpected stimuli produce a prediction error signal. Prediction error incrementally increases along the auditory hierarchy from the inferior colliculus (IC) to the auditory cortex (AC), suggesting that these regions may engage in hierarchical predictive coding. A potential substrate for top-down predictive cues is the massive set of descending projections from the AC to subcortical structures, although the role of this system in predictive processing has never been directly assessed. We tested the effect of optogenetic inactivation of the auditory cortico-collicular feedback in awake mice on responses of IC neurons to stimuli designed to test prediction error and repetition suppression. Inactivation of the cortico-collicular pathway led to a decrease in prediction error in IC. Repetition suppression was unaffected by cortico-collicular inactivation, suggesting that this metric may reflect fatigue of bottom-up sensory inputs rather than predictive processing. We also discovered populations of IC units that exhibit repetition enhancement, a sequential increase in firing with stimulus repetition. Cortico-collicular inactivation led to a decrease in repetition enhancement in the central nucleus of IC, suggesting that it is a top-down phenomenon. Negative prediction error, a stronger response to a tone in a predictable rather than unpredictable sequence, was suppressed in shell IC units during cortico-collicular inactivation. These changes in predictive coding metrics arose from bidirectional modulations in the response to the standard and deviant contexts, such that the units in IC responded more similarly to each context in the absence of cortical input. We also investigated how these metrics compare between the anesthetized and awake states by recording from the same units under both conditions. We found that metrics of predictive coding and deviance detection differ depending on the anesthetic state of the animal, with negative prediction error emerging in the central IC and repetition enhancement and prediction error being more prevalent in the absence of anesthesia. Overall, our results demonstrate that the AC provides cues about the statistical context of sound to subcortical brain regions via direct feedback, regulating processing of both prediction and repetition.

Precision in Predictive Coding: The Role of Neuromodultion in Deviance Detection
Manuel Malmierca, *Institute of Neuroscience Castilla y Leon, INCYL.*

Behaviour becomes more efficient when we can predict future stimuli. This is the basis of the enormous flexibility underlying interactions with our physical and social environment. Filtering out non-relevant stimuli is an important but understudied aspect of cognition. Today, there is rather universal agreement in neuroscience, that a major function of the brain is to constantly predict the environment on multiple levels and time scales (Friston, 2005). For example, we anticipate how a word of a friend will sound, and when and

how a sentence will end. This proposal is based on the assumption that the brain's neuronal circuitry is organized as a highly predictive machine.

Prediction errors arise as a disagreement between a prediction and the actual sensory input. The ability of the brain to recognize which prediction errors carry reliable information is critical in the process of correctly identifying unexpected stimuli. For example, if a sensor is malfunctioning because of an impairment (e.g., hearing loss or tinnitus) or because it is operating out of its appropriate physical range, the sensory input it provides is not adequately reporting on real changes in the environment, which may generate misinformative prediction errors. According to the predictive coding theory, this distinction between signal and noise is based on an important element, the precision of the prediction errors, which weights the driving power of prediction errors according to how reliable they are estimated to be (Friston, 2005).

Neuromodulatory inputs not only gate plasticity (Martins and Froemke, 2015), but also change the balance of top-down versus bottom-up influence; it is well known that neuromodulation strongly impacts sensory processing, learning and memory. Predictive coding models propose that neuromodulatory systems implement precision weighting through regulation of postsynaptic gain (Bastos et al., 2012), particularly theorizing about the involvement of acetylcholine (Moran et al., 2013). Therefore, in this talk I will show our recent work that investigates which neuromodulators are involved in the encoding of the predictions and prediction errors and how neuromodulators regulate the precision of prediction errors. We have used single neuron recordings and microiontophoretic manipulation of the cholinergic system in the rat brain to study how these neuromodulators shape the predictive responses in cortical and subcortical brain regions in the rat brain.

Prediction and Memory in the Auditory Cortex
Ryszard Auksztulewicz, *Free University Berlin*

The brain is thought to generate internal predictions, based on the memory of past stimulation, to optimise behaviour. However, it is unclear to what extent the neural correlates of mnemonic and predictive are dissociable, and whether they generalize across species. In this talk I will present results of studies combining human and rodent electrophysiology with multivariate pattern analysis, which allowed us to decode both mnemonic and predictive information from neural activity in the auditory cortex. First, in a parallel study in awake humans and anaesthetized rats, we show that mnemonic traces can be decoded from neural activity to neutral impulses presented during memory retention interval, using homologous data modalities and identical statistical methods across species and attentional states. Second, in a study using electrocorticography in anaesthetized rats, we show that neural representations related to stimulus memory and predictions could be simultaneously decoded from auditory cortical activity. Decoding of auditory predictions and memories was based on uncorrelated data features, and decoding predictions increased over the course of acoustic stimulation. Taken together, these results shed light on the evolutionary conservation of mnemonic processing across species, and show evidence for predictive processing even in passive listening under anaesthesia.

Sequence Learning and Prediction Within the Primate Central Auditory System
Christopher Petkov, *University of Iowa and Newcastle University*

There is considerable interest in understanding the neuronal transformations that occur throughout the auditory system. These are no longer thought to be simply feedforward or unidirectional. Rather, they are better conceived of as bidirectional interactions that occur throughout the broader auditory network, whereby expectancy of future events influences sensory processing. In this presentation, we first overview work in human and nonhuman primates involving the central processing of probabilistic auditory sequences that establish expectancies of upcoming sounds based on prior experience. We present data from neurophysiological recordings in nonhuman primates and human neurosurgery patients showing auditory cortical expectancy signals and those that only appear when expectations are not met. The results establish direct parallels between the neurophysiological effects in the human and nonhuman primates. These central auditory processes also involve interactions with prefrontal cortex, which we are manipulating by using optogenetic control of pyramidal projection neurons between areas in the nonhuman primates as a model system. We conclude by overviewing recent structural and functional connectivity data establishing even

closer parallels across the species, charting new pathways for research on disorders of audition reliant on animal models.

Immediate Neural Network Impact and Compensation After the Loss of a Semantic Hub
Zsuzsanna Kocsis, *University of Iowa*

The human brain extracts meaning from the world using an extensive neural system for semantic knowledge. Whether such broadly distributed systems crucially depend on or can compensate for the loss of one of their highly interconnected hubs is controversial. The strongest level of causal evidence for the role of a brain hub is to evaluate its acute network-level impact following disconnection and any rapid functional compensation that ensues. We report rare neurophysiological data from two patients who underwent awake intracranial recordings during a speech prediction task immediately before and after neurosurgical treatment that required disconnection of the left anterior temporal lobe (ATL), a crucial hub for semantic knowledge. Informed by a predictive coding framework, we tested three sets of hypotheses including diaschisis causing disruption in interconnected sites and incomplete or complete compensation by other language-critical and speech processing sites. Immediately after ATL disconnection, we observed highly specific neurophysiological alterations in the recorded fronto-temporal network, including abnormally magnified high gamma responses to the speech sounds in auditory cortex. We also observed evidence for rapid compensation, seen as focal increases in effective connectivity involving language-critical sites in the inferior frontal gyrus and speech processing sites in auditory cortex. However, compensation was incomplete, in part because after ATL disconnection speech prediction signals were depleted in auditory cortex. This study provides direct causal evidence for a semantic hub in the human brain and shows striking neural impact and a rapid attempt at compensation in a neural network after the loss of one of its hubs.

Neural Mechanisms for Tracking Uncertainty in Rapidly Unfolding Sound Sequences
Maria Chait, *UCL Ear Institute*

The brain maintains a hierarchy of models to monitor the statistics of its surroundings and inform behaviour. Determining how such models are instantiated and updated is key to understanding the brain as a statistical learning machine.

The auditory system, supported by a network of auditory, hippocampal, and frontal sources, automatically discovers regularities in rapid tone sequences even when these are not behaviourally relevant. We have previously identified specific brain signatures of sequence structure tracking in humans. The transition from a random tone pattern to a structured pattern (RAN-REG) elicits a slow increase in tonic activity that is consistent with gradual evidence accumulation and instantiation of a new model. In contrast, the opposite transition (REG-RAN) evokes an 'interrupt' response': a sharp drop in sustained activity, hypothesized to reflect immediate suppression of top-down prior expectations. The activity settles at a low sustained level, consistent with the weaker statistical constraints in the RAN pattern.

In this series of MEG experiments, we investigated how "model establishment" and "interrupt" responses are affected by information rate (by using sequences of identical statistical properties but halving tone-pip length; 25 vs 50 ms) and predictability of pattern transitions.

We examined responses to the following sequence transitions: REG1-REG2 (from one regular to a different regular pattern) REG-RAN (from a regular to a random pattern) REG1-RAN-REG1 (a regular pattern interrupted by a 500ms random pattern followed by the resuming of the original pattern). The probabilities of these transitions were varied to model a range of environmental volatilities. Naive participants performed a decoy task, while listening passively to the sounds.

We report the following key findings:
(1) The detailed dynamics of discovering, abandoning and learning new structure in sound sequences are observable in the MEG signal.
(2) The dynamics of "model establishment" roughly scaled with tone duration but with some evidence of increasing sluggishness with longer tones.

(3) In REG1-RAN-REG1 trials, post interruption model establishment occurred much faster than in REG1-REG2 trials, suggesting that a model of the original sequence was automatically preserved and re-activated.
(4) The "interrupt" response did not differ between high probably and low probability interruptions, suggesting an automatic process that (unlike what is expected from a Bayesian system) is not affected by volatility per se.

Ongoing EEG work comparing such brain responses in human and non-human primates explores how mnemonic systems may have evolved to support pattern sensitivity in humans.

Laminar and High Spatial Resolution fMRI of Human Auditory Predictive Processing.
Federico De Martino, *Maastricht University*

To deal with dynamic changes in the soundscape and adjust our behaviour accordingly, a key function of our brain is to predict future states of the world. This has led to a transformative way of thinking about brain function. That is, what we perceive does not reflect the sensory stimulus itself, but rather a combination of the stimulus and an internal (generative) model of its causes. This idea has led to several theoretical advances some of which are capitalized by Predictive Coding (PC). PC assumes that generative models are formed through the exchange of prediction errors (feedforward) and predictions (feedback) across hierarchical processing stages. In addition, PC assumes that prediction errors are modulated by the precision of currently available predictions. Results from invasive animal and human electrophysiological studies support the relevance of predictions for neural processing at different hierarchical levels. Nevertheless, especially in humans the evidence grounding PC principles onto fundamental neurocomputational units (i.e. cortical layers, subdivisions of subcortical structures) is limited and this hampers our understanding of how PC supports the processing of sounds in context in the human brain. Ultra-high field fMRI at high spatial resolution offers a unique opportunity to investigate how computations are embedded in the mesoscopic (cortical) architecture of the human brain (in vivo and non-invasively). Laminar fMRI has already been used to investigate predictive processes in the human visual cortex. In this talk I will describe recent results from studies investigating how predictions and prediction errors are processed in auditory cortical layers. We combine ultra-high field fMRI with biophysical and computational approaches to gain further insights into the computations underlying these responses.

Category Learning and Dimension-Selective Attention in Auditory Cortex
Lori Holt, *Carnegie Mellon University*

Human listeners possess rich category representations for speech sounds and words. Yet speech input exhibits complexity across multiple acoustic dimensions, and short-term speech input regularities may not match long-term norms (as in foreign accents). Theoretical accounts of speech perception often have appealed to selective attention as a means by which to balance these demands. However, we do not yet understand how – and whether - listeners learn to selectively attend to informative acoustic dimensions during category learning, how selective attention impacts cortical representations of relevant dimensions, and whether selective attention involves suppression of irrelevant dimensions as well as enhancement of relevant dimensions.

We are examining these questions using novel non-speech auditory categories. Participants complete five days of stimulus-response-feedback training during which they learn four nonspeech categories to criterion. The categories are structured to require listeners to learn acoustic patterns positioned in either a high- or low-frequency band, with simultaneous irrelevant acoustic patterns in the opposite band. Thus, category learning requires reliance on – and perhaps selective attention to – the category-diagnostic acoustic patterns. Control trials involve categorization across an orthogonal dimension, stimulus amplitude ('big' or 'small' Category A). In a single post-training MRI session, listeners categorize sounds in a 2AFC task with categories differentiated by information in either high or low spectral bands, or on relative amplitude. Combined with tonotopic mapping and "attention-o-tonotopic" mapping driven by overt endogenous attention to high and low frequency bands, we examine how dimension-selective attention driven by implicit demands of categorization impact cortical activation.

This work illuminates the cortical mechanisms supporting dimension-based auditory selective attention, providing a bridge to compare explicitly directed attention (i.e., “listen high”) and selective attention that emerges with learning. Comparison with our control (amplitude) condition allows for assessment of a putative role for suppression. Finally, the study links human studies with non-human animal studies of frequency-selective auditory attention with non-speech stimuli.

spARO Mentoring Session: Careers in Industry

12:45 p.m. - 1:45 p.m.
Canaveral 1-2

While there are similarities between working in academia and industry, pursuing a successful career in industry often requires a change in approach for former academics. Leaders from companies investigating phenomena related to ARO research will lead a discussion about how to successfully pivot from academic research into developing a career in industry.

Michelle Valero*, Akouos Inc.*
Roger Calixto*, Oticon Medical*

gEAR Workshop - Explore and Customize I: Creating Visualizations

1:00 p.m. - 1:45 p.m.
Biscayne 1

Visualization of datasets within the gEAR Portal is extremely customizable. In this session, learn how to create multiple displays from your data including line/bar charts, heatmaps, violin plots, etc. This workshop includes a hands-on component, bring your laptop along.

Ronna Hertzano*, University of Maryland School of Medicine*
Joshua Orvis*, Institute for Genome Sciences*

Podium #10 - Hair Cells: Synapses and Innervation

2:00 p.m. - 4:00 p.m.
Ocean's Ballroom 5-12

Moderators: Wang Zheng and Nicolas Grillet

The Neuronal t-Snare SNAP-25 is Required for Exocytosis at Auditory Hair Cell Ribbon Synapses

Charlotte Calvet[1], Thibault Peineau[2], Najate Benamer[1], Maxence Cornille[1], Andrea Lelli[1], Baptiste Plion[1], Ghizlène Lahlou[1], Julia Fanchette[1], Sylvie Nouaille[1], Jacques Boutet de Monvel[1], Amrit Estivalet[1], Vincent Michel[1], Martin Sachse[3], Philippe Jean[1], Nicolas Michalski[1], Paul Avan[1], Christine Petit[1], Didier Dulon[2], Saaid Safieddine*[4]
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Background: The presence of the neuronal SNARE (soluble N-ethylmaleimide sensitive factor attachment protein receptor) complex in auditory hair cell is well documented, including syntaxin 1, SNAP-25 and synaptobrevin1/2. Although, these results led to the notion that vesicle fusion at CNS and IHC ribbon synapses involves similar SNARE proteins, the study of null mutant mouse models failed to reveal a functional role for the neuronal SNARE proteins in IHC synaptic transmission. This finding led to the proposal that the IHC synapses might operate without neuronal SNARE proteins. However, existing null mutant mouse models for most of the neuronal SNARE proteins die at birth or shortly after (Verhage and Sørensen, 2020). Therefore, the IHC function in these mutants could only be analyzed indirectly using organotypic culture models, wherein the findings may not faithfully reproduce the in-vivo functioning of IHC synapses in a healthy and mature hearing organ. To untangle this issue, we focused on SNAP-25, a key component of the canonical synaptic SNARE complex. We generated a hair cell-specific Snap-25

conditional knockout (Snap-25 cKO) mouse model to study the effect of acute inactivation of Snap-25 in the IHCs, which turned out to cause deafness both when occurring at neonatal and at mature stages. We characterized these mice by in-vivo auditory tests and ex-vivo physiological recordings, combined with imaging experiments. We further performed in-vivo genetic manipulation and rescue experiments by using viral mediated transfer of the wild type or mutated Snap-25 cDNAs in the IHCs of the wild type and mutant mice.
Methods: We generated a hair cell-specific Snap-25 conditional knockout (Snap-25 cKO) mouse model to study the effect of acute inactivation of Snap-25 in the IHCs.
Results: Our results observed in the IHC subjected to SNAP-25 depletion before and after the hearing onset recapitulate several previous findings of in vitro studies using the total Snap-25 KO. We found that, the IHCs of Snap-25 cKO mice displayed a severe exocytotic defect which was associated with degeneration of ribbon synapses and hair cell loss. We verified that the defects observed were, indeed, due to IHC Snap-25 inactivation, by demonstrating that the AAV-mediated transfer of Snap-25 cDNA to the inner ear of Snap-25 cKO mice successfully rescued the IHC exocytosis, prevented the degeneration of both ribbon synapses and hair cells, and restored hearing function in the otherwise profoundly deaf mutant mice.
Conclusions: Our present results lead to the conclusion that SNAP-25 is essential for hearing function and provide strong evidence suggesting that protein is required to ensure synaptic exocytosis, maintenance of ribbon synapses and IHC survival.

Piccolino Regulates Ribbon Architecture at Cochlear Inner Hair Cell Ribbon Synapses

Rohan Kapoor*[1], Susann Michanski[2], Anna Steyer[3], Frauke Ackermann[4], Mehmet Gültas[5], Craig Garner[4], F. Kent Hamra[6], Nicola Strenzke[2], Tobias Moser[2], Carolin Wichmann[7]
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Background: Cochlear inner hair cells (IHCs) form specialized ribbon synapses with type I spiral ganglion neurons (SGNs), whereby tireless neurotransmission occurs over long periods, with extreme precision and speed. This functional specialization is essential for hearing and is attributed to the distinct molecular profile of these synapses, which employ unique players but lack several components present in conventional neuronal synapses. Ribbon synapses of ear and eye often use different isoforms of neuronal presynapse proteins. Among these is the multi-domain active zone scaffold protein piccolo (aczonin) which is only represented by its short splice variant piccolino at ribbon synapses. While the function of piccolo at neuronal synapses has been intensively investigated, the role of piccolino in IHC ribbon synapses is not yet understood.
Methods: We characterized the structure and function of IHC ribbon synapses of piccolo gene trap mutant rats to investigate the impact of loss of piccolino. We studied the morphology of the synapses using confocal, stimulated emission depletion (STED) and electron microscopy. Using electron tomography, we assessed the morphology and the tethering of SVs in highest resolution. We further performed functional analysis using systems physiology and whole-cell patch clamp of inner hair cells.
Results: Genetic disruption of piccolino results in a mild hearing deficit with elevated ABR thresholds for middle and high frequency tone bursts and a significant reduction of the amplitude of the first ABR wave reflecting an impairment in synchronous firing of spiral ganglion neurons.
Surprisingly, cell physiology revealed normal Ca2+ currents and exocytosis. Interestingly, STED microscopy showed an altered Ca2+ channel distribution. Moreover, we observed alterations in synapse morphology: smaller synaptic ribbons and enlarged postsynaptic densities. Electron microscopy uncovered two morphologically distinct ribbon categories at mutant synapses: category 1 resembling control ribbons, and category 2 ribbons which are strikingly smaller and lack synaptic vesicles at the membrane-distal apex of the ribbon.
Conclusions: Our data indicates a role of piccolino in shaping IHC ribbon morphology, and potentially organization of Ca2+ channel clusters, likely via intermediate interaction partners. The mild hearing phenotype, despite unchanged IHC Ca2+ currents and exocytosis calls for further analysis in future studies.

At present we can only speculate about the reasons for the occurrence of two morphologically distinct categories of ribbons upon piccolino disruption. One possibility is a requirement of piccolino for achieving normal ribbon size at a subset of synapses.

Probing the Function of Membrane-Proximal C2 Domains of Otoferlin, a Synaptic Protein of Sensory Hair Cells

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Background: Afferent synapses of cochlear inner hair cells (IHCs) employ an unconventional molecular machinery. Otoferlin, encoded by the deafness gene OTOF, is an enigmatic key player of this machinery. Previous work has indicated roles in vesicle tethering to the active zone membrane, Ca2+ dependent synaptic vesicle (SV) fusion, and exocytosis-endocytosis coupling.
Methods: Here we employed site-directed mutagenesis in mice to investigate the function of the membrane-proximal C2E and C2F domains of otoferlin. We performed system and cell physiology by conducting auditory brainstem responses (ABRs) and whole cell patch-clamp to evaluate the hearing impairment and measure the Ca2+ influx and exocytosis in inner hair cells of mutant mice. Protein expression level, subcellular distribution and number of synapses were estimated by confocal imaging of immunostained sections. RNAscope and qPCR were performed to check RNA level. Finally, we used purified recombinant otoferlin to do in vitro studies including nano-DSF to biochemically characterize otoferlin mutants.
Results: Substituting three aspartates of the C2E top loops thought to contribute to Ca2+ coordination (OtofD1543A, D1545A, D1550A) abolished Ca2+ influx-triggered IHC exocytosis and hearing despite substantial expression (>40%) and near-normal subcellular distribution of otoferlin. In vitro analysis of protein stability suggested a lower Ca2+ affinity of the mutant protein and both findings support the Ca2+ sensor of SV fusion hypothesis for otoferlin. Likewise, IHC exocytosis and hearing were abolished in a mouse model of a human missense mutation affecting the C2E domain (OTOFI1573T) and a mutant substituting two aspartates of the C2F top loops (OtofD1821A, D1822A). In both cases, we found greatly reduced otoferlin levels in the basal part of IHCs (~20%) to confound the analysis of the protein function.
Conclusions: Our results demonstrate a critical role of the membrane-proximal C2E and C2F domains of otoferlin and suggest a structural vulnerability of otoferlin upon mutagenesis. Moreover, our data indicate that Ca2+ binding to C2E is important for IHC exocytosis.

Does Acetylcholine (ACh) as an Efferent Neurotransmitter Directly Inhibit Outer Hair Cell Electromotility?

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Background: Acetylcholine (ACh) is a neurotransmitter of the medial olivocochlear (MOC) efferent system, which projects to outer hair cells (OHCs) and supporting cells to control OHC electromotility. The eventual effect of the MOC efferent system is to reduce active cochlear mechanics to provide a negative feedback to protect hearing from noise or other high sound stimulations. It has been long assumed that ACh will inhibit OHC electromotility to reduce active cochlear amplification. However, previous study reported that application of ACh caused isolated OHC electromotility increased rather than reduced (Dallos et al., 1997, PMID: 9045745). This seems contradictory with the efferent effect. In our previous study, we reported that the MOC efferent system has a projection to OHC supporting cells (Deiters cells and outer pillar cells) to mediate gap junctions between them to control OHC electromotility (Zhao et al., 2022, PMID: 34907797). In this study, we further examined that direct effect of ACh on OHC electromotility.
Methods: Adult mice and guinea pigs were used. OHCs were freshly isolated and OHC electromotility was assessed by nonlinear capacitance (NLC) measured by patch clamp recording.
Results: Application of 0.1 mM ACh evoked a typical inward current in OHCs. However, the NLC was not reduced. In some cases, the peak of NLC appeared slightly increased but it had no statistical significance (P>0.05). The apparent changes in NLC after application of ACh was NLC shifted to the left side, i.e., hyperpolarized direction. The peak voltage (Vpk) of NLC was significantly shifted -7.85±0.89 mV (n=16).

This shift was reversible and repeatable. We further found that the ACh-induced left-shift in NLC was large when the OHC was constrained by Deiters cells as it is in vivo. Application of ACh also evoked inward current in the cochlear supporting cells and reduced gap junctions between the cochlear supporting cells. The reduction of gap junctions between the cochlear supporting cells consequently shifted OHC electromotility to left-side as well, consistent with the direct left-shifting effect of ACh on OHC electromotility.
Conclusions: These data demonstrated that ACh does not directly inhibit OHC electromotility. However, ACh shifts OHC electromotility to hyperpolarizing direction (left-shift) via both directly and indirectly pathways to inhibit active cochlear mechanics.
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Cholesterol's Role in Synaptic Heterogeneity and Active Zone Confinement in Inner Hair Cells

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Background: Cochlear inner hair cells (IHCs) are characterized by active zones (AZs) with heterogeneous and position-dependent structural and functional characteristics. It has been revealed that this organization of IHC AZs follows a modiolar-pillar gradient. Modiolar synapses exhibit a microdomain-like coupling of Ca2+ release, whereas nanodomain-coupling and activation of Ca2+ channels at more hyperpolarized potentials seem prominent towards the pillar side (Ohn et al. 2016; Özçete and Moser 2021). How is this synaptic heterogeneity achieved and maintained in IHCs is yet largely unknown. We suggest that cholesterol is of great importance for the organization of IHCs AZs, and particularly in maintaining gradients of Ca2+ channel clusters throughout the IHC. Cholesterol is considered a key player for synaptic transmission in the CNS (Korinek et al., 2020; Mauch et al., 2001) and the retina (Mercer et al., 2011, 2012). Additionally, putative cholesterol-enriched microdomains appear to be present along the basal pole of chicken hair cells (Thomas et al., 2014). In the present study, we addressed the consequences of cholesterol manipulation on synaptic properties of IHCs, particularly on Ca2+ channel clusters.
Methods: We performed immunohistochemistry and IHC physiology on freshly dissected apical coils from organs of Corti of p14-p30 c57Bl6 wildtype mice. Manipulation of membrane cholesterol was achieved using either 1- or 5-mM methyl-β-cyclodextrin (MβCD) for cholesterol depletion or 10µg/ml cholesterol:MβCD complex (CH:MβCD) for cholesterol repletion. To study changes in the synaptic arrangement of AZs upon cholesterol manipulation, we quantified the size of Ca2+ channel clusters and ribbons using confocal and super-resolution STED microscopy. To study the effects of cholesterol manipulation on synaptic function, we performed simultaneous whole-cell patch-clamp recordings and confocal Ca2+ imaging of individual IHC AZs, as previously described (Frank et al., 2009; Ohn et al., 2016). For both microscopy and electrophysiology experiments, we determined the topographical location along the modiolar-pillar axis of individual synapses within one IHC.
Results: Our results revealed that cholesterol depletion impeded the confinement of Ca2+ hotspots and decreased the Ca2+ influx at individual AZs, particularly in modiolar synapses. In addition, cholesterol depletion shifted the voltage-dependence of Ca2+ influx of pillar synapses to more hyperpolarized potentials. Yet, maximal whole-cell Ca2+ influx and whole-cell voltage dependence of Ca2+ influx remained unchanged after cholesterol depletion.
Conclusions: Our study shows that cholesterol manipulation alters the properties of Ca2+ channels at individual IHC ribbon synapses, with distinct effects on modiolar or pillar synapses. This suggests an importance of cholesterol homeostasis in sound encoding and indicates the need to address potential contributions of synaptic alterations to hearing impairments in lipid metabolism disorders (Malgrange et al., 2015).

Nrxn3 is Required for Hair Cell Synapse Formation in the Zebrafish Lateral Line

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Background: Hair cells rely on specialized ribbon synapses to relay sensory information to the central nervous system. Molecular mechanisms of conventional synapse formation have been extensively studied in

the context of the brain, but far less is known about molecules that govern the formation of ribbon synapses between hair cells and afferent neurons. Recent RNAseq data has shown that the presynaptic cell adhesion molecule Neurexin 3 (Nrxn3) is enriched in hair cells. Whether Nrxn3 is involved in synapse formation in hair cells is unclear.
Methods: We leverage the power of zebrafish genetics to test whether Nrxn3 is important for hair cell synapse formation. For our studies we have examined Nrxn3 alleles that truncate the long, alpha form of Nrxn3. To quantify synapse numbers, we have used immunohistochemistry to label pre- and post-synapses. In addition, we have used the acoustic startle response to assess hair-cell mediated behavior in zebrafish. Lastly, we are using functional calcium imaging to quantify pre- and post-synaptic calcium responses to water jet stimuli at the ribbon synapses in Nrxn3 mutants.
Results: We find that nrxn3 mutants have a dramatic decrease in complete ribbon synapses and a concomitant increase in unpaired pre- and post-synapses. Analysis across developmental time revealed that ribbon synapses are disrupted even in young hair cells, suggesting that Nrxn3 is essential for initial synapse formation – rather than synapse maintenance – in lateral-line hair cells. In ongoing work, we are using functional imaging and behavioral assays to probe how the loss of synapses in nrxn3 mutants impacts synapse function and startle behaviors.
Conclusions: Our work has identified Nrxn3 as a novel player required for synapse formation in hair cells. In the future, we plan to perform a CRISPR-Cas-9 candidate screen to identify the binding partner of Nrxn3. Overall, understanding the molecular underpinnings of ribbon synapse formation is essential for generating novel therapies to treat hidden hearing loss caused by destruction of hair cell synapses.

Characterizing Hair Cell and Afferent Neuron Properties in Human Vestibular Organs

Olivia Kalmanson*[1], Frances Meredith[1], Mohammad Al-Amin[1], Anna Dondzillo[1], Tiffany Vu[1], Samuel Gubbels[1], Katie Rennie[2]
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Background: The vast majority of what is known about human vestibular function has been inferred from rodent models due to the inaccessibility of human inner ear organs. There are a few reports of electrophysiological recordings from cells isolated from human inner ear (Oghalai et al. 1998, Lim et al. 2014, Quinn et al. 2021), but detailed investigations of freshly harvested adult vestibular cells are still lacking. Here, we obtained vestibular neuroepithelia from adult translabyrinthine surgical patients and performed electrophysiological and immunohistochemical studies.
Methods: Colorado Multiple Institution Review Board approval was obtained (COMIRB# 19-1340). The surgical team obtained ampullae and utricles from consenting patients undergoing translabyrinthine surgical approaches. Neuroepithelia were transferred in-ice cold sterile saline to the laboratory within one hour of harvest. For electrophysiological recordings, the tissue was transferred to L-15 solution following incubation in L-15/bovine serum albumin and cells were mechanically dissociated as previously described (Rennie and Streeter 2006). Whole cell patch clamp recordings were performed on isolated hair cells and afferent terminals to characterize voltage-dependent currents. Pharmacological agents were applied via extracellular perfusion. For immunohistochemistry, end organs were fixed and stained with a combination of antibodies targeting Myosin7a, CtBP2, Tubulin3, and ChAT. Imaging was performed with a Zeiss LSM 780 confocal microscope.
Results: Five patients (ages 29-77 years, 4M, 1F) with vestibular schwannomas and non-serviceable ipsilateral hearing loss prior to surgery were studied. None reported vertigo. Recordings were obtained from type I and type II hair cells and afferent terminals up to 5 hours following dissociation. Hair cells had a mean capacitance of 8.9±5.4pF (n = 7, SD). Type I hair cells exhibited low voltage-activated currents and large outward currents in response to standard voltage protocols. Mean resting potential was -60.4±7.2 mV (n=14) and peak outward was 3.37±1.30 nA at +20 mV (n=15). Putative type II HCs exhibited higher input resistance and outward current above -50mV. A hyperpolarization-activated current (Ih) was observed in 4 hair cells. Afferents exhibited rapid transient inward Na+ current, outward current (IK) and Ih. Peak IK was 3. 77±2.51 nA (n=4) and Ih was 331±191 pA (n=3). Single action potentials occurred with depolarizing steps in current clamp. Perfusion with the K+ channel blocker 4-aminopyridine (1 mM) blocked low voltage-activated currents in type I cells and inactivating IK in calyx afferents.
Immunohistochemical labeling of cristae and utricles allowed identification of hair cells, afferent terminals, efferent synapses, and ribbon synapses.

Conclusions: We performed electrophysiological recordings and immunostaining on vestibular cells extracted during human surgical inner ear procedures. Whole cell recordings from hair cells and afferent terminals revealed ionic currents that qualitatively resemble currents in cells from rodent vestibular epithelia. Rapid access to adult human vestibular epithelia allows translational studies crucial for better understanding human peripheral vestibular function.

Homeostatic Roles of P2X4 Ionotropic Receptors in Outer and Inner Hair Cells of the Cochlea

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Background: The majority of hearing loss is sensorineural hearing loss (SNHL) due to the damage to auditory sensory hair cells and/or neurons. One of the key regulators of cochlear pathophysiology is purinergic signalling. Purinergic signalling includes P1 adenosine receptors (A1, A2A or A2B, A3) and P2 receptors (P2X1-7, P2Y1,2,4,6, 11-14) activated by extracellular nucleotides. Earlier studies have shown a broad expression of purinergic receptors in the cochlea, including sensory hair cells and supporting cells. This study aims to extend our current knowledge of purinergic signalling in the cochlea by comprehensively characterizing the expression of the P2X4 subtype in the mammalian cochlea.

Methods: Cochleas from Wistar rats (embryonic day 21 – 6-week-old, both genders) and sheep (4-6 year-old, New Zealand Romney, female) were fixed in 4% paraformaldehyde and analyzed by qPCR and immunohistochemistry. Human cochleas were obtained from tissue donors. Expression of P2X4 was examined using a commercially available anti-P2X4 antibody (Alomone, Israel) and imaged using confocal microscopy and transmission electron microscopy. Co-localization was analyzed against; cell type-specific markers for hair cells (Myosin VIIa) and supporting cells (Sox 2), and subcellular organelle markers; early endosome (EEA-1), lysosomes (LAMP-1), mitochondria (Tom20) and Golgi apparatus (GM130). To test the effects of P2X agonist (α,β,metATP, 1-500μM) and P2X4 specific inhibitor (5-BDBD, 40-160μM), cochleas from postnatal day 4-8-old rat pups were cultured in vitro in DMEM supplemented with FBS/N2 for 4-24 hours.

Results: In the rat, comparatively high P2X4 expression was found in the inner hair cell (IHC) and outer hair cells (OHC), with the level of expression increasing with cochlear maturation. No expression of P2X4 was observed in other cells of the organ of Corti after the hearing onset. P2X4 expression in both OHC and IHC was predominately cytoplasmic in nature. Substantial co-occurrences were observed between EEA-1 and P2X4 in IHC and OHC (Mander's co-localization coefficients (MC) of 0.26 and 0.42, respectively), showing localization in the trans-Golgi network in IHC. In contrast, co-occurrence of P2X4 and LAMP1 were high in OHC (MC = 0.32) but not for IHC (MC = 0.11). The expression of P2X4 in hair cells was also conserved in the cochlea of humans and sheep. Inhibition of P2X4 resulted in a significant loss of IHC ($p=<0.01$) with less impact on OHC in cochlear organotypic culture.

Conclusions: Our study provides new evidence for P2X4 as a purinergic receptor subtype selectively expressed in IHC and OHC as a feature conserved from rodents to humans. Our observation of P2X4 in trans-Golgi vesicles and lysosomes is consistent with the previous report on P2X4 in other tissues, where P2X4. Our study suggests homeostatic roles of P2X4 in both IHC and OHC, with additional implications in OHC as lysosomal P2X receptors.

Podium #11 - Inner Ear Sensory Cell Regeneration

2:00 p.m. - 4:00 p.m.
Oceans Ballroom 1-4

Moderators: Tejbeer Kaur and Jörg Waldhaus

Identifying the Gene Regulatory Network Required for Hearing Regeneration in Zebrafish

Erin Jimenez[1], Clair Slevin[1], Wei Song[2], Zelin Chen[3], Stephen Frederickson[1], Derek Gildea[1], Weiwei Wu[4], Abdel Elkahloun[1], Ivan Ovcharenko[2], Shawn Burgess*[1]
[1]National Human Genome Research Institute, [2]National Center for Biotechnology Information, [3]South China Sea Institute of Oceanology, [4]National Institute of Allergy and Infectious Diseases

Background: The capacity to regenerate tissues after injury unevenly manifests across the vertebrate lineage. Damage to the mammalian inner ear sensory epithelium is irreversible and results in permanent hearing loss or vestibular defects. Interestingly, this is a feature that sets mammals apart from most other vertebrates who can continually produce new hair cells throughout their lifetimes and/or can regenerate them in response to trauma. Because of the genetic tractability and deep genomic data available to zebrafish researchers, adult zebrafish provide an excellent opportunity to study inner ear hearing and balance regeneration after hair cell ablation. Recent advances in single cell genomics allow us to now simultaneously collect both transcriptional changes and alterations in chromatin accessibility (i.e. enhancer activation) at single cell resolution.
Methods: Using adult zebrafish inner ears as a model for sensorineural regeneration, we ablated the mechanosensory receptors and characterized the single-cell epigenome and transcriptome at consecutive time-points during hair cell regeneration. We utilized deep learning on the regeneration-induced open chromatin sequences and we identified cell-specific transcription factor (TF) motif patterns.
Results: Enhancer activity correlated with gene expression and identified potential gene regulatory networks. A pattern of overlapping Sox- and Six-family TF gene expression and binding motifs was detected, suggesting a combinatorial program of TFs driving regeneration and cell identity. Pseudo-time analysis of single-cell transcriptomic data suggested support cells within the sensory epithelium changed cell identity to a "progenitor" cell population that could differentiate into hair cells. We identified a 2.6 kb DNA enhancer upstream of the sox2 promoter that when deleted, showed a dominant phenotype that resulted in a hair cell regeneration-specific deficit in both the lateral line and adult inner ear.
Conclusions: Hearing regeneration is coordinated through a combinatorial language driven by sox- and six-family transcription factors. Sox factors activate the support cells, while both sox and six factors drive hair cell differentiation. Sox2 regulation must be tightly regulated and initiated early for hair cell regeneration to be successful.

A Cascade of Proteolytic Activities is Essential for Proliferative Hair Cell Regeneration in the Chicken Hearing Organ

Nesrine Benkafadar*[1], Amanda Janesick[1], Angela H Ling[2], Taha A. Jan[2], Stefan Heller[1]
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Background: Hearing loss is a prevalent morbidity. A major cause is hair cell (HC) loss leading to permanent and often disabling hearing loss. Lost mammalian HCs are not regenerated, unlike in non-mammals such as chickens, because supporting cells (SCs) act as facultative stem cells that can proliferate and regenerate HCs. However, the mechanism that initiates and executes avian HC regeneration is unknown, despite more than 30 years of focused research. After inducing ototoxic damage, we sought to investigate signaling effector genes that initiate and execute mitotic HC regeneration in the chicken basilar papilla.
Methods: A single injection of sisomicin into the posterior semicircular canal of 7-days-old chickens triggers complete HC loss in the BP. We performed single-cell RNA sequencing of SCs at specific time points post sisomicin. The time points were based on the time course of HC death and the SCs' regenerative responses. We detangled the temporal sequence of gene expression changes, which allowed us to focus on the earliest detectable changes in responding SCs. The major constituents of the identified candidate signaling pathway that potentially initiates HC regeneration were functionally assessed in vivo using pharmacological approaches combined with qRT PCR, in situ mRNA detection, and detection of pathway protein phosphorylation by Western blotting.
Results: Our results show that SCs display distinct gene expression profile changes as early as 12h post sisomicin infusion when the first sign of DNA fragmentation in HC nuclei is detected. In situ validation confirmed the upregulation of distinct genes in early responding SCs. We identified a signaling pathway essential for SC S-phase entry and HC regeneration, comprising a protease-activated receptor followed by a matrix metalloprotease (MMP)-mediated cascade involving EGF receptor activity, downstream MAP kinase signaling, and activation of a set of transcription factors. Ongoing work uses established inhibitors and activators in vivo to mechanistically validate key players of mitotic HC regeneration in the chicken BP. We hypothesize that sensing extracellular proteolytic activity is a key trigger of SC proliferation in the regenerating chicken basilar papilla.
Conclusions: Our results reveal an essential mechanism for mitotic HC regeneration in the avian hearing organ. Our findings inform novel therapeutic strategies aimed at reversing hearing loss in mammals.

Morphological and Regulatory Factors in Vestibular Hair Cells Differentiation and Regeneration

Shahar Kasirer*[1], Olga Loza[1], May Pery[1], David Sprinzak[1]
[1]*Tel Aviv University*

Background: The vestibular system is in charge of keeping our balance by sensing acceleration and gravitation. Each of its sensory organs consists of an alternating pattern of sensory hair cells (HCs) and non-sensory supporting cells (SCs). Many non-mammalian species have the capability to regenerate lost HCs by the proliferation and differentiation of nearby SCs. In contrast, mammals lose the capability to regenerate HCs during early development (first postnatal week in mice). A possible reason for this difference might stem from the different mechanical properties of the mammalian versus the non-mammalian systems. Recent studies have shown that inner ear development is not only regulated by biochemical signaling, but also by mechanical forces. However, the exact manner by which the coordination between these two mechanisms regulates the development and regeneration of the vestibular system is yet unknown.
Methods: We developed a live imaging assay for mouse utricle explants. To track cell morphology and differentiation, we use mice containing ZO1-EGFP (marking apical boundaries) and Atoh1-mCherry (marking differentiation of HCs). We track the dynamics of the developing utricle explants at different developmental stages and under different perturbations for 24-48 hours. We perform quantitative analysis of the morphological and regulatory events (delaminations, divisions, and differentiation) and infer the local mechanical forces in the tissue. We also track the local regeneration dynamics (proliferation and differentiation) following laser ablation of single HCs at different developmental stages. Using insights from our experimental results, we formulate a mathematical model for utricle development and regeneration, that includes biochemical signaling and mechanical forces. Our model results are then compared with experiments.
Results: Our live imaging results of E17.5 and P0 utricle explant, reveal that utricle development is initially (at E17.5) a highly dynamic process, where SCs exhibit frequent cell divisions, delaminations, and differentiation events. At P0, the number of these morphological and regulatory events is significantly reduced. Quantitative analysis of HC density and number of neighbors reveal local correlations with these events. Upon ablating a HC, we observe a renewal of the dynamics in surrounding SCs, including, proliferation and differentiations. Comparison between explants from E17.5 and P0 reveal surprising differences in the identity of the differentiating cells. Models with classical lateral inhibition can explain the E17.5 results, but cannot explain the P0 results, suggesting a strong contribution of cell mechanics to the local regeneration process.
Conclusions: The embryonic developmental of mice utricle is a dynamic process that is regulated by both Notch mediated lateral inhibition and mechanical forces. We show that local proliferation and differentiation is likely affected by local tissue mechanics, which vary with developmental time. Overall, our results can contribute to better understanding of the factors affecting regeneration of HC.

Reprogramming of Non-Sensory Cells in the Mature Mouse Cochlea via ATOH1, GFI1, and POU4F3 Expression

Melissa McGovern*[1], Yichi Niu[1], Ishwar Hosamani[1], Ken Nguyen[1], Chenhang Zong[1], Andrew Groves[1]
[1]*Baylor College of Medicine*

Background: In the mature mammalian cochlea, hair cells (HCs) detect sound from the environment. These HCs do not regenerate, and any HC loss is permanent thus leading to hearing loss. In contrast, non-mammalian supporting cells (SCs), which surround HCs, regenerate lost HCs throughout the life of the animal. Additionally, some immature SCs in the neonatal mammalian cochlea can spontaneously regenerate into HCs after damage. Furthermore, neonatal SCs and other non-sensory cells adjacent to inner HCs respond to the ectopic expression of the HC transcription factor Atoh1 by differentiating into HCs. In the mature cochlea, however, Atoh1 alone does not induce the conversion of non-sensory cells into HCs.
Methods: To determine whether mature non-sensory can convert into HCs, we have targeted the ROSA locus with a conditional allele that drives expression of the HC transcription factors Gfi1, Atoh1, and Pou4f3 (Rosa-GAP). When combined with Fbxo2CreER, this line expresses the transcription factors in non-sensory cells. Expression was induced at 3 weeks of age and cochleae were analyzed at 5 or 8 weeks of age.
Results: Expression of Gfi1, Atoh1, and Pou4f3 reprogrammed non-sensory cells within the inner and outer sulci into HC-like cells in the apex of the cochlea at 5 weeks of age. After an additional 3 weeks of reprogramming, non-sensory cells throughout the length of the cochlea were converted into HC-like cells.

These cells express the hair cell proteins Myosin VIIa and Parvalbumin, however do not upregulate mature hair cell proteins Oncomodulin or Prestin. Interestingly, cells adjacent to converted HCs began to express the SC marker Sox2. Single cell RNA-sequencing analysis of these cells revealed that converted hair cells upregulate many HC genes and signal to their neighbors through the Notch signaling pathway, thus promoting supporting cell like fate in these cells.
Conclusions: Currently, the best therapeutic for hearing loss are hearing aids and cochlear implants. While these enable users to re-gain some hearing, they provide incomplete recovery. Understanding the response of mature cochlear cells to reprograming provides insight into the molecular pathways regulating cell identity. As clinical trials for gene therapies increase, identifying genes that can produce new HCs in the mature cochlea will provide therapeutic approaches for hearing restoration in humans.

The Function of TRIM71 in Cochlear Supporting Cell Reprogramming and Hair Cell Regeneration

Charles Morgan*[1], Xiaojun Li[1], Angelika Doetzlhofer[2]
[1]Johns Hopkins University School of Medicine, [2]Johns Hopkins University
Background: Loss of cochlear hair cells (HCs) is a leading cause of hearing loss in humans. Research in mice shows that neighboring supporting cells (SCs) have some capacity to regenerate HCs at early postnatal stages. However, their regenerative potential sharply declines as SCs undergo maturation and is nearly lost by postnatal day 5 (P5). We recently reported that reactivation of the RNA-binding protein LIN28B restores the HC-regenerative potential of P5 cochlear SCs. Here, we identify the ubiquitin ligase and RNA-binding protein TRIM71 as an equally potent enhancer of SC plasticity and characterize TRIM71 target genes involved in SC de-differentiation, cell cycle re-entry, and HC formation.
Methods: To investigate the effect of TRIM71 activation, we employed a lentiviral overexpression strategy in organoid cultures generated from the cochlear sensory epithelial cells of P5 mice. Cells were infected with lentiviral particles expressing mCherry and wild-type human TRIM71 or mutant forms of human TRIM71 that lacked the RING, coiled-coil, or NHL domains, which coordinate TRIM71's ubiquitin ligase activity, protein-protein interactions, and RNA-binding activity, respectively. EdU labeling and use of an Atoh1-nGFP reporter enabled quantification of SC proliferation and HC formation. Bulk RNA-sequencing analysis was used to characterize transcriptomic changes between P5 control and TRIM71-overexpressing organoids, and the expression patterns of select target genes involved in SC de-differentiation were characterized using immuno-histochemistry.
Results: Overexpression of TRIM71 in P5 cochlear epithelial cells significantly increased organoid formation efficiency, size, and overall cell proliferation, and this effect was attenuated in the NHL deletion condition, implicating TRIM71's RNA-binding capability in organoid formation and growth. Finally, a significantly higher percentage of organoids in the TRIM71-overexpressing condition produced nascent HCs when compared to the control condition, an effect which is also dependent on RNA-binding activity. Transcriptomic profiling of P5 control and TRIM71-overexpressing organoids after 10 days in culture identified 2883 differentially expressed genes (q-value< 0.01). The list of upregulated genes includes genes that function in pro-sensory cell specification and maintenance as well as genes induced during HC formation, and the list of downregulated genes included genes that function in glial cell differentiation (e.g. Zbtb20, Nfib). In vivo characterization reveals that NFIB and ZBTB20 are highly expressed in SCs and high NFIB/ZBTB20 expression in organoids is negatively correlated with a HC fate. Pilot loss-of-function studies implicate the NFI factors in maintenance of SC identity at postnatal stages.
Conclusions: Here we identify the post-transcriptional regulator TRIM71 as novel reprogramming factor for SCs. Utilizing an organoid platform, we show that reactivation of TRIM71 primes cochlear SCs for cell cycle re-entry and HC formation by reactivating a progenitor-like state in cochlear SCs. Future investigation will characterize the role of NFI factors and ZBTB20 in SC differentiation and HC regeneration.

Sox2 is Required in Supporting Cells for Normal Levels of Vestibular Hair Cell Regeneration in Adult Mice

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[1]The University of Washington, [2]University of Montpellier, [3]Southern Illinois University School of Medicine, [4]University of Washington, Virginia Merrill Bloedel Hearing Resource Center
Background: Mammals have two types of vestibular hair cells - type I and type II - with distinct morphological, physiological, and molecular features, and different patterns of innervation. When vestibular

hair cells are destroyed in adult mice, a subpopulation of type II hair cells is regenerated. Regenerated hair cells are derived from supporting cells, which are non-sensory cells that reside alongside hair cells. We are studying molecular mechanisms regulating vestibular hair cell regeneration in mature mammals. Sox2 is a transcription factor that is necessary for development of sensory hair cells and supporting cells. Sox2 expression is maintained in vestibular supporting cells of adult mammals, but its requirement for hair cell regeneration has not been tested. Given its critical role in the developing inner ear, we hypothesized that Sox2 is necessary in vestibular supporting cells for their transdifferentiation into type II hair cells after damage.
Methods: Using adult Pou4f3DTR/+:Sox9-CreERT2/+:Rosa26tdTomato/+:Sox2loxP/loxP mice, we performed conditional knockout (CKO) of Sox2 from supporting cells prior to diphtheria toxin-mediated hair cell destruction and used fate-mapping, immunostaining, and transmission electron microscopy to assess both hair cells and supporting cells over a 12-week period. We also examined whether vestibular hair cell regeneration was impacted when one copy of Sox2 was deleted from supporting cells prior to damage.
Results: In utricles with wildtype (WT) Sox2 expression in supporting cells, mice regenerated nearly 200 hair cells by 3 weeks post-damage, which doubled by 12 weeks. In contrast, mice with Sox2 CKO from supporting cells had approximately 20 fate-mapped hair cells at 3 weeks post-damage, and this number did not change significantly by 12 weeks, indicating regeneration was dramatically curtailed. We made similar observations for saccules and ampullae. In utricles, we found no evidence that supporting cells with Sox2 deletion had altered cellular density, morphology, or ultrastructure. However, while the nuclei of some supporting cells lacking Sox2 were still able to migrate apically, they did not turn on hair cell markers (myosin VIIa or Pou4f3). Furthermore, more type I hair cells seemed to survive the diphtheria toxin ablation in Sox2 CKO mice compared to Sox2 WT mice. Sox2 heterozygotes also had reduced regeneration in utricles, but more hair cells were replaced than mice with Sox2 deletion.
Conclusions: We determined that Sox2 is required in supporting cells for normal levels of vestibular hair cell regeneration in adult mice, but it does not seem to be required for supporting cell nuclear migration during regeneration or for maintaining the phenotype of adult supporting cells.

Single-Cell Transcriptomic Analysis Reveals Increased Regeneration in Diseased Human Inner Ears

Tian Wang*[1], Angela Ling[2], Sara Billings[1], Davood Hosseini[1], Yona Vaisbuch[1], Grace Kim[1], Patrick Atkinson[1], Zahra Sayyid[1], Ksenia Aaron[1], Dhananjay Wagh[1], Nicole Pham[1], Mirko Scheibinger[1], Akira Ishiyama[3], Peter Santa Maria[1], Nikolas Blevins[1], Robert Jackler[1], Stefan Heller[1], Ivan Lopez[3], Nicolas Grillet[1], Taha A. Jan[2], Alan Cheng[1]
[1]Stanford University, [2]Vanderbilt University, [3]University of California Los Angeles
Background: Studies on human inner ear sensory cells have relied primarily on postmortem, cadaveric tissues because of the inaccessibility of the adult human inner ear. Previous works have characterized inner ear tissues from fetuses and organoids derived from human embryonic and induced pluripotent stem cells. Moreover, even though vestibular schwannoma utricles show hair cell degeneration, they have served as the primary model system to characterize hair cell morphology and regeneration capacity after aminoglycoside damage in vitro. At present, the molecular signature of hair cells in the adult human inner ear and whether the diseased human inner ear can regenerate remains unknown. Here, we have characterized the transcriptome and validated novel markers of sensory and non-sensory cells using single cell RNA sequencing (scRNA-seq) and showed evidence of hair cell degeneration and regeneration in human utricles from organ donors and vestibular schwannoma patients.
Methods: Utricles were surgically procured from 6 organ donors and 21 vestibular schwannoma patients and prepared as whole mounts or cryosections. Utricles from 4 cadavers were also examined. Three utricles (obtained from 1 organ donor and 2 vestibular schwannoma patients) underwent single cell transcriptomic analysis. Single cell RNA sequencing of purified utricular sensory epithelia was used to characterize the transcriptomic profiles of human utricles by cell clustering, differential gene expression, trajectory, and pathway analysis. Immunohistochemistry and in situ hybridization were used to validate marker genes and perform quantitative analysis.
Results: We show 25 unique transcriptional signatures among highly diverse sensory and non-sensory cells from vestibular schwannoma and organ donor utricles. Cell clusters included hair cells, supporting cells, roof cells, dark cells, stromal cells, melanocytes, vascular cells, macrophages and pericytes. We validated known and novel hair cell and supporting cell genes by immunohistochemistry and in situ in utricles from

different organ donors and vestibular schwannoma patients. Gene expression of hair cells and supporting cells is well conserved between organ donor and vestibular schwannoma utricles. However, markers of hair cell and supporting cell subtypes show divergence of between mouse and human. Lastly, vestibular schwannoma utricles displayed hair cell degeneration and 14-fold more hair cell progenitors compared to organ donor and cadaveric tissues.
Conclusions: These data constitute a foundational resource to investigate normal and diseased human inner ears and reveal an innate regenerative response in the diseased human vestibular organs.

Knockdown of Claudin 9 Levels Induces Extra Functional Inner Hair Cells

Yingying Chen*[1], Jeong Han Lee[1], Seojin Park[1], Maria C Perez-Flores[1], Braulio Peguero[2], Bruce Tempel[3], Haiwei Zhang[1], Ebenezer N Yamoah[1]
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Background: The claudins are a family of transmembrane proteins essential to the tight junction (TJ), forming barriers in epithelial and endothelial tissues. The TJs are involved in contact-mediated lateral inhibition, which plays a critical role in the rearrangement of hair cells and supporting cells in development. In the cochlea, Claudin 9 (Cldn9), one family member of claudins, is expressed highly in the organ of Corti. The roles of Cldn9 in sensory cell differentiation are unknown.
Methods: The mouse lines, doxycycline (dox)-tet-OFF-Cldn9 transgenic, were used under CBA/CaJ background and the wild-type controls. Additionally, we performed adenovirus Cldn9 gene knockdown in CBA/CaJ mouse line. Cldn9 -GFP-virus was delivered into the round window at the different postnatal stages, and then cochlea was collected for morphological analyses. For the Cldn9 transgenic mouse line, two concentrations of dox (1.0 mg/ml and 4.0 mg/ml) were used, and structural and functional analyses were performed comparing the wild-type littermates. ABR and DPOAE were tested at one month old. Immunostaining of Myo7a as HCs, and Sox2 as supporting cell (SCs) markers were used to assess HCs and SCs count. CtBP2 are pre-synaptic ribbons, and Homer 1 are post-synaptic markers used to assess synaptic integrity and functions. Other TJ proteins, including Cldn6 and ILDR1, were co-labeled with Myo7a to identify the location and expression levels.
Results: Dox intake resulted in the knockdown and alterations of Cldn9 expression in the organ of Corti of the Cldn9-HET mice. The ABR and DPOAE experiments on the auditory phenotype of the Cldn9-HET mice show that the administration of dox results in profound changes in hearing functions. This change is limited to mice with the Cldn9-TetOFF promoter and not wild-type littermates. ABR wave I amplitude was reduced in the Cldn9-HET mice. The HCs count showed extra inner hair cells (IHCs) in the Cldn9-HET mice and sh-RNA knockdown of Cldn9 induced extra-IHC but not outer hair cells (OHCs). By contrast, the synapse number per IHC decreased in the Cldn9-HET mice. Cldn9-HET mice treated with 1.0 mg/ml dox showed decreased Cldn6 and ILDR1 expression.
Conclusions: Cldn9 expression in the cochlea is tightly regulated with other TJ proteins to control the hearing function and induce the extra IHCs.

Symposium #12 - Novel Approaches to Ototoxicity Management across the Life Course

2:00 p.m. - 4:00 p.m.
Crystal D-E

Novel Approaches to Ototoxicity Management Across the Life Course

Crystal D-E
Chair: Katharine Fernandez, *NIDCD*
Co-Chair: Gayla Poling, *Mayo Clinic*
Co-Chair: Laura Dreisbach, *School of Speech, Language, and Hearing Sciences, San Diego State University*

Drug-induced ototoxicity is an adverse event to life-saving therapeutic drugs that results in irreversible damage to the inner ear and auditory nerve, presenting as hearing loss and/or balance/vestibular dysfunction. Research has shown that early detection of toxicity through prospective ototoxicity monitoring provides the opportunity to consider modifications to treatment that may minimize or prevent permanent hearing loss or balance impairment. However, routine implementation of ototoxicity management in the clinical setting is

often omitted from practice due to a lack of accepted standard protocols, largely driven by the lack of consensus on reliable monitoring tools and patient perceived benefit, as well as the lack of clinical resources for implementation of an effective program.

This symposium will provide a much-needed opportunity to present current research highlighting the effectiveness of monitoring tools for use in a variety of clinical settings across diverse populations while discussing issues related to the implementation of these measures into current clinical programs. Specifically, this symposium will convey the scope of cochleotoxicity and vestibulotoxicity in clinical practice with considerations of the range of treatment exposures, adverse events, and patient populations to address the complexities of ototoxicity management that inspire novel approaches to current and developing clinical practices. Emerging research efforts in clinical manifestations of ototoxicity on neurocognition. Applications of pharmacodynamic modeling for early detection, and approaches to prevention of hearing loss will be presented. Technological considerations for measurements made in the ear canal will be discussed as well as the application of various tools and interpretations to identify significant changes in the presence of ototoxic exposures. These contributions and considerations along with the understanding of the global burden of ototoxic hearing loss can be used to advance development of feasible and effective ototoxicity management programs, direct research efforts, and prioritize a patient-centered focus across the continuum of care.

Complexities of Ototoxicity Management in Clinical Practice
Carmen Brewer, *National Institute on Deafness and Other Communication Disorders/NIH*

Medications commonly prescribed for anticancer treatments and some infections are known to cause auditory and vestibular/balance dysfunction known as ototoxicity. While ototoxicity is recognized to accompany the life-saving impact of these treatments, a parallel effort to manage ototoxicity has not become standard of care. Despite the well-established physical, socio-economic, and psychological consequences of hearing and balance dysfunction, clinical practice in management of patients receiving ototoxic agents is not consistent within or across countries. Early detection of ototoxicity through serial monitoring provides multidisciplinary care teams opportunities for identification of adverse effects, modifying treatment plans to mitigate hearing loss, and timely interventions. Preventing or minimizing ototoxicity is critical in order to preserve quality of life for patients receiving these treatments and to reduce the societal burden of hearing loss.

Ototoxicity management includes the full scope from diagnosis, monitoring, and rehabilitation to therapeutic treatment of individuals who experience hearing loss, tinnitus, or balance/vestibular difficulties following treatment exposures. Moreover, growing demands for audiologic care related to early detection of hearing loss and prevention, require design and implementation of new pathways that leverage advanced clinical tools to promote timely accessibility to individualized hearing health care while balancing important public hearing perspectives and care delivery models. Enhancements in clinical approaches to known practice gaps offer opportunities for innovation and research to further expand the audiologic practice with prevention of ototoxicity. This is essential for the earliest identification of ototoxicity or treatment-induced auditory and vestibular dysfunction. Timely detection can provide the patient/family and care teams opportunities to identify adverse effects and mitigate their subsequent impact. Moreover, emerging approaches for earliest detection and prevention of ototoxicity can be incorporated in current practice to advance ototoxicity management from monitoring to diagnosis to interventions.

The primary objective of this presentation is to 1) convey the scope of ototoxicity in clinical practice (i.e., range of exposures and populations), and 2) summarize the complexities of ototoxicity management that inspire novel approaches to current and emerging clinical practice. These considerations can be used to advance development of feasible and effective ototoxicity management programs, direct research efforts, and prioritize a patient-centered focus across the continuum of care.

Global Estimates of Ototoxic Hearing Loss Associated With Exposure to Multidrug-Resistant Tuberculosis, Malaria, and Cancer Treatments
Lauren Dillard, *Medical University of South Carolina, College of Medicine*

Multidrug-resistant tuberculosis (MDR-TB), malaria, and cancer are highly prevalent conditions worldwide and are commonly treated with ototoxic medications, placing many individuals globally at risk for ototoxic hearing loss (HL). Understanding the global burden of ototoxic HL can inform the policies, research, and clinical care needed to promote its primary prevention and management. The purpose of this study was twofold. First, to estimate the prevalence of ototoxic HL associated with treatment for MDR-TB (with aminoglycoside antibiotics), malaria (with antimalarials) and cancer (with platinum-based compounds cisplatin and/or carboplatin). Second, to estimate the annual global number of individuals i) exposed to ototoxic drugs to treat these conditions, and ii) HL cases associated with exposure.

Three separate systematic reviews and meta-analyses were conducted to estimate pooled prevalence (95% confidence interval [CI]) of HL associated with MDR-TB, malaria, and cancer treatments. To estimate the crude number exposed to ototoxic medications, we used global estimates of disease incidence, treatment, and mortality, provided by the World Health Organization, GLOBOCAN, and other relevant sources. For each condition, we estimated the crude global annual number of HL cases by multiplying the estimated number of exposed individuals (after accounting for mortality) by pooled prevalence estimates of ototoxic HL ascertained from meta-analyses. Sensitivity analyses present upper and lower estimates of annual HL cases for each condition. Sensitivity analyses were conducted by simultaneously varying several assumptions to create high and low estimates of exposures, which were combined with 95% CIs of pooled prevalence estimates of HL from meta-analyses.

For each condition, we present the crude estimated i) global annual number of individuals exposed to treatment, ii) pooled prevalence of HL associated with exposure to treatment with ototoxic drugs, and iii) global annual number of HL cases associated with exposure: MDR-TB exposed: 187,000; MDR-TB HL prevalence estimate: 40.6% (CI 32.8-66.6), MDR-TB HL cases: 76,000 (sensitivity analysis 59,000-211,000); Malaria exposed: 134 million, malaria HL prevalence estimate: 9.2% (CI 7.1-11.6), malaria HL cases: 12.3 million (sensitivity analysis 5.4-13.7 million); Cancer exposed: 1.02 million, cancer HL prevalence estimate: 43.2% (CI 37.9-48.6), cancer HL cases: 441,000 (sensitivity analysis 387,000-496,000).

Results demonstrate the high global caseload of potentially preventable HL and highlight the urgent need to prioritize primary and secondary global HL prevention associated with exposure to commonly used ototoxic medications. There exists uncertainty in global estimates that may be clarified by future research.

Clinical Presentation and Management of Ototoxicity Due to Aminoglycoside Treatments

Angela Garinis, *Oregon Health and Science University, Department of Otolaryngology*

Aminoglycosides (e.g., gentamicin, amikacin, tobramycin) are highly potent, broad spectrum antibiotics widely and routinely used as a first-line treatment in patients with severe bacterial infections. Aminoglycoside antibiotics are well-documented, particularly when administered intravenously, to produce ototoxicity symptoms. Although aminoglycosides are effective at combating infections, they also have well-documented adverse events such as nephrotoxicity (kidney damage) and ototoxicity, including both vestibulotoxicity (balance/vestibular manifestations such as oscillopsia) and cochleotoxicity (tinnitus, hearing loss, difficulties listening in noise). It is not currently possible to predict which patient will ultimately develop ototoxicity after one or more courses of aminoglycoside treatment. Thus, early identification, prevention and mitigation of ototoxicity-related symptoms are recommended through the routine implementation of ototoxicity monitoring protocols.

Patients with cystic fibrosis (CF) are frequently prescribed antibiotics with known ototoxic adverse events. Clinical recommendations for implementing routine and guideline adherent ototoxicity management in patients with CF will be highlighted as an illustration of novel approaches to ototoxicity management of aminoglycoside treatments in the clinic. These are: 1) including questions about hearing, tinnitus and balance problems as part of the routine CF case history for all patients; 2) utilizing timely point-of-care measures; 3) establishing a baseline and conducting post-treatment evaluations for each course of intravenous ototoxic drug treatment; and 4) repeating annual hearing and vestibular evaluations for all patients with a history of ototoxic antibiotic exposure.

Evaluating the Vestibulotoxic Potential of Aminoglycosides in Patients Treated With Amikacin
John Lee, *National Institute on Deafness and Other Communication Disorders, National Institutes of Health*

Aminoglycosides are broad-spectrum antibiotics used to manage recurrent respiratory infections and treat serious bacterial infections including multidrug-resistant tuberculosis and cystic fibrosis. Despite their robust antimicrobial efficiency and widespread clinical use, many of the FDA-approved aminoglycosides can induce toxic side effects including cochleotoxicity (i.e., outer hair cell death, permanent sensorineural hearing loss) and vestibulotoxicity (i.e., type I vestibular hair cell death, chronic disequilibrium).

While increasing attention has been given to identification, monitoring, and prevention of aminoglycoside-induced hearing loss, the vestibulotoxic potential of these drugs remains unclear. Reported incidences of vestibulotoxicity are highly variable, ranging from 0% to 60%, and a lack of comprehensive, routine vestibular testing has inhibited understanding of the vestibular changes induced by different aminoglycosides. Severe vestibular symptoms (i.e., vertigo) are not reported in most patients with vestibulotoxicity, due to both ears being equally affected. Symptoms more commonly associated with bilateral vestibular dysfunction (i.e., disequilibrium, postural instability) are often underappreciated and attributed to general deconditioning of patients during/after aminoglycoside treatment. As a result, many patients experiencing aminoglycoside-induced vestibulotoxicity likely go unevaluated. In addition, objective vestibular testing used to evaluate vestibulotoxicity is frequently limited to assessment of horizontal semicircular canal function. Clinical findings are often extrapolated to reflect the status of the entire vestibular periphery, and effects of ototoxic drugs on utricular, saccular, and anterior/posterior semicircular canal function remain largely unknown. Histological analyses of temporal bones from patients exposed to aminoglycosides and animal studies suggest different aminoglycosides may preferentially affect different vestibular end organs. As a result, testing only horizontal canal function likely underestimates these drugs' vestibulotoxic potential.

The purpose of this clinical project was to develop a vestibulotoxicity monitoring protocol composed of functional tasks, objective tests, and questionnaires to comprehensively assess vestibulotoxic changes associated with aminoglycosides. Patients treated with IV and inhaled amikacin at the NIH underwent vestibular testing prior to treatment onset and at various post-treatment timepoints to evaluate the effects of amikacin on vestibular function. By monitoring vestibular function before and after all amikacin treatments, this study will ensure vestibular losses are properly identified and managed to minimize patients' risk of injury and falls. Results of this clinical study will also provide insight into the differential consequences of aminoglycosides on all vestibular end organs.

Importance of Monitoring Tools and Pharmacodynamic Modeling for Aminoglycoside Ototoxicity
Lisa Hunter*, Cincinnati Children's Hospital Medical Center*

Patients treated with life-saving aminoglycoside antibiotics frequently experience adverse side effects of ototoxicity – permanent hearing loss and degraded speech communication. Cystic fibrosis (CF) is the most common life-threatening genetic disease in Caucasians and causes persistent lung infections in childhood that are frequently treated with aminoglycoside (AG) antibiotics, thus is an important patient group to target for prevention of ototoxicity. Currently, most patients with CF at risk are not monitored for ototoxic hearing loss. The lack of monitoring is primarily due to lack of availability and awareness of early detection methods, as well as treatment alternatives that can preserve hearing. There are critical gaps in our understanding of individual susceptibility for ototoxicity and access to effective tests that identify those at higher risk.

The long-term goal of our research program is to develop predictive models using novel auditory tests and pharmacodynamics (PD) for early detection and prevention of sensorineural hearing loss (SNHL) in at-risk individuals receiving aminoglycoside (AG) antibiotics. Newer methods to detect onset of ototoxicity include extended high frequency (EHF) transient otoacoustic emissions (TEOAE) and digits in noise (DIN) tests. DIN tests that can be automated or delivered remotely via the internet or through smartphones could fundamentally improve access to ototoxicity monitoring. Aims of this study are to (1) Optimize accurate detection of existing hearing loss at baseline and shifts that are due to ototoxicity using EHF chirp TEOAEs;

(2) Determine accuracy of remotely delivered DIN to detect EHF hearing loss due to ototoxicity; (3) Determine if EHF hearing is related to higher cumulative AG exposures and set optimal dosing cut-off levels using PD models validated in CF to detect ototoxicity risk.

Results from prospective longitudinal monitoring with EHF TEOAE and DIN measures reveal temporal relationships to hearing threshold shifts. Pharmacodynamic models quantify individual differences in drug exposures that effectively predict hearing levels. Outcomes from improved monitoring will have an important positive impact because they will provide a better understanding of ototoxicity mechanisms, timing and risk factors that can be translated into improved ototoxicity monitoring. Clinical trials of drugs to protect the inner ear could be facilitated by expanded knowledge and availability of improved diagnostic and monitoring tools.

Navigating Complexities of Ear Canal Acoustics in Ototoxicity Monitoring
Shawn Goodman, *Department of Communication Sciences and Disorders, The University of Iowa*

One of the longstanding challenges associated with measuring high frequency (> 8 kHz) otoacoustic emissions (OAEs) is the effect of ear canal acoustics on measured sound pressure levels. This issue directly impacts test-retest variability, a major determining factor in the sensitivity of ototoxicity monitoring protocols. When an OAE probe is sealed in the outer ear, the canal acts as a tube closed at one end and open at the other, resulting in standing wave resonances. As a result, at certain frequencies sound pressures measured at one end of the canal (the probe microphone near the ear canal entrance) do not match the sound pressures at the other end (the ear drum). The problem affects measurements of both stimulus levels as well as OAE levels.

Over the years, several solutions to this problem have been proposed, including use of a constant voltage, the depth compensation method, use of long “reflectionless” calibration tubes, and Thevenin-based source separation (to estimate forward pressure level and emitted pressure level). A brief overview of each method will be presented, along with references providing details of implementation. Advantages and disadvantages of the various methods will be discussed, including their relative theoretical accuracy and issues with practical implementation in ototoxicity monitoring.

Maximizing Measurements to Identify Significant Change in Ototoxicity Monitoring
Laura Dreisbach, *School of Speech, Language, and Hearing Sciences, San Diego State University*

Most therapeutic treatments known to cause hearing loss initially damage basal cochlear regions. Identifying the tools to best reflect this damage across the lifespan are critical to the identification and management of these patients. One such tool, distortion-product otoacoustic emissions (DPOAEs), have the potential to quantify cochlear damage that has not yet been observed on the audiogram at both conventional (< 8 kHz) and extended high (> 10 kHz) frequencies.

DPOAE levels across the range of human hearing are repeatable over time in healthy newborns, children, and young adults, as well as a patient population rendering this metric an acceptable monitoring tool. The repeatability of DPOAE levels is enhanced with improved calibration techniques which provide more control over stimulus levels. Additionally, DPOAE paradigms utilizing varied stimulus levels to determine a threshold or varied ratios to calculate group delays are repeatable over time and have been used in individualized serial monitoring protocols in patients undergoing chemotherapy treatments with various platinum derivatives. To this end, the earliest signs of underlying cochlear damage were found at the highest frequencies with a response using a DPOAE concentrated discrete frequency sweep with high stimulus levels and detection thresholds.

While most efforts primarily focus on the repeatability of the DPOAE level, there are other attributes of DPOAE measures that are typically used for interpretation, namely the signal-to-noise ratio (SNR). Thus, the repeatability of DPOAE SNR values needs to be established to determine which attribute of DPOAEs should be used in monitoring programs. To answer this question DPOAE SNR repeatability was assessed in the same populations where DPOAE level repeatability had been determined. While DPOAE SNR values

were repeatable across four sessions, DPOAE levels were less variable allowing earlier indicators of cochlear damage.

Exploring various DPOAE paradigms and attributes across the lifespan and at the highest frequencies affords the clinician the most sensitive tools for the earliest detection of ototoxicity. As DPOAEs are a complex measure and minimally comprise two cochlear sources, further examinations are warranted to determine if these sources are differentially influenced by ototoxic exposures. Emerging DPOAE applications including targeted monitoring protocols to assess cochlear function at the highest frequencies and improved calibration techniques to ensure stable measurements have the potential to enhance clinical practice.

spARO Special Initiative Workshop: Networking as a Tool for Resilience

4:15 p.m. - 6:15 p.m.
Canaveral 1-2

Networking as a Tool for Resilience

Lina Reiss and Tanvi Thakkar
Zoe Owrutsky and Sean Anderson
Bobby Gibbs and Stephen Dennison
Ruth Litovsky
Seba Ausili and Kristina DeRoy Milvae
Amanda Lauer and Jonathan Peelle

The COVID-19 pandemic severely limited the ability of students and trainees to network. While networking is often recognized for its effectiveness in career advancement, it also plays a key role in building resilience to navigate challenging times in the field, particularly as much work remains to create an inclusive and equitable environment. This workshop will consist of six break-out groups themed after networking and resilience. They will focus on: (1) race, gender, and ability, (2) mental health and loss, (3) navigating career changes, (4) the roles of different mentors, (5) growing one's family, and (6) using social media to promote one's work. This workshop is meant to complement ARO's efforts regarding equity and inclusion. This workshop will be a space where participants can discuss issues relevant to their lives and form connections with other members of ARO. While the workshop will be primarily aimed at students and trainees, it will benefit the ARO community at large, and any conference attendees are welcome to join. This workshop will take place over 1 hour. It will begin with a brief introduction as to the purpose of the event and a description of resources that exist inside and outside of our field related to each topic. Then, participants will be invited to join a break-out group. Each break-out group will function as a roundtable discussion, where participants are invited to ask questions or share, and discussion leaders from the field can share experiences or guide the conversation. Each group will have one to two discussion leaders. Participants will be able to move from one topic to another at will during the session.

spARO Special Initiative Workshop: Networking as a Tool for Resilience

4:15 p.m. - 6:15 p.m.
Canaveral 1-2

The COVID-19 pandemic severely limited the ability of students and trainees to network. While networking is often recognized for its effectiveness in career advancement, it also plays a key role in building resilience to navigate challenging times in the field, particularly as much work remains to create an inclusive and equitable environment. This workshop will consist of six break-out groups themed after networking and resilience. They will focus on: (1) race, gender, and ability, (2) mental health and loss, (3) navigating career changes, (4) the roles of different mentors, (5) growing one's family, and (6) using social media to promote one's work. This workshop is meant to complement ARO's efforts regarding equity and inclusion. This workshop will be a space where participants can discuss issues relevant to their lives and form connections with other members of ARO. While the workshop will be primarily aimed at students and trainees, it will benefit the ARO community at large, and any conference attendees are welcome to join. This workshop will take place over 1 hour. It will begin with a brief introduction as to the purpose of the event and a description

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Sean R. Anderson, *Cochlear Americas*
Tanvi Thakkar, *University of Wisconsin-La Crosse*
Ruth Litovsky, *University of Wisconsin*
Lina Reiss, *Oregon Health and Science University*
Kristina DeRoy Milvae, *University at Buffalo*
Bobby Gibbs, *Univesity of Maryland College Park*

Neil Segil Memorial Symposium

4:15 p.m. - 6:15 p.m.
Oceans Ballroom 1-4

Chair: Andrew Groves, *Baylor College of Medicine*
Presenter: Angelika Doetzlhofer, *Johns Hopkins University School of Medicine*
Presenter: Litao Tao, *Creighton University*
Presenter: Ksenia Gnedeva, *USC Keck School of Medicine*
Presenter: Tatjana Piotrowski, *Stowers Institute for Medical Research*
Presenter: Jennifer Stone, *University of Washington, Virginia Merrill Bloedel Hearing Resource Center*
Presenter: Edwin Rubel, *University of Washington*
Presenter: Andrew Groves, *Baylor College of Medicine*

spARO Science Communication Workshop

6:00 p.m. - 7:00 p.m.
Merritt 2
A part of what makes science so exciting is being able to share our passion with those around us, and communicating our work effectively to diverse audiences allows science to make a positive impact in society. The spARO 2023 Science Communications workshop is focused on helping scientists gain comfort and confidence to engage in, or instigate, scientific conversations beyond our field. We aim to provide a relaxed and interactive environment for attendees to diversify their science communication skills and promote constructive two-way scientific conversations with both scientists and non-scientists of various backgrounds.

Allison Coffin, *Washington State University*
Matsya Thulasiram, *Sunnybrook Research Institute*
Braulio Peguero, *NIH/NIDCD*

Monday, February 13, 2023

Podium #13 - Synapse and Auditory Nerve in Hidden Hearing Loss

8:00 a.m. - 10:00 a.m.
Ocean's Ballroom 5-12
Moderators: Hainan Lang and Douglas Fitzpatrick

Spectral Approach to Electrocochleographic Analyses: Implication for Hidden Hearing Loss

Viacheslav Vasilkov*[1], Charles Liberman[1], Stéphane Maison[1]
[1]*Harvard Medical School / Mass Eye and Ear*

Background: In mouse studies, cochlear nerve degeneration (CND) is correlated with the suprathreshold amplitude of ABR wave 1, as long as cochlear thresholds remain normal. However, diagnosing CND in humans is challenging, as wave I amplitude is small and waveforms are noisier than in anesthetized animals. Alternative methods have been proposed including the use of electrocochleography (EcochG) to obtain larger responses that comprise the summating potential (SP), including currents from hair cell receptor potentials and the action potential (AP or N1), representing the summation of cochlear nerve action potentials. However, it remains difficult to separate hair cell and neural components, as they overlap in time. Here, we propose an objective method 1) to extract the inferred neural spiking components of the EcochG from other sources of currents in the frequency domain and 2) to assess the relevance of these measures to the identification of CND in "normal-hearing" participants, as measured indirectly by their performance on difficult tasks of speech-in-noise recognition.
Methods: 122 native speakers of English with normal audiometric thresholds who passed the Montreal Cognitive Assessment were recruited. Word recognition scores were assessed at 55 dB HL using time-compressed words (65%) with added reverberation or a modified version of the QuickSIN™.
EcochG responses were evoked by 100 µs-clicks delivered at 125 dB pSPL presented at 9.1 or 40.1/s. Response waveforms were exported into MATLAB, zero-padded and transformed into the frequency domain, resulting in 3 main spectral peaks near 85 Hz, 260 Hz and 550-810 Hz. Since the latter peak is likely dominated by action potentials, we then bandpass filtered the EcochG waveforms from 0.47 - 3 kHz to study neural components and from 0 to 0.47 kHz to study the other contributions to the response.
Results: The amplitude of the inferred neural contribution to wave 1, i.e. the wave 1 peak after band-pass filtering, was better correlated with word-recognition scores than any wave 1 measure extractable without filtering. Consistent with neural adaptation, the bandpass filtered wave 1 was also significantly reduced by increasing stimulus presentation rate. Interestingly, the amplitude of the low-pass [0-.47 kHz] component was attenuated as well, suggesting that non-spiking neural generators contribute to these low frequency components, possibly in the form of PSPs.
Conclusions: Consistent with a role of CND in the degradation of word recognition in challenging listening environments, the inferred neural component of the wave 1 peak extracted from the EcochG waveform by bandpass filtering was significantly correlated with speech intelligibility performance.
Research supported by a grant from the NIDCD (P50 DC015857).

Transtympanic Delivery of Soluble Fractalkine Peptide Repairs Cochlear Synapses and Function After Acoustic Trauma

Vijayprakash Manickam*[1], Andrew Stothert[1], Elyssa Pereyra[1], Lyudmila Batalkina[1], Tejbeer Kaur[1]
[1]*Creighton University*

Background: Cochlear ribbon synapses between inner hair cells (IHCs) and spiral ganglion neurons (SGNs) are vulnerable to damage and/or loss due to acoustic trauma. Such damaged synapses can repair spontaneously in mouse (Kaur et al., 2019, Kim et al., 2019 and Shi et al., 2015) and guinea pig (Shi et al., 2013 and Hickman et al., 2021). However, mechanisms underlying synaptic repair are unknown. We have demonstrated a critical role for fractalkine signaling (CX3CL1-CX3CR1) in synaptic repair after acoustic trauma where in the presence of fractalkine receptor (CX3CR1) on cochlear macrophages the damaged synapses recover however, in the absence of CX3CR1 synaptic repair is impaired (Kaur et al., 2019). Here we examined if fractalkine ligand (CX3CL1 or FKN), expressed by IHCs and SGNs, is also necessary for synaptic repair and whether transtympanic delivery of FKN isoforms (membrane or soluble) repairs cochlear synapses after acoustic trauma.
Methods: FKN wild type (WT) and knockout (KO) mice (5-6 weeks of age) of both sexes were exposed for 2 hours to a synaptopathic noise level of 93 dB SPL at 8-16 kHz octave band. Hearing function was assessed prior to, at 1 and 15 days after acoustic trauma. Following function assessment at 1 day after trauma, mice were unilaterally trans-tympanically (TT) injected with 5ml volume of either vehicle (sterile Poloxamer 407), control peptide, membrane bound FKN peptide (mFKN, 312 amino acids (aa)), soluble FKN peptide (sFKN, 83 or 80 aa). Mice were euthanized at 15 days post exposure and isolated cochleae were processed for multilabel fluorescent immunohistochemistry to examine hair cells, ribbon synapses, and neurons.
Results: Absence of FKN does not affect hearing function and IHC synapse density in steady state. However, at 15 days after acoustic trauma FKN KO mice displayed significant elevation in hearing thresholds whereas FKN WT mice showed nearly complete recovery of elevated hearing thresholds. Auditory brainstem response (ABR) peak 1 amplitudes were reduced and ~ 50 % synapses were lost in both

exposed FKN WT and KO mice. Remarkably, FKN WT and KO mice that were TT injected with the sFKN peptide (80 aa, 50 ng/ml) at 1 day after acoustic trauma showed significant recovery of hearing thresholds and ABR wave 1 amplitudes to baseline levels and increased IHC synapse density when compared to mice injected with vehicle, control peptide or mFKN. FKN ELISA detected the presence of sFKN (80 aa) peptide inside the cochlea up to 24 hours at 1/10th of the total concentration (250 ng) injected into the middle ear.
Conclusions: Together, these data imply that intact endogenous fractalkine signaling axis plays a critical role in synaptic repair and that soluble isoform of FKN is most effective to repair cochlear synapses after acoustic trauma.

Compound Action Potentials of the Gerbil Auditory Nerve Recover After Round Window Infusion of Kainate Despite Persistent Synapse Loss

Artem Diuba*[1], Jerome Bourien[1], Sharon Kujawa[2], Jean Luc Puel[1]
[1]INM - Inserm U1298, [2]Eaton-Peabody Laboratories, Massachusetts Eye and Ear and Harvard Medical School
Background: Noise overexposure can induce a loss of synapses between the inner hair cells (IHCs) and auditory nerve fibers (ANFs) without destroying the hair cells. Recent studies suggest that synapses regenerate spontaneously during the months following exposure (Hickman et al, Front Cell Neurosci, 2021 Aug 9;15:684706). Here, we investigate whether synapses can recover after round window infusion of kainate (KA) in gerbils.
Methods: Artificial perilymph (AP) containing 25 mM kainic acid was infused into the round window niche for 1 hour in young adult female Mongolian gerbils. AP alone was infused in control gerbils. Cochlear function was assessed measuring distortion product otoacoustic emissions (DPOAEs) and compound action potentials (CAP) of the auditory nerve. Synapses were quantified using CtBP2 and GluA2 immunolabeling and confocal imaging. Morphological and functional assessments were performed at different cochlear locations from 2 to 16 kHz and different stages after drug application from 3 hours to 4 months.
Results: Although completely abolished 3 hours after the KA application, the CAP thresholds and amplitudes progressively recovered starting from the 1st day to 4 months, especially at 8 and 16 kHz where the CAP amplitudes reached their control values. Whatever the time point recorded, the amplitude of the DPOAEs remained mostly unaffected. Ultrastructural examination of the cochlea 3 hours after KA infusion showed swelling of the afferent terminals and disruption of the ribbon synapses. One day after KA infusion, less than 25% of cochlear ribbon synapses (re: control) were observed all along the tonotopic axis. Two weeks after, the partially recovered synapse count was still under 50% at all cochlear locations. In contrast to CAP amplitudes and thresholds, synapse count did not recover 4 months post-KA, reaching 50% in the regions of 2, 4 and 8 kHz and 70% in the 16 kHz.
Conclusions: Our results show for the first time that the compound action potentials of the gerbil auditory nerve can fully recover from KA-induced excitotoxicity, despite persistent synapse loss. Further investigations, including single-fibers recordings from the auditory nerve are required to examine the phenotype of remaining ANFs.

Does Auditory Nerve Loss Cause Hidden Hearing Deficits for Short Tones in Noise?

Catherine Loftus*[1], Kenneth Henry[2], Kristina Abrams[2], Margaret Youngman[2], Paul Allen[2]
[1]University of Rochester Medical Center, [2]University of Rochester
Background: Loss of auditory nerve afferent innervation of the cochlea is commonly seen with aging, but remains poorly understood. While hair cell damage is frequently detectable with the pure tone audiogram and presents with clinically apparent deficits in listening abilities, auditory nerve fiber loss is more difficult to detect. Theoretical models predict “hidden hearing loss” for detection of brief signals in noise in individuals with auditory nerve fiber loss, because these stimuli provide limited opportunity for temporal integration; however, experimental evidence remains controversial. While a previous study in mice found that short tone perception was adversely affected by auditory-nerve loss in comparison to long tones, testing was limited to a period of several weeks after lesioning and therefore allowed limited perceptual adaptation.
Methods: Selective lesioning of auditory nerve fibers without disrupting hair cells was accomplished with intracochlear kainic acid infusions. Behavioral experiments were conducted using operant conditioning procedures. Auditory nerve lesions were characterized physiologically with auditory brainstem response and through histological analyses conducted at the conclusion of behavioral studies. Testing was performed in 4

kainic acid exposed and 4 age-matched controls. The overshoot phenomenon was studied by moving the tone onset to the beginning of the noise.
Results: Thresholds for detecting tones in noise increased by approximately 10 dB for 20-ms tones compared to 200-ms tones, and were similar between 1.4 and 2.8 kHz test frequencies. There was surprisingly little threshold elevation observed when the short tone was moved to the beginning of the noise (i.e., minimal overshoot phenomenon). Results were compared between kainic acid exposed animals and age-matched controls to evaluate the impact of auditory-nerve loss on perception of brief masked signals.
Conclusions: Use of experimental auditory-nerve lesions in this animal behavioral study provide new insight into the aspects of auditory perception in noise impacted by this common cochlear pathology.

Peripheral Myelin Disorders and the Mechanisms of Hidden Hearing Loss

Luis Cassinotti*[1], Lingchao Ji[1], David Kohrman[1], Beatriz Borges[1], Mary Caroline Yuk[1], Aditi Desai[1], Nathan Cass[1], Zahara Amir[1], Guoqiang Wan[1], Gabriel Corfas[1]
[1]Kresge Hearing Research Institute University of Michigan Medical School
Background: Hidden hearing loss (HHL) is an auditory neuropathy believed to contribute to deficits in speech discrimination and intelligibility in people with normal audiological tests. In animal models, HHL presents as normal auditory thresholds with defective cochlear neurotransmission, i.e., reduced auditory brainstem response (ABR) peak 1 amplitudes. Work in animal models has shown that neuronal pathologies can cause HHL. Specifically, there is strong support for loss of synapses between the inner hair cells (IHCs) and spiral ganglion neurons (SGNs) being a cause of age-related and noise-induced HHL. Thus, in many instances, cochlear synaptopathy and HHL have been discussed as a single pathology.
Methods: Since peripheral neuropathies can be also caused by myelin impairments, we explored whether different myelin disorders can also cause HHL using three mouse models.
Results: In the first model, Schwann cell underwent ablation by genetic means, causing a near total loss of auditory nerve myelin within one week. Remarkably, this demyelination does not alter auditory thresholds, yet induces permanent reduction in ABR peak 1 amplitudes and longer peak 1 latencies. Furthermore, these alterations persist even after auditory nerve remyelination through the proliferation and differentiation of new Schwann cell precursors. Importantly, transient Schwann cell ablation does not change IHC-SGN synapse density, but it causes a permanent disorganization of cochlear heminodes, the nodal structures closest to the IHCs. Moreover, HHL caused by Schwann cell ablation and noise-exposure are additive, supporting the notion that HHL can be caused by at least two distinct mechanisms.
We then analyzed a mouse model of Charcot Marie Tooth disease type 1A (CMT1A), the most common hereditary motor and sensory peripheral neuropathy; and a mouse line that has peripheral nerve hypomyelination due to loss of Schwann cell ErbB receptor signaling. Remarkably, despite the different molecular mechanisms leading to the peripheral myelin defects, all models present with the same phenotypes, i.e., HHL and disorganized heminodes.
Conclusions: Together, our results show 1) that peripheral myelopathies can cause HHL that is very similar to that seen with synaptopathy except that it also affects ABR peak 1 latencies; 2) that the heminodes play a critical role in the generation of the auditory nerve compound action potential, possibly acting as the action potential initiation site; and 3) that patients with peripheral myelin disorder might also suffer from HHL.
This work was supported in part by R01DC018500 (GC)

Hybrid Optogenetic and Electrical Stimulation Elicits Responses With High Temporal Precision in the Auditory Nerve

Elise Ajay*[1], Alex Thompson[1], Andrew Wise[1], David Grayden[2], James Fallon[1], Rachael Richardson[1]
[1]Bionics Institute, [2]University of Melbourne
Background: Cochlear implants artificially restore hearing to people with hearing loss through electrical stimulation of the auditory nerve. The device has restored speech understanding to users all over the world, but many still struggle to appreciate music and recognise speakers. These limitations are believed to result from the broad spread of electrical stimulation throughout the saline environment of the cochlea. Optogenetics has been demonstrated as an alternative to electrical stimulation, using light instead to stimulate nerves, which can achieve higher spatial precision within the cochlea. Unfortunately, the high rates of stimulation used in contemporary cochlear implants cannot be achieved with optogenetics. Hybrid stimulation, using concurrent optogenetic and electrical stimulation, can improve the spatial precision of cochlear implants, and may be capable of achieving high stimulation rates.

Our study first aimed to confirm if concurrent optogenetic stimulation could reduce the activation threshold of electrical stimulation. Secondly, we compared the temporal characteristics of the auditory nerve response across three modes of stimulation: electrical, optogenetic, and hybrid.
Methods: Auditory nerve responses to three modes of stimulation – light/optogenetic, electrical, and hybrid – were obtained from acutely deafened transgenic mice expressing the channelrhodopsin ChR2-H134R in auditory neurons. Stimuli were presented at 4 Hz or as a burst over 300 ms at 100 pulses per second via a laser-coupled optical fibre and/or platinum wires inserted into the cochlea. Light stimuli (1 ms) were presented at 100-150% of threshold, and 58 µs biphasic current pulses were presented at 0-2 mA, delayed by 942 µs relative to the start of the light pulse. Thresholds and temporal characteristics of the response were recorded using electrodes positioned on the bony wall of the cochlea and the back of the neck.
Results: Addition of suprathreshold optogenetic stimulation lowered electrical activation thresholds by 258 ± 72 µA ($n=6$, $p<0.05$) when light was at 110% threshold, and 395 ± 44 µA ($n=6$, $p<0.001$) when light was presented at >140% threshold. Responses at the end of optogenetic-only burst stimuli had an increased latency of 308 ± 62 µs ($n=7$) compared to responses at the start of the burst. Responses to electrical-only stimulation showed little to no latency changes over the burst. Hybrid responses demonstrated electrical-like temporal precision when the concurrent optogenetic stimulus was close to activation threshold but increasing the optogenetic power worsened temporal precision.
Conclusions: Our results show that hybrid stimulation can achieve higher temporal precision than optogenetic-only stimulation. Furthermore, our data supports previous studies demonstrating lower electrical activation thresholds with concurrent optogenetic stimulation, which may improve spatial precision of cochlear implants, as previous studies show. Future research in larger animal models will provide greater insight into the full extent of the benefits that hybrid stimulation can provide in the cochlea.

Detecting Cochlear Synaptopathy Using Envelope Following Responses in the Budgerigar

Leslie Gonzales*[1], John Wilson[1], Sarah Verhulst[2], Kenneth S. Henry[1]
[1]University of Rochester Medical Center, [2]Ghent University
Background: Loss of auditory-nerve-fiber (ANF) afferents is common in aging, and may occur in individuals with acoustic overexposure. Previous studies have used the amplitude of the wave-I of the auditory brainstem response (ABR) to estimate cochlear synaptopathy, which is a robust measure of ANF damage in animal models including the budgerigar. In contrast, ABR wave I may be less informative of ANF status in humans due to its low amplitude. The envelope-following response (EFR) is a scalp potential evoked with amplitude-modulated stimuli, that may be more sensitive to cochlear synaptopathy in humans due to its greater amplitude. EFRs are typically measured using sinusoidally amplitude-modulated (SAM) tones. However, a recent modeling study suggests that square wave modulated (SWM) tones provide more synchronous ANF responses than SAM stimuli, and therefore may be more sensitive for detecting cochlear synaptopathy. The current study in budgerigars, a parakeet species, tested whether SWM tones provide a better indicator of cochlear synaptopathy in animals with experimentally induced auditory-nerve lesions.
Methods: EFRs to SWM tones and SAM tones were recorded in budgerigars before and after kainic acid exposure. Intracochlear infusions of 1-2 mM kainic acid, the glutamate analog, were performed to produce substantial ANF loss in both ears while sparing hair cells. Modulation frequencies were 80-120 Hz and the carrier frequency was 2.75 kHz. EFR amplitude was estimated by combining response energy at the first several harmonics of the modulation frequency. ABR responses to clicks were also obtained for quantification of wave-I amplitude (i.e., the compound auditory-nerve response). Budgerigars were chosen because their hearing range includes lower frequencies that humans rely on for speech comprehension. Furthermore, budgerigars are widely used in behavioral auditory research, including ongoing behavioral studies of cochlear synaptopathy in our lab, and show performance comparable to humans for various complex auditory discrimination tasks.
Results: Both ABR wave-I amplitude and EFR amplitude showed a dramatic reduction after kainic-acid exposure followed by slight recovery over the weeks following auditory-nerve injury. Compared to SAM tone EFRs, EFRs evoked by SWM stimuli were greater in amplitude and showed a more consistent reduction after kainic acid exposure. Furthermore, EFRs evoked by SWM tones showed a stronger correlation to click-evoked ABR wave-I amplitude than EFRs evoked by SAM tones.
Conclusions: The results suggest that EFRs evoked by SWM stimuli provide a better indicator of ANF damage in an animal model of cochlear synaptopathy than EFRs evoked by SAM tones. Future studies can

explore the use of EFRs evoked by SWM tones as a potential clinical measurement of ANF damage in humans.
This research was supported by R01-DC017519

Differential Expression of miRNAs and Their Predicted Target Pathways in Cochlear Nucleus Following Chronic Noise Exposure in Rats

So Young Kim*[1], So Min Lee[2], Jiwon Jeon[2], Chang Ho Lee[1]
[1]CHA University, Bundang CHA Medical Center, [2]CHA University

Background: Several recent preclinical studies have reported that dynamic changes in miRNA expression contribute to hearing function. This study aims to investigate miRNA expression changes in the cochlear nuclei (CN) of rats following chronic noise exposure.
Methods: Eight-week-old rats (n = 14) were exposed to noise for 4 weeks. The control rats (n = 14) were raised under identical conditions without noise. Two months after noise exposure, the auditory brainstem response (ABR) was examined, and the cochlea and CN were harvested. In the CN, the expression levels of arc, neurocan, and brevican were measured (n = 6 per group). Furthermore, the expression levels of miRNAs and their predicted target genes were measured in the CN (n = 8 per group). ABR thresholds were elevated after 4 weeks of noise exposure, which were main-tained for 3 months.
Results: . In CN, the protein expression of arc and brevican was higher in the noise-exposed group than in the control group (0.95 [standard deviation (SD) = 0.53] vs. 3.19 [SD = 1.00], $p < 0.001$ for arc and 1.02 [SD = 0.10] vs. 1.66 [SD = 0.24], $p < 0.001$ for brevican). The noise-exposed rats exhibited lower expression levels of miR-758-5p, miR-15b-5p, miR-212-3p, miR-199a-5p, and miR-134-3p than the control rats (all $p < 0.001$). The AMPK signaling pathway was predicted to be regulated by these miRNAs. The predicted target genes AKT3, SIRT1, and PRKAA1 were highly expressed in noise-exposed rats.
Conclusions: In CN of noise-exposed rats, the miRNAs of miR-758-5p, miR-15b-5p, miR-212-3p, miR-199a-5p, and miR-134-3p were reduced and related to AMPK signaling including AKT3 and SIRT1 expres-sion. These modulation of signaling pathways could mediate the increased expression of brevi-can in the CN of noise-exposed rats.

Podium #14 - Speech Perception: Imaging and Modelling

8:00 a.m. - 10:00 a.m.
Oceans Ballroom 1-4

Moderators: Hanin Karawani and Zilong Xie

Processing Speech in Noise, Verbal Working Memory, and Cognitive Inhibition Converge in Bilateral Insula

Jaimy Hannah*[1], Madison Tutton[1], Ingrid Johnsrude[1]
[1]University of Western Ontario

Background: When speech is heard in the presence of background noise, cognitive processes are recruited to compensate and maximize intelligibility. This results in elevated activity in frontal cortex when compared to clear speech. Performance on speech-in-noise tasks is correlated with performance on tasks of cognitive control and working memory, which both also rely on frontal cortex. We conducted three meta-analyses of imaging studies related to verbal working memory, speech-in-noise listening, and cognitive inhibition - in order to identify functional overlap among these, thus helping to clarify the cognitive architecture of speech listening in adverse conditions.
Methods: Activation likelihood estimation (ALE) meta-analyses identify regions that are consistently activated across several neuroimaging studies by treating each activation focus as a probability distribution and determining the overlap of these distributions. We examined the convergence in brain regions identified across three ALE meta-analyses of fMRI studies of healthy adults. The speech-in-noise (SiN) meta-analysis included studies in which naturally produced speech was masked in some way by noise. Studies were included if they contrasted a lower signal-to-noise ratio (SNR) with a higher SNR (or clear speech). The cognitive inhibition (CI) meta-analysis consisted of studies using the Stroop task, in which the incongruent condition was contrasted against a neutral condition. The verbal working memory (VWM) meta-analysis consisted of “n-back” studies, in which participants had to press a button when a visually presented stimulus

was the same as the one presented n trials previously. Studies were included if the n-back condition was contrasted against a vigilance control task.
Results: The SiN meta-analysis revealed consistent activation of bilateral primary auditory cortex, left inferior frontal gyrus, left pre-supplementary motor area, and bilateral insula. The CI meta-analysis revealed consistent activation in several bilateral frontal and parietal regions, and in bilateral insula. The VWM meta-analysis revealed an extensive bilateral frontoparietal network, including bilateral insula. Activation patterns for the three task domains converged uniquely in the anterior insula bilaterally. In addition, overlap for the SiN and VWM ALE analyses was observed in left anterior cingulate cortex and left pre-supplementary motor area.
Conclusions: The overlapping regions across SiN, VWM, and CI tasks – bilateral anterior insula – is part of the multiple demand network (MDN) – a domain-general frontal network involved in the organization and control of several cognitive processes. The insula has been implicated in numerous studies across several cognitive domains, becoming active when a challenging task condition is contrasted against an easier condition. Although the overlap between SiN and VWM was more extensive, it consisted only of regions in the MDN that have been implicated in both verbal and nonverbal cognitive tasks. These results are consistent with the idea that frontal involvement during SiN processing is due to recruitment of domain-general cognitive resources.

Measuring Neural Responses to Audiovisual Speech Processing During Movie Viewing Using Optical Neuroimaging

Jonathan Peelle*[1], Arefeh Sherafati[2], Aahana Bajracharya[2], Michael Jones[2], Joseph Culver[2]
[1]Northeastern University, [2]Washington University in St. Louis
Background: When trying to understand speech in background noise, listeners benefit from visual speech information. However, the mechanisms through which visual and auditory information inform perception are still unclear. One challenge for understanding audiovisual speech processing is that the processes contributing to perception in everyday conversation (connected, meaningful speech) may differ from those involved in typical experimental paradigms. A related problem is that activity related to continuous speech in fMRI is difficult to interpret because of the acoustic scanner noise. To circumvent these challenges we measured cortical hemodynamic activity using HD-DOT high-density diffuse optical tomography (HD-DOT) while participants viewed a clip from a movie. HD-DOT is an optical neuroimaging method that provides uniform sensitivity and accurate source localization over the field of view, which covers most of the superficial cortical surface. HD-DOT is acoustically silent and thus ideally suited for studying neural mechanisms of speech perception.
Methods: Participants (n=48) viewed a 10-minute clip from The Good, The Bad, and the Ugly containing a variety of communication contexts. Participants were instructed to minimize movement but given no other tasks. For each second of the movie, we coded speech (if present) as being auditory-only or auditory visual. (Auditory-only speech occurs when a character speaks off camera, with their back to the camera, or with their mouth obscured.) We used these events to create regressors which we we analyzed using a standard GLM-based framework in automatic analysis (aa) and SPM12.
Results: As expected from studies using single words and sentences, auditory-only speech was associated with increased activity in bilateral superior temporal cortex, as well as inferior left frontal gyrus and middle frontal gyrus. In comparison to auditory-only speech, audiovisual speech was associated with greater activity in visual cortex and portions of left frontal cortex.
Conclusions: In the current study we demonstrate the feasibility of using naturalistic stimuli (movies) to study audiovisual speech perception using functional brain imaging. We identify two regions of the frontal lobe - inferior frontal gyrus and premotor cortex - that are more active for audiovisual speech than for auditory-only speech. These findings suggest a distributed network for audiovisual speech processing in naturalistic conditions that may bring us closer to understanding processes guiding everyday conversation.

Cortical Encoding of Phonetic Features of Both Attended and Ignored Speech in Hearing Impaired Individuals

Sara Carta*[1], Emina Aličković[2], Alejandro López Valdes[1], Johannes Zaar[2], Giovanni Di Liberto[1]
[1]Trinity College Dublin, [2]Eriksholm Research Centre, Oticon A/S, Denmark
Background: Speech comprehension involves the simultaneous processing of increasingly more abstract properties, from acoustics to phonetic and semantic features. Previous studies probed the hierarchical nature

of speech processing, by measuring neural activity with electroencephalography (EEG), resulting in objective neural indices of speech and language comprehension. Importantly, these neural metrics have been found to be impacted by the attentional focus of the listener, in realistic noisy and multi-talker scenarios. Building up on this body of knowledge, we assessed how attentional selection impacts cortical representations of speech in environments with competing talkers.
Methods: This study investigates cortical responses to acoustic and phonetic features of speech, in a cohort of older participants with hearing impairment. Participants were immersed in a multi-talker auditory scene, which is particularly challenging for people with hearing impairment, even when using hearing-aids. Specifically, participants were asked to selectively attend to one speaker, while ignoring other competing speakers.
We fit linear models to characterise the brain encoding of both attended and ignored speech streams. By doing so, we could determine how strongly acoustic and phonetic features are encoded in the listeners' EEG signals, across two different hearing-aid noise reduction schemes.
Results: Cortical signals were shown to reflect phonetic features of both attended and unattended speakers, regardless of the noise-reduction strategy used.
Conclusions: As such, these results indicate that categorical perception of phonetic features contributes to the neural representation of speech.

Perceptual Warping Exposes Categorical Representations For Speech In Human Brainstem Responses

Jared Carter[1], Gavin Bidelman*[2]
[1]University of Nottingham, [2]Indiana University
Background: The brain transforms continuous acoustic events into discrete category representations to downsample the speech signal for our perceptual-cognitive systems. Such phonetic categories are highly malleable and heir percepts can change depending on surrounding stimulus context. Previous work suggests these acoustic-phonetic mapping and perceptual warping of speech emerge in the brain no earlier than auditory cortex.
Methods: Here, we examined whether these auditory-category phenomena inherent to speech perception occur even earlier in the human brain, at the level of auditory brainstem. We recorded speech-evoked frequency following responses (FFRs) during a task designed to induce more/less warping of listeners' perceptual categories depending on stimulus presentation order of a speech continuum (random, forward, backward directions). We used a novel clustered stimulus paradigm to rapidly record the high trial counts needed for FFRs concurrent with active behavioral tasks.
Results: We found serial stimulus order caused perceptual shifts (hysteresis) near listeners' category boundary confirming identical speech tokens are perceived differentially depending on stimulus context. Critically, we further show neural FFRs during active (but not passive) listening are enhanced for prototypical vs. category-ambiguous tokens and are biased in the direction of listeners' phonetic label even for acoustically-identical speech stimuli.
Conclusions: Our data expose FFRs carry category-level information and suggest top-down processing actively shapes the neural encoding and categorization of speech at subcortical levels. These findings suggest the acoustic-phonetic mapping and perceptual warping in speech perception occur surprisingly early along the auditory neuroaxis, which might aid understanding by reducing ambiguity inherent to the speech signal.

Hierarchical Neural Organization in the Auditory Brain Revealed During Naturalistic Speech Listening by a Dynamic, Spatial Attention Task

Kelsey Mankel*[1], Daniel Comstock[1], Brett Bormann[1], Doron Sagiv[1], Hilary Brody[1], Lee M. Miller[1]
[1]University of California, Davis
Background: Naturalistic, continuous speech stimuli open new windows into how the auditory brain processes complex sounds and may shed light on why some listeners report speech perception difficulties despite otherwise normal hearing. Yet, the nature of ongoing speech makes studying lower-level processes in the brainstem difficult, let alone simultaneous measurement of both brainstem and cortical responses. To understand the role of bottom-up and top-down interactions during real-life speech listening—and how they might break down in noisy environments or with hearing impairments—we need consider auditory function at all levels along the hierarchy.

Methods: We used EEG and "chirped speech" (Cheech), synthetic frequency sweep chirps blended with continuous speech narratives, to rapidly assess auditory system function from brainstem to cortex during our novel, dynamic spatial attention switching task. Normal hearing, young adult listeners tracked short story narratives either in quiet or in the presence of a competing talker separated in virtual space. Participants were evaluated on several aspects of listening performance including narrative comprehension, embedded target word identification, ignoring competing talkers, listening effort, and fatigue. Given evidence that factors such as executive functions or temporal fine structure sensitivity may influence speech-in-noise perception, we also measured cognitive (e.g., working memory, inhibitory control), auditory-perceptual (e.g., speech in noise, temporal fine structure, pitch discrimination), demographic (e.g., noise exposure history, subjective SES), and audiological factors (e.g., pure tone thresholds, tympanometry, otoacoustic emissions) to determine their contributions to individual differences in speech listening performance.
Results: Cheech-evoked brainstem (ABR), early cortical (P1-N1), and later linguistic event-related potential components were extracted from the continuous speech narratives. Preliminary data indicate neural encoding and behavioral performance were both degraded for the dual talker compared to the single talker condition. Neural differences were also observed for the target speaker relative to the distractor voice, but only at the cortical level. The data also revealed sensitivity to individual differences associated with speech listening performance across multiple neurobehavioral metrics on the task.
Conclusions: The Cheech method described here demonstrates promise as a tool to understand speech encoding and processing within the auditory hierarchy. Cheech-evoked neural measures obtained during our dynamic auditory attention task, combined with cognitive, auditory-perceptual, audiological, and self-reported demographic measures, provide a snapshot along multiple levels of speech processing. By examining data across all these levels, we can develop neurobehavioral "profiles" that, in addition to distinguishing good versus poor speech listeners, lays the foundation for better identification and treatment of current—or future—"hidden" hearing difficulties.

Progression of Acoustic, Phonemic, Lexical and Sentential Neural Features Emerge for Different Speech Listening

I.M Dushyanthi Karunathilake*[1], Christian Brodbeck[2], Shohini Bhattasali[1], Philip Resnik[1], Jonathan Z. Simon[3]
[1]University of Maryland - College Park, [2]Department of Psychological Sciences, University of Connecticut, [3]Electrical and Computer Engineering; Biology; Institute for Systems Research, University of Maryland
Background: Understanding speech requires analyzing acoustic waveforms via intermediate abstract representations including, phonemes, words, and ultimately meaning along with other cognitive operations. Recent neurophysiological studies have reported that the brain tracks acoustic and linguistically meaningful units. However, since the speech representation units are usually correlated with each other, and often a small subset of features are analyzed, it is unclear whether these neural tracking accounts for uncaptured variance that has not been modeled, hence, causing the feature responses to be less accurate. Additionally, the way these feature responses are modulated by top-down mechanisms and speech comprehension is not well understood.
Methods: To address these limitations, we recorded magnetoencephalography (MEG) data from 30 healthy, younger participants while they listened to four types of continuous speech-like passages: speech-envelope modulated noise, narrated English-like non-words, word-scrambled narrative, and true narrative. Using multivariate temporal response function (mTRF) analysis, we show that the cortical response time-locks to emergent features from acoustics to linguistic processes at the sentential level as incremental steps in the processing of speech input occur.
Results: Our results show that when the stimulus is unintelligible, the cortical response time-locks only to acoustic features, whereas for intelligible speech, the cortical response time-locks to both acoustic and linguistics features. For the case of narrated non-words, phoneme-based lexical uncertainty generates less activation than for true words, suggesting a lack of predictive coding error. Temporal analysis shows that the non-word onsets do generate smaller early responses than word onsets, but they also generate stronger late responses than word onsets suggesting different neural mechanisms associated with accessing lexico-semantic memory traces. For the scrambled word passages, we find additional responses based on context-independent (unigram) word surprisal, but for true narrative, the responses are additionally driven by context-based word surprisal. The unigram word surprisal responses show strong late peaks for the scrambled word passage, consistent with an N400-like response. The results also show that most language-

dependent time-locked responses are left lateralized, whereas lower-level acoustic feature responses are right lateralized or strongly bi-lateral.
Conclusions: Taken together, our results show that brain responses to certain linguistic units are influenced by the speech content, the level of processing and speech features that could be attributed to evaluate perception and comprehension.

Perception of Artificial-Intelligence (AI) Based Synthesized Speech Under Different Listening Conditions in Younger and Older Adults

Björn Herrmann*[1]
[1]*Rotman Research Institute*

Background: Artificial intelligence (AI) based synthesized speech has become almost human-like, ubiquitous in everyday live (e.g., smart phones, grocery self-checkouts), and relatively easy to generate using modern text-to-speech synthesizers. This opens opportunities to use AI speech in research and clinical areas, such as hearing sciences, audiology, and speech pathology, where recordings of speech materials by voice actors can be time- and cost-intensive and may not be available for different languages or accents. However, much research thus far has focused on technological developments towards more human-like voices evaluated by younger adults. How older adults perceive AI speech under different listening conditions is unclear.
Methods: Using Google's Wavenet text-to-speech synthesizer, the current study explores whether AI speech can be used to investigate common speech-in-noise perception phenomena in younger and older adults, and the degree to which individuals recognize AI speech as such. Speech intelligibility was recorded for human speech and synthesized speech masked by a 4-Hz modulated or an unmodulated multi-talker babble noise.
Results: For both human and AI speech, speech intelligibility was better for the modulated than the unmodulated masker (masking release), and this masking-release benefit was reduced in older adults. Release from masking effects were comparable between human and AI speech, suggesting that modern AI speech could be useful for hearing and speech research. The data further suggest that older adults recognize the presentation of AI speech less frequently, rate AI speech as more natural, and are less able to discriminate between human and AI speech compared to younger adults. Research on speech perception in older adults may thus especially benefit from modern AI-based synthesized speech because, to them, AI speech feels much like spoken by a human.
Conclusions: In sum, the current study highlights that modern AI-based synthesized speech may provide a useful alternative to human-recorded speech materials. Common speech-in-noise perception phenomena are similarly captured by AI and human speech, and especially older adults perceive modern AI speech as being spoken by a human voice. AI speech can easily be generated in different languages and with different accents. The current research thus delivers the foundation towards new research opportunities for basic and applies scientists.

Self-Supervised Redundancy Reduction Results in De-Noised Representations of Target Speech

Sonia Yasmin*[1], Ingrid Johnsrude[1], Jonathan Michaels[1]
[1]*The Brain and Mind Institute, Western University*

Background: Understanding speech in noise can be challenging for people with hearing loss. Hearing aids are the current treatment of choice for hearing loss but even those with advanced features, such as noise reduction and directional microphones, provide limited benefit in noise. New approaches to auditory signal de-noising may help improve hearing aids. One such approach is based on the concept of redundancy reduction. In sensory processing, it is efficient for a system to extract meaningful structures in signals and code them independently, reducing redundancy. We evaluate the feasibility of redundancy reduction in auditory signal de-noising by exploiting an innovative self-supervised learning method known as Barlow Twins (Zbontar et al., 2021), previously used for image de-noising
Methods: We develop a novel computational model which takes a cochlear model (Lyon, 1982) of degraded audio as an input and outputs a lower-dimensional representation that reduces the influence of noise. Our model uses the Barlow Twins loss function in which paired distortions of the same core signal (i.e. two different maskers on the same signal) are represented by the network and then compared against one another in an embedding space to maximize similarity between the embeddings while reducing redundancy between

the components of the embedding. This model allows us to implement auditory de-noising in a biologically meaningful way. Using 1000 samples of naturalistic audio taken from the Moth Podcast, which we distorted using multi-talker babble at a broad range of SNRs, we trained our model to represent degraded speech signals as a low redundancy embeddings.
Results: The same speech signals that underwent different degradations were highly correlated, reflecting similar information content between distorted versions of the same signal. The learned parameters from the training phase were successfully used to produce similar redundancy-free embeddings for 460 novel samples of distorted speech, demonstrating generalizability of our model to un-seen data.
Conclusions: Our work tests a method for auditory de-noising based on a new redundancy reduction approach, Barlow Twins, used in computer vision. Rapid auditory signal de-noising may help to meet the every-day needs of hearing-impaired individuals. We are currently reconstructing de-noised auditory signals using their redundancy-free representations, and using these signals to evaluate neural tracking of the attended acoustic signal.

Symposium #15 - Multiomics Approaches to Further the Understanding of Hearing Loss and Tinnitus: Integrating different omics data types to identify biomarkers, unravel causal pathways, and reveal treatment targets

8:00 a.m. - 10:00 a.m.
Crystal D-E

Multiomics Approaches to Further the Understanding of Hearing Loss and Tinnitus: Integrating Different Omics Data Types to Identify Biomarkers, Unravel Causal Pathways, and Reveal Treatment Targets

Chair: Sharon Curhan, *Brigham and Women's Hospital/Harvard Medical School*

High-throughput technologies that enable analyses of whole genomes, transcriptomes, proteomes, metabolomes and metagenomes have revolutionized investigative opportunities for research in otolaryngology. The “omics” suffix transforms a molecular term to describe a comprehensive global assessment of a set of molecules. Advances in technology enabling cost-efficient, high-throughput analysis of biologic molecules are driving exciting growth in omics research. Individually, each type of omics data can reveal differences associated with disease that are useful as biomarkers of disease processes and provide insight on biological pathways or processes that may differ among those with and without disease. Expanding on associations identified in single omics analyses, the integration of different omics data types can then be used to elucidate potential causative changes that lead to disease and identify treatment targets that can be tested in further molecular studies. Integrating multi-omics data can help us connect genotype to phenotype, lead to discovery of shared or distinct mechanisms between diseases, and can inform development of personalized treatments. Multiomics profiling enables a comprehensive landscape view of molecular changes contributing to cellular responses and normal physiology, augments biologic understanding of disease onset and progression, and facilitates drug discovery. The 2021 NIH “Multiomics in Health and Disease” strategic planning workshop prioritized research that extends genomics beyond the DNA sequence and synthesizes these multiomics data with clinical data. Ultimately, the goals in the clinical setting are to integrate these multiomics data with electronic health records and clinical decision support tools, and to extend the use of biomarker testing beyond diagnosis and treatment to include comprehensive health and wellness. This symposium will present an overview of several omics data types and approaches, provide examples of their implementation in otolaryngology research, and propose how integration of multi-omics data in large-scale studies can be a powerful and promising new approach to understand ear and hearing health and disease.

Metabolomics of Hearing Loss and Tinnitus
Oana Zeleznik, *Brigham and Women's Hospital, Harvard Medical School*

The development of omics tools has shifted metabolic research from a reductionist approach focused on specific metabolic pathways to a discovery-based approach that provides an unbiased and comprehensive view of an individual’s metabolic status. The metabolome is defined as the collection of all small molecule

metabolites (e.g., organic acids, amino acids, nucleotides, sugars, acylcarnitines and lipids) in a biological sample (e.g., plasma, urine, saliva and tissues). Currently, multiple metabolomics approaches and technologies are used to measure the metabolome. Targeted approaches measure a distinct set of previously characterized metabolites while untargeted approaches measure all metabolites in a sample, including known and unknown metabolites. Nuclear magnetic resonance-based technologies are suitable for detection of a smaller number of metabolites with M-μM concentrations while mass-spectrometry-based technologies are superior for the detection of a large number of known and unknown metabolites with lower concentrations (up to fM). Metabolomic profiles can be used to characterize healthy tissue, to study tissue metabolism and disease pathology, and to identify biomarkers and risk factors for disease. Metabolomics also offers a valuable approach for drug discovery and evaluation of treatment efficacy, side effects and toxicity. Metabolite levels are 'downstream' of transcriptional and translational processes and reflect direct input from the genotype, diet, environment and microbiome. Thus, the systematic analysis of metabolites can uncover valuable insights into complex otolaryngologic conditions, including hearing loss, tinnitus, vestibular disorders, sinusitis, and head and neck cancers. In this presentation, we will provide an overview of methods for investigating the human metabolome and illustrate their implementation in recent metabolomics investigations of hearing loss and of tinnitus. For example, using examples from recent investigations of plasma metabolomic profiles among individuals with persistent tinnitus, I will describe the use of metabolomics to better understand the diverse etiologies that underlie tinnitus, help identify subtypes and reveal factors associated with tinnitus susceptibility, I will show how the metabolome is uniquely suited to evaluate hearing loss and tinnitus, measure the impact of environmental factors, and how metabolomic assays can be powerful tools to identify biomarkers and pathoetiologic processes.

Genomics of Hearing Loss and Tinnitus

Renato Polimanti, *Yale University School of Medicine Department of Psychiatry*

Hearing loss and tinnitus are complex disorders due to the interplay of multiple genetic and environmental factors. Indeed, although rare monogenic forms of hearing loss (i.e., caused by mutations in a single gene) are present, most hearing loss cases are linked to the cumulative effect of many common genetic variants with small individual effects (i.e., polygenic risk) that act together with several environmental risk factors (such as noise exposure, tobacco smoking, drugs). Large-scale genomic studies based on hundreds of thousands of participants recruited by many biobanks around the world are permitting investigators to study the molecular basis of human diseases and traits at an unprecedented depth. In recent years, genome-wide association studies of hearing loss and tinnitus identified dozens of risk loci involved in the pathogenesis of these disorders, also showing that there is a large correlation between their genetic risk. This strongly supports that the pathogenesis of these disorders is partially shared. The present talk will provide an overview of the most recent advancement in our understanding of the genomics of hearing loss. This will include the discovery of risk loci mapped in genes involved in biological pathways that could be targeted for the development of novel drugs or repurposing of existing molecular compounds. Another important aspect is related to the characterization of the polygenic risk of hearing loss and tinnitus across diverse ancestral backgrounds to stratify disease risk and prioritize possible interventions for individuals at high risk. The effect of genetic variants on hearing loss and tinnitus has also been used to conduct genetically-informative causal inference analyses to distinguish possible causes and consequences among comorbid traits and diseases (e.g., depression, immunological conditions, and neurologic disorders). Beyond genetic associations, analyses of the transcriptomic regulation of specific tissues and cells in the context of their effect on hearing loss and tinnitus showed that several genes previously implicated in HL pathogenesis because of their potential involvement in the peripheral structures of the auditory system may also be involved in the pathogenesis of these disorders through transcriptomic changes in multiple brain regions. In summary, the attendees will learn about how the application of Big Data analytics to genomic information is leading our field towards novel insights into the complex pathogenesis of hearing loss and tinnitus. However, they will also learn about the challenges that investigators still need to address to make sure the translation of genomic findings will lead to reliable healthcare tools.

Investigating the Microbiome to Study Hearing Loss and Tinnitus

Jacqueline Starr, *Brigham and Women's Hospital, Harvard Medical School*

In the human-microbe superorganism, the number of genes in a person's microbiome has been estimated at 200 times the number of genes in the human genome. Humans depend on bacterial activity for the normal development of immunity and digestion. In all resident body sites—the gut, oral cavity, skin, nares, and ear—microbes mediate our responses to diet, medications, and other exogenous exposures. Emerging evidence supports a gut microbial contribution to neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease. Molecules derived from the gut microbiome, such as amyloid-like peptides and short-chain fatty acids, influence neuroinflammation and blood-brain barrier permeability modulation in animal models, and these molecules may act as neurotransmitters. Rigorously proven examples are accumulating of microbial mediation of pharmacologic efficacy or toxicity. It is highly plausible that microbial activity could mediate the otoxicity of medications or other chemical exposures. Yet almost nothing is known about microbial effects on hearing health or auditory dysfunction. Such information could point to novel approaches to design targeted interventions for prevention and/or treatment of hearing loss and tinnitus. We will provide an overview of methods for studying the human microbiome as an etiologic agent. We will describe established techniques and some current challenges. We will illustrate with examples relevant to the study of hearing loss and tinnitus.

Using Omics to Uncover Mechanisms at the Bench: A Comparative Transcriptomic Analysis of Human Vestibular Schwannomas From Archival FFPE Tissue Reveals Inflammatory Pathways Involved in Tinnitus

Konstantina Stankovic, *Department of Otolaryngology Head and Neck Surgery, Stanford School of Medicine*

Similar to the way genomics expanded the focus of genetics from individual variants and genes to encompass large-scale interrogations of the entire genome, other omics approaches have rapidly been developed to investigate entire pools of transcripts, proteins, metabolites, microbiota, and exposures. These multiple omics data types afford a powerful discovery opportunity to better understand disease pathogenesis, uncover new treatment targets and inform molecular studies. For example, transcriptome profiling is an increasingly used approach to investigate human diseases at the molecular level. Transcriptomics examines RNA levels across the genome, identifies the presence of specific transcripts, novel splice sites and RNA editing sites, and quantifies the expression of transcripts. Numerous expression studies have identified molecular biomarkers and therapeutic targets for a variety of pathologies. In this presentation, I will discuss how evaluating transcriptome profiles can be a powerful approach to gain insights into the molecular mechanisms underlying complex auditory disorders and illustrate with a specific example of an investigation of tinnitus. Although the etiology of tinnitus is heterogenous, tinnitus is present in over 70% of patients with a vestibular schwannoma (VS). VSs are histologically benign intracranial tumors that arise from Schwann cells of the vestibular nerve, and typically lead to hearing loss, tinnitus and dizziness. To gain molecular insight into VS-associated tinnitus, we performed next generation sequencing on RNA extracted from archival formalin-fixed paraffin-embedded (FFPE) VS tissue from patients with and without tinnitus. A comprehensive bioinformatic analysis revealed inflammatory pathways, such as the allograft rejection pathway, as differentially expressed. Our study demonstrates the feasibility of next-generation sequencing from archival FFPE VS tissue and identifies novel molecular players in VS-associated tinnitus. Further, future studies that integrate transcriptomics with other omic strategies could provide a more comprehensive understanding of alterations related to tinnitus and ultimately inform clinical testing and the development of tailored therapeutics.

Podium #16 - New Structural and Functional Perspectives of the Auditory Brainstem

10:30 a.m. - 12:30 p.m.
Ocean's Ballroom 5-12

Moderators: Philip Smith and Ruili Xie

Circuitry of Unipolar Brush Cells in the Dorsal Cochlear Nucleus

Christian Candler[1], Tim Balmer*[1]
[1]*Arizona State University, School of Life Sciences*

Background: The dorsal cochlear nucleus (DCN) receives acoustic signals from the auditory nerve, as well as multisensory (non-auditory) signals from various sources, to perform complex functions such as

canceling self-generated sounds and localizing sounds relative to the head and body. Understanding how this cerebellum-like circuit processes information requires knowledge about how the different cell types in DCN are connected and how their synapses transform signals. Granule cells receive multisensory signals and project parallel fiber axons to fusiform cells, the principal output neurons of the DCN. Unipolar brush cells (UBCs) also receive multisensory input and are presumed to project to granule cells and other UBCs. However, their location in the deep layer of DCN, relatively distant from the granule cell domains, suggests the possibility that they target additional cell types.
Methods: Whole-cell patch-clamp recordings of fusiform cells and cartwheel cells were made in acute brain slices from mice. UBCs that expressed channelrhodopsin-2 (ChR2) were optogenetically stimulated to investigate their connectivity and synaptic effects.
Results: Our data shows that UBCs may synapse directly onto fusiform cells and cartwheel cells. Stimulation of UBCs evoked EPSCs in fusiform and cartwheel cells with short latencies, in addition to the expected longer-latency polysynaptic EPSCs. These short latencies were similar to known monosynaptic EPSC latencies recorded in granule cells, the major target of UBCs, via the same UBC optogenetic stimulation. Confocal imaging of biocytin-filled cells revealed potential anatomically defined synaptic contacts between UBC axons and fusiform cells.
Conclusions: Multimodal signals that are conveyed to UBCs may have a more direct influence on DCN excitability than previously appreciated. Future studies will examine how UBCs contribute to auditory responses in DCN neurons.

Identification of Three Different Subtypes of Auditory Nerve Synapses in the Cochlear Nucleus Using Pou4f1-TdTomato Mouse

Shengyin Lin[1], Meijian Wang[1], Chuangeng Zhang[1], Ruili Xie*[1]
[1]*The Ohio State University*

Background: Sound information is encoded by different subtypes of spiral ganglion neurons (SGNs) and transmitted to cochlear nucleus (CN) neurons via auditory nerve (AN) synapses. Despite decades of research, it remains a challenge to investigate the morphology and physiology of different subtypes of AN synapses. Recent studies using the single-cell RNAseq technique identified selective molecular markers that label different subtypes of type I SGNs in the cochlea. Of particular interest, calretinin is selectively expressed in type Ia SGNs corresponding to high spontaneous rate/low threshold neurons, whereas Pou4f1 is selectively expressed in type Ic SGNs corresponding to low spontaneous rate and high threshold neurons. Our preliminary study using immunohistochemistry showed no Pou4f1-labeling in the CN, suggesting that this transcription factor does not localize in AN fibers and synapses, and therefore cannot be used as a marker in the CN to selectively identify different AN synapses. To solve this problem, we developed a new approach using Pou4f1-tdTomato mice to identify and characterize all three subtypes of AN synapses in the CN.
Methods: We crossed the Pou4f1-cre mouse with a tdTomato reporter line and generated the Pou4f1-tdTomato mice. To characterize tdTomato expression in SGNs, cochlea was cryo-sectioned and immunostained using antibodies against calretinin (type Ia marker), calbindin (type Ib marker), and TuJ-1 (type I SGN marker). Acute brain slices containing the CN were also prepared for whole-cell recording to characterize electrophysiological properties of synaptic transmission, and post hoc staining against calretinin and VGluT1 to identify different AN synapses.
Results: Immunohistological experiments in the cochlea confirmed that tdTomato is primarily expressed in a subset of type I SGNs without calretinin- (type Ia) or calbindin- (type Ib) expression, except a small population of SGNs that co-express multiple markers. In the CN, tdTomato also labeled a subset of AN fibers and AN synapses. Based on the expression patterns of tdTomato and calretinin, we identify three subtypes of AN synapses in the CN including (1) calretinin+tdTomato-; (2) calretinin-tdTomato-; and (3) calretinin-tdTomato+ synapses that presumably correspond to type Ia, Ib, and Ic SGNs. We further showed that type Ic AN synapses with tdTomato expression showed lower synaptic efficacy during high-rate synaptic transmission than calretinin-expressing type Ia synapses.
Conclusions: The results showed that Pou4f1-tdTomato mice selectively label a subgroup of SGNs in the cochlea and can be used to identify three subtypes of AN synapses in the CN. We conclude that this strain is a valid model to study the morphology and function of different subtypes of AN synapses.

In Vivo Single-Cell Recordings From Octopus Cells in Mouse Cochlear Nucleus

Hsin-Wei Lu*[1], Philip Smith[2], Philip Joris[1]
[1]KU Leuven, [2]University of Wisconsin
Background: Research in the mouse auditory brainstem has gained attention thanks to the wide availability of genetic tools and extensive in vitro data. However, there is a paucity of in vivo single-cell physiological data. In all mammals studied, the spike output of octopus cells in the posteroventral cochlear nucleus (PVCN) is remarkably transformed relative to their auditory nerve (AN) excitatory inputs. Genetic manipulation in mice of these cells and their inputs, combined with in vitro and in vivo physiology, holds the promise of gaining exquisite mechanistic insight into this transformation. Our aim is to first obtain in vivo spike and intracellular recordings from octopus cells and their inputs from the AN in unmanipulated mice.
Methods: Experiments were performed on anesthetized adult (~3 months) C57BL/6 mice. Recordings were made via sharp and whole-cell patch pipettes. Biocytin was included in the pipette to label recorded cells. Stimuli include pure tones, sinusoidally amplitude modulated (SAM) tones, click trains and harmonic complex tones with Schroeder phases varying from curvature (C) -1 to 1 with fundamental frequency (F0) at 100 Hz.
Results: Both extracellular and intracellular recordings were obtained from PVCN neurons that fit the classical description of octopus cells, i.e. they showed pure onset (Oi) response to short tone pips at their characteristic frequency (CF), and exhibited small spikes (< 15 mV) when recorded intracellularly. Two cells were labeled in the octopus cell area, and the axon of one cell could be traced projecting through the intermediate acoustic stria. Like in other species, spike responses from mouse octopus cells entrained to click trains up to at least 300 Hz (max 500 Hz) with jitter <= 100 µs. All cells lacked spontaneous spiking and were tuned to high frequencies (80% had CF > 10 kHz) with high thresholds (median: 62 dB SPL). In some cells, thresholds to SAM tones were ~5-10 dB lower than those to pure tones. Unlike other species, spiking showed strong adaptation during SAM stimuli and thus no entrainment despite strong phase-locking (vector strength ~ 0.9 – 0.95 at Fmod 300 Hz). Like gerbil octopus cells, mouse octopus cells showed Schroeder phase sensitivity, with some tuned to positive C and some negative, and could fire entrained spikes to F0 (at the preferred phase) even when temporal delay across frequencies spanned 5 - 10 milliseconds.
Conclusions: We have obtained, to the best of our knowledge, the first in vivo single cell recordings from mouse octopus cells. Their physiology is largely in line with that described in other species but differs in some respects. Consistent with our observations in gerbils, mouse octopus cells tolerate large temporal dispersions across frequencies, arguing against the hypothesis that they are broadband coincidence detectors.

Microstructural Changes in the Brainstem Regions of the Auditory Pathway Among Children With Hearing Loss

Peter Moon*[1], Kristina Ward[2], Taseer Din[2], Sara Saki[2], Alan Cheng[2], Kristen Yeom[3], Iram Ahmad[2]
[1]Stanford University School of Medicine, [2]Stanford University Department of Otolaryngology-Head and Neck Surgery, [3]Stanford University Department of Radiology
Background: Sensorineural hearing loss (SNHL) is the most common congenital sensory deficit in children. Diffusion MRI is used to identify microstructural changes in the brain, and can serve as an important tool to gain insight into neural development and potentially guide therapies that optimize language development. While previous studies have characterized the thalamo-cortical tracts of the auditory pathway in pediatric patients with hearing loss, no study has assessed brainstem regions of this pathway. The goal of our study was to investigate MRI-based microstructural changes along the brainstem regions of the auditory pathway in pediatric patients with SNHL.
Methods: We retrospectively reviewed cohort of pediatric patients with SNHL who obtained MRI at 3T between 2011 and 2019. We identified 16 pediatric patients (age <18 years) with at least moderate asymmetric/bilateral SNHL, and gender-matched controls without neurological, developmental, or MRI-based brain macrostructural abnormalities. Diffusion tensor imaging (DTI) was used to evaluate microstructural alterations. The following brainstem regions and tracts of the auditory pathway were assessed: superior olivary nucleus (SON), inferior colliculus (IC), ipsilateral tracts between the inferior colliculus and superior olivary nucleus (IC-SON). Diffusion values for bilateral regions and tracts were generated, then averaged to calculate a mean value for fractional anisotropy (FA) and mean diffusivity (MD) for each subject.

Results: We identified significant differences in FA values of the SON between the SNHL cohort and controls (0.377±0.056 vs 0.422±0.052; p=0.009). No other FA or MD values were significantly different between the two groups. Among younger children (≤5 years), MD was significantly decreased in the SNHL cohort compared to controls in the IC (0.918±0.051 vs 1.120±0.142; p<0.001). However among older children (>5 years), there were no significant differences in MD (1.124±0.198 vs 0.997±0.103; p = 0.119). There were no significant differences in MD or FA in the white matter fibers of the IC-SON tract.
Conclusions: This study is the first to assess microstructural changes in brainstem auditory pathway regions among children with SNHL. Longitudinal studies are warranted to assess the predictive value of these MRI-based findings for long-term outcomes and the efficacy of intervention.

Modulation of Voltage-Gated Calcium Channels Following Noise Exposure: Impact of Synaptic Activity in the Auditory Pathway

Selin Yalcinoglu*[1], Rod Braun[1], Amaar Wattoo[1], Avril Genene Holt[1]
[1]*Wayne State University School of Medicine*

Background: A temporary noise-induced hearing loss (TTS) can result in sustained changes in spontaneous neuronal activity in auditory related nuclei. Voltage-gated calcium channels (CaVs) function to moderate neuronal activity and are distributed throughout the auditory pathway. We have begun to examine their role in central auditory nuclei plasticity following TTS exposure. L-Type calcium channel blockade (verapamil), Auditory Brainstem Response waveforms (ABR), and sensory gating (gap inhibition of Acoustic Startle Reflex-giASR) were used to assess the effect of noise on peripheral and central CaV function.
Methods: Twenty-five male Sprague-Dawley rats were divided into four groups and given either verapamil (30 mg/kg) or saline intraperitoneally. The treatment groups were no noise exposure plus saline (n=6) or verapamil (n=5) and noise exposure plus saline (n=7) or verapamil (n=7). The noise groups were unilaterally exposed to a 16 kHz, 106 dB SPL tone for one hour. ABR and giASR were performed at same, one and five day[s] after treatment at 12 and 20 kHz with the addition of 4, 8, 16, 24 kHz for giASR. ABR amplitudes (wave I and V) and thresholds were evaluated and giASRs were measured at 45 and 60 dB SPL. Each group was subjected to a 20 ms startle noise during silence, in the presence of a background tone, and in a background tone with a gap prior to startle.
Results: Verapamil did not affect hearing thresholds in the no noise group but did reduce the time to recover from the noise induced TTS. In the noise group, wave I amplitude decreased compared to controls on the same day of noise exposure (p<0.03 and p<0.009, 12 and 20 kHz, respectively). On the same day of verapamil administration wave V amplitude decreased in the no noise group compared to the control at 12 and 20 kHz (90% p<0.006 and 94% p<0.02, respectively). Before the noise exposure and treatment, all rats could inhibit their startle. After noise exposure, a trend towards enhanced ability to inhibit startle response was observed. However, five days after treatment the noise verapamil group had a reduction in the ability to inhibit startle compared to same and one day after (26.6% p=0.0116 and 29.8% p=0.0051 respectively)
Conclusions: Our results demonstrate that CaVs throughout the auditory pathway impact noise-induced changes. CaVs regulate peripheral and central synaptic function differentially. In the periphery, reduced CaV function prevented prolonged TTS. After the blockade of CaVs resolved, the synaptic activity of central nuclei remained low, suggesting increased susceptibility to changes in function. Changes in sensory gating may be due to alterations in the localization and expression of CaVs in central nuclei such as the inferior colliculus after noise exposure. Future studies should focus on further understanding the relationship between changes in CaVs, and noise-induced central plasticity.

NR2D-Containing NMDA Receptors Enhance Temporal Summation in VIP Neurons in the Inferior Colliculus

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[1]*University of Michigan*, [2]*The University of Michigan, Kresge Hearing Research Institute*

Background: The inferior colliculus (IC) is a hub of integration for ascending auditory information. VIP neurons are glutamatergic stellate neurons found throughout the IC and that project to a range of brain regions including the auditory thalamus and superior colliculus. We previously found that excitatory commissural inputs to VIP neurons can elicit EPSPs that include an NMDA receptor (NMDAR) component at resting membrane potential, however, the mechanism for NMDAR activation at resting potential and the role of these receptors in synaptic processing remained unclear. We hypothesized that VIP neurons express NMDARs containing NR2C/D subunits, which are less susceptible to Mg2+ block than the more common

NR2A/B-containing receptors, thus allowing them to activate at rest. In addition, we hypothesized that the kinetics of NMDA receptors would facilitate temporal summation in VIP neurons, a process that may enhance the transformation of temporal codes to rate codes in the IC.
Methods: To test these hypotheses, we targeted whole-cell patch-clamp recordings to VIP neurons in VIP-IRES-Cre x Ai14 mice. We first used puffs of glutamate to activate NMDARs on VIP neurons and then applied PPDA, an NR2C/D selective antagonist, and CIQ, an NR2C/D selective positive allosteric modulator, to determine whether the NMDAR component was mediated by receptors containing these subunits. We next used optogenetics to investigate whether commissural inputs activated NMDARs on VIP neurons at resting potential and whether NR2C/D-containing receptors contributed to the temporal summation of EPSPs elicited by trains of optogenetic stimuli. Additionally, we performed single molecule fluorescent in situ hybridization (RNAscope) to determine whether NR2C and/or NR2D mRNA was present in VIP neurons.
Results: We found that puffs of glutamate reliably elicited EPSCs in VIP neurons in the presence of the AMPA receptor antagonist NBQX, indicating NMDAR activation at resting membrane potential. These EPSCs were sensitive to NR2C/D-specific pharmacology. We next used a Mg2+-free ACSF to allow activation of all NMDARs on VIP neurons and found that PPDA almost completely blocked the NMDAR-elicited EPSC in many VIP neurons, suggesting that most NMDARs on VIP neurons contain an NR2C/D subunit. Our optogenetics experiments show that NR2C/D containing NMDARs on VIP neurons are activated by commissural projections, and activation of these receptors enhances temporal summation of trains of commissural inputs. Lastly, our RNAscope results show that 92% of VIP neurons express NR2D mRNA while only 8% express NR2C mRNA.
Conclusions: Our study demonstrates that VIP neurons express NMDARs containing NR2D subunits, which allow NMDARs to activate at resting membrane potential. Due to the slow kinetics of NMDARs, this mechanism expands the time window for synaptic integration in VIP neurons, suggesting that VIP neurons may play a computational role that involves integrating auditory information over periods of tens of milliseconds.

The Influence of Inhibition on Coincidence Detection in Octopus Cells of the Mouse Cochlear Nucleus

Lauren Kreeger*[1], Lisa Goodrich[2]
[1]Harvard Medical School, [2]Harvard University
Background: Octopus cells (OCs) of the mouse cochlear nuclues receive excitatory inputs from ~60 auditory nerve fibers (ANFs) representing 1/3 of the tonotopic axis. Temporally precise ANF activation is required for OCs to fire, suggesting OCs detect coincident arrival of broad stimuli. Little is known about the distribution of ANF synapses on OC dendrites, especially when considering the genetic diversity of ANFs. Additionally, while immunohistological stains have revealed inhibitory synapses on OCs, there has been no physiological evidence of functional inhibitory inputs. By combining anatomical, physiological, and genetic approaches, we are investigating how the integration of excitation and inhibition contributes to coincidence detection in OCs.
Methods: We used Foxg1Cre, Ntng1Cre, and Myo15iCre mice to target ANFs. We also used Glyt2Cre and VgatCre mice to target inhibitory inputs. Putative synaptophysin-positive synaptic puncta from cre-expressing populations were labeled with the Ai34D line and OCs were sparsely labeled with a Thy1-YFP-H line. We made 100µm parasagittal sections in young adult (P28-35) mice and made reconstructions of synapses on OCs. We also targeted OCs for in vitro whole-cell current clamp recordings while electrically and optically activating synaptic inputs (P35-50 mice, 35°C). To analyze post-synaptic potentials (PSPs) mediated by specific inputs, we used an Ai32 (ChR2-EYFP) line, which enabled optical activation of pre-synaptic inputs during recordings from OCs.
Results: We found that ANFs densely innervate proximal OC dendrites (0.27 synapses/µm2) and make fewer synapses at distal dendrites (0.04 synapses/µm2). Additionally, OC inputs are dominated by type Ia ANFs (~64% of all ANF synapses), with very few inputs from type Ic ANFs (~9% of all ANF synapses). GABAergic innervation to OCs is sparse however there is unexpectedly dense glycinergic innervation of OC dendrites (~25% of all synapses). Glycinergic innervation is evenly distributed along OC dendrites (0.04 synapses/µm2); we find very few glycinergic synapses on OC somas (<0.01 synapses/µm2) compared to ANF synapses (0.19 synapses/µm2).

Optically-evoked inputs from different ANF subpopulations had similar temporal properties. Additionally, we found that optical activation of only type Ib and Ic ANFs is sufficient to produce spikes in OCs, even though their synapses account for 36% of ANF inputs. We measured spontaneous IPSPs in adult OCs and demonstrated that optical-activation of GlyT2+ inputs evokes IPSPs that are blocked by strychnine. Ongoing experiments are investigating how optically-evoked inhibition impacts temporal summation of electrically-evoked ANF inputs. Preliminary data suggest that both the hyperpolarizing and shunting components of glycinergic inhibition play a role in OC function.
Conclusions: We are working towards a single-cell model of the ANF-OC circuit. This model includes anatomical and physiological data and accounts for molecularly distinct ANF populations and glycinergic inhibition. New evidence for the role of inhibition in OC coincidence detection suggests there could be separate domains between OC somas and dendrites.

The Projections of the Medial Superior Olive of the Rat

Héctor Rincón*[1], Mario Gómez[1], Marcelo Gómez-Álvarez[1], Enrique Saldana[1]
[1]*University of Salamanca*
Background: Many animals use the difference in the time of arrival of the sound at the two ears (interaural time difference [ITD]) as a useful cue to locate low frequency sound sources in the horizontal plane. ITDs are encoded in the medial superior olive (MSO), one of the main nuclei of the superior olivary complex. Compared to mammals that hear low-frequency sounds, such as the cat, the guinea pig and the gerbil, the MSO of rats and mice has been seldom studied, for two main reasons. Rats do not use ITDs to localize sounds (Wesoleck et al, 2010. Hearing Research 265:54–62). Moreover, the purported small size of the MSO in these species has acted as a powerful deterrent to experimental approaches. This situation is paradoxical, as rats and mice are used extensively in auditory research, and the physiology of the mouse MSO shares many of the features of the MSO of mammals that do hear low-frequency sounds (Fischl et al., 2016. Journal of Neurophysiology 116:2676–2688).
Methods: We used tract-tracing techniques to investigate the projections of the MSO of albino rats. We injected unilaterally into this nucleus the bidirectional tracer biotinylated dextran amine (BDA) and analyzed the trajectory, morphology, and distribution of the labeled axons.
Results: Our results are based on eleven successful cases with satisfactory anterograde labeling and whose injection site was centered in the MSO and minimally affected neighboring nuclei. As expected, spherical bushy cells were retrogradely labeled in the ventral, low-frequency region of the anteroventral cochlear nucleus on both sides, demonstrating that our injections targeted the MSO.
Anterogradely labeled axons formed a very dense terminal field in the dorsolateral, low-frequency region of the central nucleus of the ipsilateral inferior colliculus, along the border with the external cortex. Dense terminal fields were also found in the central, low-frequency region of the ipsilateral dorsal nucleus of the lateral lemniscus. Less dense terminal fields were found in the ipsilateral intermediate nucleus of the lateral lemniscus and the ipsilateral medial geniculate body of the thalamus. Many of the axons labeled in the thalamus had large en passant and terminal varicosities,
considerable larger than those found in the other targets of the MSO.
Conclusions: Our results demonstrate that the projections of the rat MSO are similar to those described in species that use ITDs to localize low-frequency sounds. This conclusion raises and interesting question: If rats do not use IDTs, what is the function of their MSO?

Podium #17 - Aging: Animal Models

10:30 a.m. - 12:30 p.m.
Oceans Ballroom 1-4

Moderators: Rick Friedman and Shinichi Someya

ACOU082: A Unique, Systemically Administered Kv7.4 Activator Drug Candidate for the Treatment and Prevention of Age-Related Hearing Loss

Mathieu Petremann[1], Barbara Peixoto Pinheiro[1], Youssef Adel[1], Marcus Müller[1], Michael Bös[2], Hubert Löwenheim[1], Jonas Dyhrfjeld-Johnsen*[2]
[1]*Translational Hearing Research, Department of Otolaryngology, Head and Neck Surgery, University of Tübingen, Tübingen, Germany,* [2]*Acousia Therapeutics*

Background: Growing aging populations are a major driver behind the increasing prevalence of hearing loss (WHO, 2021). Age-related hearing loss (AHRL) is most clearly predicted by degree of outer hair cell loss (Wu et al., 2020), which is also strongly associated with reduced speech-in-noise comprehension (Parker, 2020). We recently published the first data demonstrating the capacity of a clinical stage, locally delivered Kv7.4 activator (ACOU085) to reduce ARHL and outer hair cell (OHC) loss in the SAMP8 mouse model of accelerated senescence (Pinheiro et al., 2022). Here we report that the systemically delivered Kv7.4 drug candidate ACOU082 has the potential to not only significantly reduce age-related hearing loss progression, but also to enhance hearing function relative to baseline after short term treatment.
Methods: Following baseline auditory brainstem response (ABR, 2-45.2 kHz) recordings at 45 days of age, groups of n=10 SAMP8 mice were treated bi-weekly with ACOU082 or vehicle for 5 months using topical application with follow-up ABR recordings performed monthly. After final ABR recordings with 5 months of treatment (age of 195 days), mice were sacrificed for subsequent histological analysis of the organ of Corti. In the ACOU082 treated group all mice (n=10) completed the entire study protocol, in the vehicle treated control group n=9 completed all ABR recordings, while only n=7 organs of Corti could be exploited for histology.
Results: Comparison of ABR threshold shifts relative to individual baselines between ACOU082 treated (n=10) and vehicle treated control (n=9) animals revealed statistically significantly lower ABR threshold shifts for the ACOU082 treatment group at all monthly assessments (2-way ANOVA, $p \leq 0.001$-0.018). Group differences of ABR threshold shift means ranged from 1.2 dB SPL (2 kHz) to 20.1 dB SPL (45.2 kHz) after 5 months of treatment, with largest differences at individual frequencies (17.2-20.1 dB SPL, 8/16/45.2 kHz) also reaching statistical significance in the post-hoc Holm-Sidak test. Cochleogram analysis revealed a statistically significant reduction of OHC loss in ACOU082 treated cochleae (overall average improvement ~4%, range 0.7-12.2%) within the frequency region of 4-45.2 kHz identified by responder analysis. Uniquely, ACOU082 treatment improved ABR thresholds at early time-points vs baseline: after 1 month of ACOU082 treatment, threshold reductions across frequencies ranged from 3.6 to 12.6 dB SPL, reaching statistical significance at 4, 8 and 32 kHz (2-way RM ANOVA, p=0.004-0.047, Holm-Sidak post-hoc test).
Conclusions: In summary, chronic systemic ACOU082 treatment not only significantly reduces age-related hearing loss progression in SAMP8 mice with a clinically relevant effect size compared to vehicle controls, but also significantly enhances hearing sensitivity after short term treatment, consistent with the Kv7.4 target mechanism of action. This unique dual benefit of both enhancing and preserving hearing supports the high potential of developing ACOU082 as a drug candidate for chronic and progressive hearing loss disorders.

Age-Related Auditory Brainstem Slowing Does Not Affect the Stability of Complex Sound Representations in the Aged Auditory Midbrain

Rüdiger Land*[1], Andrej Kral[1]
[1]*Institute of Audioneurotechnology and Department of Experimental Otology, Hannover Medical School, Germany*
Background: The potential impact of central auditory aging on hearing ability in the elderly - independent of peripheral hearing loss - is an important issue in aging societies. Self-reported hearing problems of elderly with ‘clinically normal’ peripheral hearing are often explained with age-related changes of the central auditory pathway. However, ‘pure’ central presbycusis remains difficult to demonstrate due to co-occurring ‘hidden’ cochlear or cognitive decline. For true ‘central’ presbycusis to cause perception deficits - independent of other factors - it needs to be shown that central aging effectively degrades complex sound representations at some point along the auditory pathway on the neuronal level.
Methods: Inaccessible to psychophysical methods, the underlying age-related neural changes remain poorly differentiated in terms of effect size and the level of the auditory pathway. Here we studied, how small age-related changes of simple parameters such as gap detection threshold and ABR wave latencies affect complex sound representations along the central auditory pathway. Specifically, we measured the effect of age-related changes in the auditory brainstem on the stability of complex spatiotemporal multiunit ‘speech-like’ sound representations in the auditory midbrain of aged normal hearing CBA/J mice.
Results: We found that, although brainstem conduction speed slowed down with age, the change was limited to the sub-millisecond range and only minimally affected temporal processing (i.e. gaps-in-noise sensitivity) in the midbrain of old mice. Importantly, besides a small delay, multiunit complex temporal sound representations in the auditory midbrain did not differ between young and old mice. This indicates

that although small age-related neural effects in simple sound parameters in the brainstem may be present in aging in normal hearing mice they do not effectively deteriorate complex neural population representations at the level of the auditory midbrain.
Conclusions: The result challenges the widespread belief of 'pure' central auditory processing deficits as an automatic consequence of aging, at least up the the level of the auditory midbrain. However, this finding emphasizes the potential role of undetected 'hidden' peripheral damage and accumulating effects toward higher cortical auditory-cognitive processing to explain perception deficits in 'normal' hearing elderly.

Age-Related Loss of Olivocochlear Efferent Innervation in Gerbils

Friederike Steenken[1], Christine Koeppl*[2]
[1]Carl von Ossietzky University Oldenburg, [2]Carl Von Ossietzky University
Background: Efferent neurons send feedback innervation from the brainstem to outer hair cells (medial olivocochlear efferents, MOC) and to auditory-nerve afferents beneath inner hair cells (lateral olivocochlear efferents, LOC).
Quantification of LOC innervation in young and old cochleae revealed mixed results. In aged CBA/CaJ mice, overall losses of LOC terminals that spared mid-frequency cochlear locations were observed (Grierson et al, 2022). However, changes at the level of individual surviving IHC appeared restricted to basal cochlear regions (Kobrina et al., 2020; Grierson et al, 2022). In humans, there was no clear evidence for any age-related decline of LOC innervation density (Liberman and Liberman, 2019). In contrast, age-related loss of MOC innervation density was more consistently apparent both in humans and mice, and also at the level of individual surviving OHC.
Here we both quantified the basic efferent innervation pattern along the cochlear tonotopic gradient in young-adult gerbils and explored changes with ageing.
Methods: Bullae from pentobarbital-euthanised young-adult (3-8 months) gerbils were rapidly extracted and fixed with 4% PFA in PBS. After decalcification, cochleae were immunolabelled with primary antibodies against MyoVIIa, labelling hair cells, synaptotagmin, labelling all efferent terminals, and NKAa3, labelling both type1 afferent and MOC efferent fibres and terminals. Finally, cochleae were microdissected and confocal stacks obtained at seven locations, corresponding to 0.5, 1, 2, 4, 8, 16, and 32 kHz (Müller, 1996).
Quantification of MOC and LOC innervation density followed Liberman and Liberman (2019) as the synaptotagmin-labelled area from 2D maximum-intensity z-projections, divided by the number of HC in the stack (11-13 IHC or 43-45 OHC).
Results: In 5 young-adult (3-8 months) cochleae analysed, the innervation area of MOC fibres (OHC region) peaked broadly between 2 and 16 kHz, while LOC contact areas in the IHC region showed little variation across tonotopic locations.
A further 4 old (>=36 months) cochleae indicated a significant reduction of efferent contact area, for both MOC ($F(1,21) = 5.826$, $p = 0.023$) and LOC ($F(1,40) = 23.604$, $p < 0.0005$), with an overall loss of innervation area of 24% and 20% per surviving HC, respectively.
Conclusions: Both MOC and LOC showed reduced innervation areas in old gerbils, at the level of individual HC. The loss was less pronounced than the reduction in brainstem efferent neurone numbers (Radtke-Schuller et al., 2015).
Evidence from mice suggested that during aging, LOC efferent fibres also form functional axosomatic synapses on IHCs (Lauer et al., 2012; Zachary and Fuchs, 2015; Jeng et al., 2021), something not typical for normal-hearing young adults, implying a significant change of efferent function. To estimate whether such a shift happens in gerbils, we aim in future to quantify the labels and their co-localization in 3D.
Funded by the DFG, EXC 2177

Auditory Temporal Processing in Aging Male and Female C57BL/6N Mice

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Background: Increasing age can lead to numerous challenges for the auditory system, such as problems with speech intelligibility. Altered temporal processing is one means by which such issues can arise. To assess neural synchrony and temporal processing in mice, we have measured Inter-Trial Coherence (ITC) and Amplitude-Modulation Following Responses (AMFRs) in male and female C57BL/6N mice

homozygous for the Cdh23ahl allele at increasing ages (1, 2, 6 and 9 months) to assess sex- and age-differences.
Methods: ITC stimuli were comprised of 12kHz, 18kHz and 24kHz pure tones (5ms duration, 1ms rise/fall time), presented at 0-90dB SPL. Single sweep responses were recorded and analysed to yield an ITC value (McClaskey et al. 2020), representing the neural synchrony associated with wave 1 of the ABRs. AMFR stimuli were comprised of 12kHz carrier tones presented at 80dB SPL (100ms duration, 5ms rise/fall time), which were 100% sinusoidally amplitude-modulated at rates (modulation frequency, fm) from 100-1750Hz. We calculated AMFR magnitude, phase, group delay and synchrony coefficient (phase locking) as a function of fm (Shaheen et al. 2015). The AMFR magnitude transfer function was generally multi-peaked, and the peak fm near 1100Hz was used to present a series of AMFR stimuli from 0-90dB SPL. From these level series, AMFR magnitude and synchrony coefficient were calculated.
Results: No obvious sex differences were noted across age in ITC measurements. However, age- and frequency-dependent reductions in auditory nerve synchrony were noted in 9 months old mice compared to 1 month old mice. Synchrony in 12kHz responses were comparable, while increasingly pronounced reductions in synchrony were noted for 18kHz and 24kHz responses, respectively, with increasing age. No obvious sex differences were noted in the AMFR vs fm transfer functions recorded at 80dB SPL. However, AMFR magnitudes were reduced in 6-9 months old mice compared to 1 month old mice. When comparing AMFRs across 0-90dB SPL, male mice 1 month old showed enhanced AMFR magnitudes at high SPLs, but this switched to reduced high SPL AMFR magnitudes at 6-9 months old, compared to age-matched females. Across all ages tested, males showed reduced AMFR phase locking across levels from 50-70dB SPL compared to females, but phase-locking become comparable in both sexes at higher levels (80-90dB SPL).
Conclusions: Age-related changes in auditory nerve response synchrony appear to follow the progressive high frequency hearing impairment seen in C57BL/6N mice. However, it seems that even in regions of the cochlea that demonstrate little ABR threshold elevation at 9 months old (12kHz), AMFR temporal processing deficits become apparent with increasing age. These data contribute to the growing knowledge of aging related changes in the mammalian auditory system.

Characterization and Phenotypic Analysis of Age-Related Hearing Loss in Outbred CFW Mice

Olivia La Monte*[1], Ely Boussaty[2], Eric Du[2], Peter Dixon[2], Thomas Zhou[2], Clara Draf[3], Andrew Woodhouse[2], Rick Friedman[2]
[1]University of California, San Diego School of Medicine, [2]Department of Surgery, Division of Otolaryngology – Head and Neck Surgery, University of California, San Diego, [3]University of California, San Diego and VA Medical Center, La Jolla
Background: A major concern for the global population is age-related hearing loss (ARHL), also known as presbycusis. Human genome-wide association studies (GWAS) have identified several hearing loss risk loci but lack sufficient replication and power. A substantial fraction of patients with ARHL have no identifiable mutation despite this, suggesting unidentified monogenic or polygenic causes. For these reasons, direction has turned to mouse GWAS for hearing traits with basis in support of heredity in hearing loss shown by strain specific auditory brainstem response (ABR) impairment patterns described in inbred mouse strains. While some success has been attained in gene discovery, these investigations often have an early-onset phenotyping bias and extending this approach to include a screening of aged diverse mice is needed to understand the genetic basis of ARHL. In order to do so, our study aimed to phenotypically characterize the auditory function of young and aged Crl:CFW(SW)-US_P08 (CFW) outbred mice.
Methods: Outbred mice stocks provide fine-scale mapping resolution and the CFW stock specifically has demonstrably less linkage disequilibrium than other commercially available stocks. Male and female CFW mice were obtained in a 1:1 sex ratio and arrived at 3 weeks of age. The hearing function of these mice was evaluated through auditory brainstem response (ABR) testing after acclimatization for two weeks (n = 306). These mice were subsequently aged to six months and ten months, and ABR retesting was done at both time points (n = 781/687). Hearing thresholds were determined through visual inspection by an independent observer and defined as the minimum sound intensity at which a distinctive ABR wave I waveform could be distinguished in the response evoked potential. Patterns of ABR thresholds between frequencies assessed were determined to categorize and further characterize ARHL within the CFW mice.
Results: In total, we characterized 1765 ABRs comprising three different aging timepoints of the CFW mice. We observed significant variation in both absolute thresholds and in the effect of aging among the

stock. From these ABRs, we were able to further identify eight distinct patterns of hearing loss and were able to categorize nearly all data (93-96%) within these categories. Proportions within each category varied immensely between aging time points. When evaluating sex-based differences, we note a small but consistent hearing deficit in female CFW mice.
Conclusions: There has been no comprehensive phenotypic study of ARHL in CFW mice to date. We are aiming to vastly accelerate the pace of discovery of polygenic loci and pathways for ARHL to create the foundation for a systems genetic approach. The phenotypic data in this study are essential to map ARHL associations at a higher resolution than has been previously achieved and will form the basis for a comprehensive association study.

Differential Outcomes of High Fat Diet on Age-Related Rescaling of Cochlear Frequency Place Coding Revealed the Role of TRPV1 and Lipid Homeostasis in Stabilization of Pitch Perception

Yu Zhang*[1], Guotong Lin[2], Na Xue[3], Yi Wang[4], Tingting Du[2], huihui liu[2], Wei Xiong[5], Hao Wu[2], Lei Song[2]
[1]Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, [2]Shanghai No. 9 People's Hospital, [3]Shanghai Ninth People's hospital, [4]Tsinghua University, [5]Chinese Institute for Brain Research

Background: Our recent finding suggested age-related outer hair cell (OHC) surface area reduction altered cochlear frequency place coding. We hypothesize that the genetic background and metabolic state participate in age-related rescaling of cochlear frequency place coding. C57BL/6J (B6) and CBA/CaJ (CBA) mice had contrasting age-related hearing and cellular profiles that could test whether age-related frequency rescaling follows distinct morphological and functional differences. High fat diet (HFD) potentially influences lipid metabolism, which was proposed to serve a crucial role in OHC survival and could shift Prestin's function through its interaction with lipids. We hereby comprehensively characterized molecular, cellular, and functional changes in the cochlear mechanics using HFD models of B6 and CBA backgrounds to elucidate the underlying mechanism of age-related cochlear frequency place rescaling
Methods: In this study, we explored age-related OHC changes and how HFD affected hearing. Auditory brainstem responses and cochlear morphology were followed throughout the whole dietary period. OHC nonlinear capacitance (NLC) was measured by whole-cell patch clamp to validate cellular changes underlying frequency place rescaling. Distortion product otoacoustic emission (DPOAE) group delays verified the consequential changes in cochlear mechanics. The differential impact of HFD between CBA and B6 mice was compared by RNA-seq experiments to reveal the role of lipid and TRPV1 in maintaining cochlear frequency place coding.
Results: We first tested whether altering metabolic state would affect age-related changes in cochlear mechanics. We found differential otological outcomes from treatments: In B6 mice, over-the-board protection against age-related hearing loss (ARHL) is supported by cellular measurement of OHC NLC and DPOAE group delay; In contrast, CBA mice, which do not develop progressive ARHL, did not benefit from HFD. The cellular changes in CBA mice were also distinct from B6 mice. Such changes helped elucidate what drives the frequency-place rescaling. Next, to determine the cellular mechanism of the strain-dependent differential outcomes of HFD, we performed RNA-seq. Through differentially expressed genes analysis and Gene set enrichment analysis of lipid metabolism, we found that differential TRPV1 expression in the cochlea and lipid homeostasis are the keys to rescuing B6 hearing but not CBA mice. Acute trans-tympanic injection of capsaicin recapitulated DPOAE group delay prolongation, further validating functional changes of OHCs would alter cochlear frequency place coding.
Conclusions: HFD attenuates age-related rescaling of cochlear frequency place coding in B6 but not CBA mice. TRPV1 and lipid metabolism differentially activated in B6 but not CBA mice, effectively ameliorate the progression of age-related hearing changes. Taken together, metabolic state-derived changes in OHC morphology, function, and genetic background could all contribute to the performance of cochlear mechanics, leading to redefined frequency place coding and potentially improving pitch perception. To prevent ARHL, lipid and Prestin interaction may serve as a promising intervention target.

Interrogating Epigenetic Mechanisms in Hearing Loss Using a Genome-Wide Methylation Analysis

Marie Roche*[1], Denise Yan[1], Pei-Ciao Tang[1], Juan Young[1], Susan Blanton[1], Feng Gong[1], Xuezhong Liu[1]
[1]University of Miami School of Medicine

Background: Hearing loss affects a substantial proportion of the population; the World Health Organization (WHO) estimated that 466 million people worldwide are disabled with hearing impairment. Numerous factors, including genetics and chronic diseases, are linked to hearing loss. However, age-related hearing loss (ARHL), presbycusis, is the most common form of acquired hearing loss in the adult population. As a complex disease, presbycusis is typified by unrefined epigenetic susceptibility and necessitates both extrinsic and intrinsic factors. Nonetheless, data on the contribution of aberrant epigenetic regulation to hearing loss are scarce. Here we present a study examining whether DNA methylation, an epigenetic modifier, could be identified as a biomarker to presbycusis
Methods: We assembled a hospital-based cohort comprised of 55 ARHL subjects and 79 aged-matched controls. Hearing measurements were used to determine the audioprofiles. A quantitative interrogation of methylation sites across the genome was achieved using the Illumina Infinium® Methylation EPIC Beadchip array. This assay measures CpG loci across relevant genomic regions including CpG islands and promoters. Methylation-specific PCR was used to confirm methylation levels at the identified site in ARHL patients.
Results: Our data demonstrate a strong correlation between patients' hearing measurements and CpG sites methylation in known hearing loss genes including CDH23, TMPRSS3, SOD1, and FAM65B. Additionally CpG sites located in ESPN and TNFRSF25 show an increase in methylation at each tested frequency as the patient's hearing deteriorates. We are in the process of screening sequence variants in TNFRSF25 in ARHL patients and characterizing the expression patterns of Tnfrsf25 in the inner ear of wildtype and Tnfrsf25 knockout mouse model.
Conclusions: Interrogation of methylation in our ARHL case-control dataset has identified altered methylation in known hearing loss genes. Epigenetic modifications correlate with hearing impairment suggesting a role in the auditory system with potential opportunities in identifying novel deafness genes.

Long-Term NAD+ Supplementation Prevents the Progression of Age-Related Hearing Loss in Mice

Mustafa Okur*[1], Risako Kimura[2], Burcin Duan Sahbaz[2], Uri Manor[3], Jaimin Patel[2], Leonardo Andrade[3], Chandrakala Puligilla[2], Deborah L. Croteau[2], Vilhelm A. Bohr[2]
[1]University of California Irvine, [2]National Institute on Aging, [3]The Salk Institute for Biological Studies
Background: ARHL is the most common type of sensorineural hearing loss. Current treatment approaches for ARHL have failed, including those targeting reactive oxygen species reduction. The slow progression of ARHL adds to the challenge for intervention because it requires treatment approaches safe for long-term use. Our previous findings showed that NAD+ supplementation using NR effectively prevented the progression of hearing loss in premature aging models (8). NR can be administered orally and has no known serious side effects, and many safety studies have been done (12), making it a good candidate for long-term administration. Given these promising findings, we studied the impact of long-term NR administration on ARHL progression and investigated the potential underlying biological mechanisms of NR's benefit on hearing loss.
Methods: We assessed hearing capacity in two different wild-type mouse strains pre- and post- NR treatment using the techniques of ABR and DPOAE We then performed transcriptomic, biochemical and histological analysis to investigate potential underlying biological mechanisms of NAD+ repletion.
Results: We showed that long-term NR administration prevents the progression of ARHL. We also found that NR administration restores age-associated reduction in cochlear NAD+ levels, upregulates biological pathways associated with synaptic transmission and PPAR signaling, and reduces the number of orphan ribbon synapses between afferent auditory neurons and inner hair cells. Finally, we found that NR targets a novel pathway of lipid droplets in the cochlea by inducing the expression of CIDEC and PLIN1 proteins that are downstream of PPAR signaling and are key for lipid droplet growth.
Conclusions: Taken together, our results demonstrate the therapeutic potential of NR treatment for ARHL and provide novel insights into its mechanism of action.

Symposium #18 - Putting Tinnitus Theories to the Test

10:30 a.m. - 12:30 p.m.
Crystal Ballroom D-E

Putting Tinnitus Theories to the Test

Crystal Ballroom D-E
Chair: Calvin Wu, *University of Michigan, Otolaryngology - HNS*
Co-Chair: Alice Burghard, *University of Connecticut Health Center*
Co-Chair: Amarins Heeringa, *Carl Von Ossietzky University*

Long-Duration Sound Induced Plasticity is Altered in Mice With Tinnitus

Alice Burghard, *University of Connecticut Health Center*

A presentation of a long-duration sound (LDS) can lead to a change in both spontaneous activity as well as sound-driven activity in the inferior colliculus (IC) in mice. While the majority of sound-driven responses are suppressed, a subset is potentiated after the LDS. This potentiation is more likely in units with higher spontaneous activity. Since tinnitus is associated with increased spontaneous activity in the auditory system, we hypothesized that tinnitus animals will have more facilitation and less suppression than animals without tinnitus. Exposing awake CBA/CaJ mice to a unilateral noise trauma resulted in mice with and without behavioral signs of tinnitus. Using multichannel electrodes, we recorded the activity in the inferior colliculus contra- and ipsi-lateral to the sound exposed ear. The prevalence of LDS induced afterdischarge activity (LSA, a significant increase in spontaneous activity) in the contralateral IC was higher in the tinnitus group than in the sound-exposed non-tinnitus and the control (not sound-exposed) group in channels tuned to the sound exposure frequency (16 kHz). When comparing LDS-driven plasticity in tone evoked activity between mice with and without behavioral signs of tinnitus, we find that the sound exposed non-tinnitus animals show more suppression in overall spiking activity than tinnitus animals exposed to the same sound. The tinnitus animals show a response that is more similar to control (not sound-exposed) animals. When comparing the change in baseline activity during the tone presentations, we find in channels tuned to the sound-exposure frequency that tinnitus animals show a reduced facilitation compared to the non-tinnitus and control animals. Taken together this indicates a difference in LDS-induced plasticit in sound-exposed tinnitus vs non-tinnitus animals that might serve as an objective test to differentiate between hearing loss with or without tinnitus.

Sound Evoked Changes After Long Duration Sound as a Test for Tinnitus

Emily Fabrizio-Stover, *Uconn Health*

An objective, non-invasive, electrophysiological test is needed for efficient tinnitus research. In wild type, CBA/CaJ mice, a long-duration sound (LDS) can alter both spontaneous firing rate and responses to sound in the inferior colliculus (IC). Specifically, the majority of sound-driven responses are suppressed while a subset are facilitated after the LDS. We believe that because tinnitus animals show increased spontaneous activity in the auditory system, the LDS-generated changes will be less apparent than in non-tinnitus animals. Here, we recorded auditory brainstem responses (ABRs) before and after the LDS and show that there are tinnitus-specific differences. Awake CBA/CaJ mice received a unilateral sound exposure that resulted in mice with and without behavioral evidence of tinnitus. ABR responses to tone pips at three or more frequencies were collected from tinnitus, non-tinnitus, and unexposed control mice. We quantified the effect of LDS-changes and calculated a tinnitus score based on peak-trough amplitudes for each ABR wave. The tone-pip ABRs evoked by sounds in the exposed ear for tinnitus and non-tinnitus mice showed that non-tinnitus mice had significantly lower scores than tinnitus mice. That is, non-tinnitus mice had more suppression after LDS than tinnitus mice. At higher frequencies at later waves, the effect was more significant. However, there was no significant difference between tinnitus and the control. A correlation analysis of pre-LDS and post-LDS waveforms showed a significantly bigger difference in non-tinnitus mice than in tinnitus mice. A differential time frequency analysis analyzing the spectrum of the ABR waveforms over time showed tinnitus specific ‘hotspots’ at tinnitus frequencies, but not at non-tinnitus frequencies. Responses to the LDS show tinnitus specific changes that may be a basis for an electrophysiological test for tinnitus.

Emergence of Tinnitus in a Bayesian System of Signal and Noise

William Sedley, *Newcastle University*

Many contemporary frameworks characterise the brain's perceptual systems as engines for Bayesian inference, which generate, maintain and update internal models of the sensory environment so as to optimise the detection of meaningful signals whilst minimising noise and error within the system. As such, sensitivity can be maximised for sensory information that is salient, familiar, anticipated, sought, contextually relevant or reliable, whilst information that is irrelevant, unfamiliar or unreliable can be minimised or ignored altogether. In some ways, this can be considered a way of distinguishing signal from noise, with signals being incorporated into generative models and prior predictions, and noise being explained away as prediction error. However, prediction errors generated by sensory information with sufficiently high precision lead to the modification of existing priors or the formation of new ones. Here, I summarise existing arguments and models for how tinnitus can appear as an emergent property of an otherwise normally functioning perceptual system acting to compensate for hearing loss. I then consider the question of whether tinnitus is a 'signal' that is detected with excessive sensitivity, or whether it is 'noise' misinterpreted as a signal, and therefore whether tinnitus is the sign of a better or worse functioning perceptual inference system. I go on to consider how tinnitus due to hearing loss might compare to tinnitus without hearing loss that occurs as part of the visual snow syndrome, and whether this informs the debate about central noise vs. central gain as the origin of the tinnitus signal. Finally, I discuss a range of research avenues (some already underway) that might support, refute or refine Bayesian models of tinnitus, ranging from psychophysical testing through neurophysiological oddball responses to biologically informed computational models.

Towards a Unified Theory of Auditory (Phantom) Perception
Achim Schilling, *Neuroscience Lab, University Hospital Erlangen*

“What are the neural mechanisms of auditory (phantom) perception and how could this complex set of various neuronal mechanisms be meaningfully understood by humans?” The answer of this question needs a highly interdisciplinary approach based on computational neuroscience, experimental neuroscience and artificial intelligence. We argue that the most promising way of understanding tinnitus, is to tackle the problem on an algorithmic level, which means that we try to understand tinnitus mechanisms on an intermediate level between the molecular mechanisms (implementational level) and the computational level (formulation of the task to be solved).

To do so, we created a hybrid computational model of the auditory pathway consisting of a simple cochlear and DCN model, which we combined with a deep neural network. Thus, the deep neural network could be interpreted as a model of the higher auditory processing stages up to the cerebral cortex, but is also a tool to quantify meaningful information in the DCN output. We trained the deep neural network on speech recognition and used the accuracy as an objective function of speech comprehension ability of the auditory pathway. Finally, we distorted the system by adding a simulated hearing loss and fed in intrinsic neural noise to the DCN.

We were able to show that indeed the addition of neural noise can partly compensate the hearing loss and can increase the speech comprehension ability by a factor of 2, an effect called stochastic resonance (SR). We hypothesize that the origin of that neural noise is the somato-sensory system innervating the dorsal cochlear nucleus. Despite of the explanatory power of the model, one crucial question remains unsolved: Why does (nearly) everyone with tinnitus suffer from hearing loss, but not everyone with hearing loss suffers from tinnitus?

We argue that the bottom-up model described above is only the first part of the big picture. A second part, namely a complementary top-down model is needed to account for the influence of stress, attention, and experience on tinnitus perception. We argue that potentially the increase of sensory precision due to Bayesian inference as described by Sedley and Friston could be caused by intrinsic neural noise and lead to a prediction error in the cortex. The combination of our bottom-up model with this top-down model based on predictive coding provides a unified framework of the neural algorithms underlying tinnitus perception.

Attentional Deficit in Tinnitus—Symptom or Cause?
Madan Ghimire, *SIU School of Medicine*

Incidence of chronic tinnitus has progressed to impact more than 15 percent of the global population. Tinnitus pathology is believed to be initiated by damage to the auditory periphery resulting in maladaptive plastic changes, altering central auditory, limbic and attentional systems. Individuals most disturbed by their tinnitus, show bimodal abnormalities of selective attention. For example, Norena and colleagues (2004) showed that individuals with tinnitus are bound to their tinnitus percept and are unable to divert their attention away from the sound in their heads. Similar attentional deficits were observed in an animal model of tinnitus (Brozoski et al., 2019). Based on this hypothesis, several therapeutic approaches employing sound were devised to divert attention away from tinnitus and have been used in tinnitus patients with modest success. Later, Roberts and colleagues (2013) proposed that a partial deafferentation induced loss of signal, creates a mismatch between the predicted and experienced inputs into the auditory cortex (A1), recruiting attentional resources and reinforcing the phantom signals. It is yet unclear what role attentional systems play in the tinnitus pathology. Present studies were designed to examine the tinnitus-related changes in attentional resources in the A1. The role of nicotinic signaling in the regulation of attention in the central nervous system has been well studied. When attention is required, cholinergic neurons of basal forebrain are found to increase release of acetylcholine (ACh) to the target cortical region and administration of nicotinic agents are found to heighten attention. Using a well-established animal model of tinnitus, we examined tinnitus-related changes in nicotinic acetylcholine receptors (nAChR) signaling in A1 layer 5 pyramidal neurons (PNs) and vasointestinal peptide positive (VIP) neurons. In vitro whole-cell patch-clamp studies revealed a significant tinnitus-related loss of nAChR signaling in layer 5 PNs. In contrary, puffed ACh evoked a significantly greater number of action potentials in VIP neurons from animals with behavioral evidence of tinnitus. Since, increased VIP neuron activity favors excitation of PNs through disinhibition, tinnitus-related increases in nAChR evoked excitability of VIP neurons may tip the balance toward increased excitability of principle cortical neurons.

The Elusivity of an Objective Test for Tinnitus in Humans

Joel Berger, *Dept. Neurosurgery, University of Iowa Hospitals and Clinics*

Tinnitus assessment in humans currently still relies on self-report, often based on frequency matching procedures. Contrastingly, in animals, objective assessment of tinnitus is the only method for detecting the presence of a phantom percept following either noise exposure or administration of a drug such as sodium salicylate. Development of objective assessments that can be used in both animals and humans would allow bridging of the oft-mentioned gap between animal and human studies, thus linking potential theories of tinnitus to the human experience. Previous data have demonstrated that the most commonly-used behavioral test for tinnitus in animals - the gap pre-pulse inhibition of the acoustic startle paradigm – can be adapted to neural recordings in awake animals (Berger et al., 2017; 2018). I will discuss these studies, along with attempts to bring this paradigm to humans and report rare data that we recorded from an intracranially-implanted epilepsy patient with intermittent tinnitus. Ultimately, although a clinically-useful objective test for tinnitus in humans remains elusive at present, the development and validation of one would provide corroboration of animal behavioral studies and allow for more accurate assessment of a phantom percept.

Central Gain: A Closer Fit to Hyperacusis Than to Tinnitus?

Elouise Koops*, Department of Radiology, Massachusetts General Hospital/Harvard Medical School, Boston, USA*

Central gain refers to the increase of spontaneous activity observed in hierarchically higher auditory pathway areas after hearing loss induction (Schaette and Kempter, 2006). The upregulation of neuronal activity in central auditory regions is interpreted as a homeostatic plasticity response to decreased peripheral input. In the context of hyperacusis (Auerbach et al., 2014; Diehl and Schaette, 2015) and tinnitus (Norena, 2011; Schaette and McAlpine, 2011), the central gain framework has been extended to include sound-evoked activation. Whereas tinnitus is the most extensively studied condition co-occurring with hearing loss, 59% of those with hyperacusis have co-occurring hearing loss (Paulin et al., 2016), and the majority of those with hyperacusis also report tinnitus (Anari et al., 1999; Dauman and Bouscau-Faure, 2005; Schecklmann et al., 2014). Even though hyperacusis frequently co-occurs with hearing loss and tinnitus, it is often not taken into account in experimental studies, hampering adequate characterization of the neural signatures of these conditions.

In an fMRI study, we investigated the subcortical and cortical BOLD-responses in a group (n=35) that often reports hyperacusis: individuals with hearing loss and tinnitus. Additionally, we characterized the frequency tuning of cortical voxels in the primary auditory cortex of those with and without hyperacusis. In this study, hyperacusis was indicated by a cut-off score of 22 on the Hyperacusis Questionnaire (HQ).

In the group with hyperacusis, sound-evoked activity was higher in both cortical and subcortical auditory structures. This increase in responsivity extended to frequencies not affected by hearing loss, and the higher subcortical and cortical activity in response to sound appears to be a marker of hyperacusis. The frequency tuning of auditory cortical voxels was not significantly different in those with hyperacusis. In contrast, the auditory cortex BOLD signal was reduced in response to the presentation of the tinnitus frequency in those with higher hyperacusis scores. Overall, the heightened subcortical and cortical activity can reflect an increase in neural gain along the auditory pathway in those with hyperacusis, but may not capture cortical responses that are involved in tinnitus.

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Funding Your Scientific Genius!

12:45 p.m. - 2:00 p.m.
Merritt

Larry Hoffman, *Geffen School of Medicine at UCLA*
Ronna Hertzano, *University of Maryland School of Medicine*
Joan Wincentsen, *American Hearing Research Foundation*
Catherine Perrodin, *RNID*
Tian Wang, *Congressionally Directed Medical Research Programs*
Clare Thibodeaux, *Cures Within Reach*
Amy Poremba, *NIH-NIDCD*

Short Course- Part 1: Single Cell “Omics”; A Practical Introduction to Molecular Analysis at the Single Cell Level

12:45 p.m. - 2:00 p.m.
Biscayne 1

This course will provide a practical introduction to molecular analyses at the single cell level. Part 1 will feature short presentations on current state-of-the-art approaches for analysis of mRNA, Protein and epigenetic state. Part 2 will include presentations on bioinformatic packages that can be used to analyze and synthesize the large data sets that are generated from single cell studies. Part 2 will also include additional information on useful websites and online tutorials.

Introduction: Single Cell "Omics" Applications and Limitations
Lisa Goodrich*, Harvard University*

Single Cell and Single Nucleus RNA-Sequencing– Platforms, Data, Options
Ronna Hertzano*, University of Maryland School of Medicine*

ATAC Seq and ATAC Plus Single Cell
Litao Tao*, Creighton University*

Long Read Sequencing – PacBio, Nanopore
Matthew Kelley*, NIH/NIDCD*

Single Cell Proteomics
Stefan Heller*, Stanford University School of Medicine*

Podium #19 - Binaural and Spatial Hearing: Behavior and Electrophysiology

2:00 p.m. - 4:00 p.m.
Ocean's Ballroom 5-12

Moderators: Lindsey Van Yper and Pierre Apostolides

Limits on Coincidence Detection in MSO Neurons

Philip Joris*[1], Tom Franken[1]
[1]*KU Leuven*

Background: Neurons in the medial superior olive (MSO) compare the timing of acoustic signals at the two ears. This neural computation enables the remarkable sensitivity of humans and animals to interaural timing differences (ITDs). The actual process underlying this computation, generically described as "coincidence detection", is not understood in detail.
Methods: Responses to binaural beat stimuli were obtained over a range of frequencies in two types of preparations. Whole-cell recordings were performed in the MSO of gerbils and yielded both postsynaptic potentials and spike output. Axonal recordings from fibers in the lateral lemniscus obtained in chinchilla were classified as MSO-like or DNLL-like based on a number of response criteria. The responses were graphed as a function of instantaneous ipsi- and contralateral phase.
Results: Both within and across identified and putative MSO neurons, there was large variability in the ability of either ear to influence spiking. In some neurons at some frequencies, the distribution of spikes reflected a restricted range of ipsi- and contralateral phases, consistent with a simple coincidence mechanism. However, in many cases there was a marked difference between the two ears such that spikes were locked to a narrow range of phases at one ear but to a broad range at the other ear. Even more surprising was that, particularly at very low frequencies, both ears could be locked over a narrow range of phases without showing actual binaural interaction, indicating that coincidence is not a sufficient requirement to generate ITD-sensitivity.
Conclusions: A simple process of coincidence detection poorly describes the process underlying ITD-sensitivity. The data are interpreted in terms of differences in convergence of monaural inputs onto MSO neurons from the two sides, interacting with intrinsic membrane properties.

Cortical Patch and Map of Auditory Space

Chenggang Chen*[1], Xindong Song[1], Yueqi Guo[1], Xiaoqin Wang[1]
[1]*Johns Hopkins University*

Background: Despite four decades of research, the nature of the neural representation of sound location in the auditory cortex remains unclear. Previous studies have failed to identify any maps or patches of spatial representation in the mammalian auditory cortex (Middlebrooks and Pettigrew, 1981, J Neurosci; Middlebrooks, 2021, J Neurosci). A prevailing hypothesis of cortical spatial processing is the distributed population coding, supported by the evidence that neurons respond broadly to sound locations on the contralateral hemifield (Ortiz-Rios et al., 2017, Neuron; van der Heijden et al., 2019, Nat Rev Neurosci). However, electrophysiology and fMRI methods have limited spatial resolution to evaluate the cortical representation of sound locations.
Methods: In the present study, we took advantage of the flat brain of the marmoset, a highly vocal New World monkey, and used wide-field calcium imaging methods to investigate the neural representations of sound location in the auditory cortex and neighboring multisensory region (fundus of superior temporal, FST) in awake condition.
Results: Most cortical areas preferred contralateral sound locations, but regions tuned to the front and ipsilateral locations formed five to ten patches. We found those patches in both primary and nonprimary, rostral and caudal auditory cortex.
Next, we investigated whether spatial tuning of patches depends on interaural time and level differences (ITD and ILD) cues. We found patches that prefer low frequency were ITD cue dependent. In contrast, patches that prefer high frequency were cue independent. Patches identified with spatial and binaural stimuli were relatively stable across sound levels.
Furthermore, a neighboring multisensory FST has weak sound-driven responses and was not topographically organized by sound frequency. Surprisingly, FST was organized topographically by sound locations that range from far-contralateral to front.
Finally, a horizontal visual stimulus will evoke a strong response only in the FST but not in the auditory cortex. We also identified a retinotopic map in the FST. Notably, multisensory auditory and visual-spatial maps in the FST largely overlapped.
Conclusions: In summary, we found that auditory space is represented in the cortex by patch and map.

Early-Life Development Shaped by Cue Reliability in the Barn Owl's Auditory Midbrain

Keanu Shadron*[1], Jose Luis Pena[1]
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Background: In order to accurately perceive the world and respond accordingly, the brain has to deal with noise inherent in sensory cues. One method is for the brain to learn which cues are reliable across contexts and rely on these cues in the future. Previous studies have demonstrated that sensory systems are able to actively learn recent statistics underlying cue reliability and adapt to short term changes. However, relatively little is known about whether and how sensory systems adapt to natural statistics of sensory cues that are expected to be stable over time. To address these knowledge gaps, we investigated whether the anticipated reliability of binaural spatial cues drives the tuning of neurons that compute sound location in barn owls. Barn owls use the interaural time difference (ITD) to determine azimuthal location. Previous work showed that the variability of ITD cues in natural acoustic scenes vary across frequencies in a location dependent manner, based on the acoustical properties of the head. Thus, for a given location, certain frequencies convey ITD cues more reliably, and the neural tuning in the midbrain external nucleus of the inferior colliculus (ICx) reflects this pattern. We hypothesize that if the frequency tuning of the ICx is driven by cue reliability across locations, then altering the pattern of cue reliability should adjust frequency tuning.
Methods: We found that the absence of the facial ruff, the primary determinant for ITD reliability, led to a specific decrease in the reliability of high frequencies originating from frontal space. To test if the owl's ICx frequency tuning is driven by permanent changes in the pattern of ITD reliability, ICx neurons were recorded from two adult owls that had the facial ruff removed as juveniles.
Results: Results indicate that ICx neurons tuned to frontal locations are tuned to lower frequencies than previously reported in normal owls, consistent with the change in ITD reliability induced by the facial ruff removal. Additionally, recordings immediately upstream of the ICx, show normal tuning to frequencies across the owl's normal hearing range. This suggests that the ruff-removed owls' midbrain is still encoding ITD for these higher frequencies but aren't using them for sound localization. Recordings from two juvenile owls, before the facial ruff fully developed, show similar tuning to ruff-removed owls, suggesting that the high frequency tuning is learned once the facial ruff develops. Comparisons to owls that underwent facial

ruff removal during adulthood further suggest that the shaping of ICx frequency tuning is largely confined to early-life development.
Conclusions: These data suggest that the tuning properties of the midbrain map are developmentally adapted to the permanent statistics of spatial cues.

Neural Coding of Sound Localization Cues in Rabbits With Mild, Permanent Noise-Induced Hearing Loss

Emili Garretson[1], Joshua Mencsik[1], Mitchell Day*[1]
[1]*Ohio University*
Background: Human listeners with sensorineural hearing loss are known to exhibit a deficit in sound localization ability, but the physiological mechanisms underlying this deficit are unknown. We previously showed that rabbits with severe noise-induced hearing loss (75-dB threshold shift) had degraded neural coding of sound localization cues: neurons in the central nucleus of the inferior colliculus (ICC) were completely insensitive to interaural time difference (ITD) of noise stimuli, but encoded interaural level difference (ILD) of noise stimuli the same as those of normal-hearing, unexposed rabbits. We also showed that noise overexposure resulting in a 75-dB shift in the auditory brainstem response (ABR) threshold led to near complete loss of cochlear outer hair cells and some loss of inner hair cells. In the present study, we tested whether mild, permanent noise-induced hearing loss (~25-dB threshold shift) degrades neural coding of sound localization cues in rabbit ICC neurons. Unlike our previous study, this noise overexposure is expected to leave cochlear outer and inner hair cells intact.
Methods: Neural responses were measured to several stimuli presented over earphones: noise stimuli filtered with individualized head-related transfer functions and presented from different directions within the horizontal plane (azimuth); noise stimuli with ITD varying over a large range; low-frequency tones varying in ITD; and high-frequency, amplitude-modulated tones varying in ITD of the amplitude modulation. Stimuli were presented at sound levels relative to either the individual neuronal threshold or to the rabbit's ABR thresholds to prevent changes in neural sensitivity simply due to elevated thresholds. For each stimulus, neural coding of sound localization cues was quantified as the d-prime between minimum and maximum firing rates.
Results: Based on analysis of data from ICC neurons with characteristic frequencies greater than 4 kHz, there were no clear differences in neural coding of sound localization cues between hearing-impaired and normal-hearing rabbits. Importantly, we found many examples of ICC neurons in mildly hearing-impaired rabbits whose firing rates were sensitive to changes in ITD of noise or tonal stimuli, unlike our result for severely hearing-impaired rabbits.
Conclusions: Our present results suggest that degradation of neural coding of sound localization cues following noise overexposure may require a threshold degree of hearing loss—a threshold not reached by the 25-dB ABR threshold shifts in the present study.

On the Neuronal Encoding of Stimuli With Reduced Interaural Coherence

Jörg Encke*[1], Mathias Dietz[2], Torsten Marquardt[3], David McAlpine[1]
[1]*Macquarie University*, [2]*University of Oldenburg, Department of Medical Physics and Acoustics*, [3]*University College London*
Background: The ability to localize sources of sound in the horizontal plane relies on location-specific differences between the stimuli at the two ears. In natural listening environments, these stimulus features are rarely static, and sounds from multiple sources interact, reducing interaural coherence, the similarity of the sound at the two ears. This reduction in coherence is caused by random temporal fluctuations, and thus uncertainty, in localization cues. Perceptually, this uncertainty is reflected by a widening in the perceived location of an auditory object. However, the underlying mechanism by which the auditory system encodes fluctuating binaural cues is still a matter of debate.
Methods: Most models of binaural hearing rely on a cross-correlation function when detecting changes in interaural coherence. However, evidence for a neural substrate resembling a cross-correlation has proved elusive in mammals. Here, we employ a different approach and make use the temporal fluctuations of the localization cues instead of the cross-correlation function. Employing a biophysically plausible two-channel model, we show that this mechanism codes for stimulus coherence and thus for the degree of temporal fluctuation in interaural parameters. We evaluate this approach by comparing model predictions to human behavioral sensitivity to interaural coherence. Then, using single-neuron recordings from ITD-sensitive

neurons in the inferior colliculus, we directly compare the performance of our fluctuation-based approach to that of a cross-correlation-based approach. We also investigate the precision with which neurons encode the temporally fluctuating localization cues.
Results: Our computational model can account for an extensive range of psychoacoustic performance limits, achieving and even exceeding the performance of cross-correlation-based models. The neural data also demonstrate that temporal fluctuations in binaural cues are encoded with high precision and that encoding of coherence in terms of fluctuations in binaural cues far outperforms performance based on the cross-correlation approach.
Conclusions: Results from this study suggest that the auditory system does not have to rely on a cross-correlation function to encode interaural coherence. Instead, uncertainty in binaural cues is encoded by neurons that follow the temporal fluctuations in the localization cues.

Parallel Pathways for Interaural Time Difference Processing in the Inferior Colliculus is Partially Maintained After Early Auditory Deprivation in Rats

Woonhoe Goo[1], Dae Woong Kang[1], Woongsang Sunwoo*[2]
[1]Seoul National University Hospital, [2]Gachon University Gil Medical Center
Background: Selective stimulation of frequency-specific pathways demonstrates differences in neural interaural time difference (ITD) sensitivity in the inferior colliculus (IC) depending on the tonotopic place of intracochlear stimulation. Binaural neurons in the IC are sensitive to ITDs with a high resolution when electrical pulse trains are presented through a low-frequency pathway when compared to a high-frequency pathway. However, despite the potential importance of intracochlear stimulating site for improving the ITD sensitivity, it is unclear at present whether these functionally segregated pathways can be preserved following early auditory deprivation.
Methods: To examine this issue, we used neonatally deafened rats with ototoxic drugs and recorded the responses of single neurons in the IC to electrical pulse trains with ITDs delivered at two intracochlear locations representing the maximum difference in their tonotopic axes, and compared with previous data from the acutely deafened rats as adult (Sunwoo and Oh, J Neurosci Res. 100:461) which had normal hearing experience during development. Twenty-five Wistar Albino rats were deafened as neonates with daily injection of kanamycin and then bilaterally implanted at 10–12 weeks of age. Stimuli were periodic trains of biphasic electric pulses with varying pulse rates (20–1280 pps) and ITDs (-1200 to +1200 μs).
Results: We found that early auditory deprivation has a major impact on patterns of ITD tuning in low-characteristic frequency IC neurons which responded selectively to electrical stimulation with the apical electrode pair (apical units). The prevalence of ITD-sensitive neurons with a peak-shaped ITD tuning curve, which may reflect predominant input from the medial superior olivary complex, in neonatally deafened rats was diminished compared to that in acutely deafened rats (40% vs 67%). Conversely, monotonic-type responses rarely occurred in acutely deafened rats but were most prevalent in neonatally deafened rats (42% vs 14%). We also demonstrated that deprivation of auditory experience deteriorated the degree of ITD sensitivity, as measured using the signal-to-total variance ratio (STVR) metric, in apical units to a level comparable to that in high-characteristic frequency IC neurons activated by the basal electrode pair (basal units). However, the predicted ability of apical units to discriminate ITDs based on a signal detection theory was still higher than that of basal units, even in the absence of binaural hearing experience. Moreover, in apical units from the neonatally deafened animal, the distribution of their tuning parameters was well adapted for providing good discrimination within the physiological ITD range.
Conclusions: These findings suggest that functionally segregated pathways for ITD processing from the cochlea to the IC is partly maintained after neonatal deafness. Thus, the selective electrical stimulation of low-frequency pathways can still be an important factor for achieving an improved sensitivity to ITDs in bilateral CI users, even after early auditory deprivation.

Pupil-Linked Arousal System Reflects Bayesian Surprise in Spatially Volatile Environment

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Background: Bayesian theory has been applied successfully to explain human perception in noisy and volatile environments. It prescribes that sensory signals are to be integrated with prior beliefs, weighted by

their relative reliabilities, unless changes render irrelevant the prior beliefs. Mechanistically, Krishnamurthy, Nassar, et al., (2017, Nat Hum Behav) suggested that the arousal system plays a mediating role: Arousal is high whenever prior beliefs are unreliable or irrelevant and so the brain prioritizes processing of novel sensory signals. Specifically, they found that baseline pupil size was inversely related with prior reliability and that evoked pupil dilation negatively correlated with prior relevance. However, alternative links between the pupillometry data and latent variables of their Bayesian inference model were not considered. Moreover, spatial prediction responses based on foregoing audiovisual stimuli preceded the sounds that were subsequently localized, thereby potentially confounding perceptual biases towards Bayesian priors with consistency biases during cognitive decision making.
Methods: Therefore, we set out to confirm the hypothesized relation between the arousal system and Bayesian inference in two ways. 1) We tested two alternative sets of predictors for baseline and evoked pupil sizes in a reanalysis of the original data. We obtained latent variables from an improved Bayesian inference model that was jointly fitted to the behavioral and pupillometry data of each individual, thereby explicitly accounting for the mutual information between the two data sources. 2) We redesigned the experiment using auditory-only volatile sequences wherein 32 participants localized the last sound without intervening prediction responses while we continuously monitored their pupil size.
Results: Reanalysis results indicated that surprisal predicted evoked pupil sizes better than prior relevance or learning rate. Moreover, our reanalysis did not reproduce a trial-by-trial correlation between baseline pupil size and prior reliability, nor did we find a significant relation with other measures of prior uncertainty. Results of the new experiment are yet to be analyzed.
Conclusions: Our work so far corroborates evidence that the arousal system plays a crucial role in adjusting auditory perception under conditions of uncertainty. Evoked pupil dilation being best predicted by momentary levels of surprisal suggests a causal relationship between arousal modulation and the integral evaluation of sensory likelihood and prior beliefs in line with Bayesian inference. [Supported by Austrian Science Fund (FWF, ZK66)]

Representation of Auditory Space in the Shell of the Inferior Colliculus

Meike Rogalla*[1], Gunnar Quass[2], Deepak Dileepkumar[2], Alex Ford[2], Gunseli Wallace[1], Harry Yardley[2], Pierre Apostolides[2]
[1]University of Michigan, [2]Kresge Hearing Research Institute, University of Michigan
Background: Spatial hearing enables humans and animals to localize sounds in their vicinity, which contributes to survival. Unlike vision or touch, the peripheral auditory system lacks a spatial map at the sensory receptor level. Sound source location is therefore derived centrally from mainly binaural cues, as well as from monaural cues. In the case of unilateral hearing loss, binaural cues are no longer available, which limits spatial hearing. However, monaurally occluded humans and animals can regain sound localization following perceptual training. It is assumed that the observable re-learning of sound localization relies on the context-dependent re-calibration of auditory space representation by monaural cues. Thus, central experience-dependent auditory plasticity mechanisms must exist to re-calibrate sound localization circuits. The “shell” nuclei of the inferior colliculus (shell IC) are hypothesized to act as plasticity loci for sound localization cues. However, the neural population coding of spatial information in the mammalian shell IC remains poorly understood.
Methods: We developed an acoustic delivery system to present sound stimuli from distinct spatial positions within the horizontal frontal field by moving a speaker around the animals’ head while performing cellular resolution 2-photon Ca2+-imaging in the shell IC of awake, head-fixed mice.
Results: We found that neurons in the murine shell IC are spatially tuned, and that the population coding follows a surprisingly different pattern as previously shown for other auditory regions: In contrast to the central IC, where spatial tuning shows a contralateral dominance, we found both contra- and ipsi-lateral selective neurons, such that a single hemisphere contained a representation of the entire horizontal field. Although previous data suggested a monotonic code for spatial representations in the mammalian auditory system, many shell IC neurons were tuned to discrete contra- and ipsi-lateral positions. Tuning required binaural integration and seems impervious to representational drift: tuning broadened or shifted towards the contralateral hemifield after inserting an ear plug into the left ear.
Conclusions: To our knowledge, these results are the first insight into spatial population codes of the mammalian shell IC. Future studies will test if active engagement in a localization task is required for plasticity of spatial tuning during monaural hearing loss.

Symposium #20 - Music to our Ears: Does Musical Training Improve Auditory and Speech Processing and Perception?

2:00 p.m. - 4:00 p.m.
Oceans Ballroom 1-4

Music to Our Ears: Does Musical Training Improve Auditory and Speech Processing and Perception?

Oceans Ballroom 1-4
Chair: Deniz Başkent, *University of Groningen, University Medical Center Groningen, Department of Otorhinolaryngology/Head and Neck Surgery, The Netherlands*
Co-Chair: Andrew Oxenham, *University of Minnesota*

Music is found in every human culture. Performing involves fine-grained motor, cognitive, multimodal-integration, and aesthetic skills; listening critically involves almost as many. Perhaps most importantly, music is fun. The enjoyable and social aspects of music could make it a powerful and motivating vehicle for auditory training, particularly in a clinical setting among those with hearing loss and cochlear implants. But does musical training improve skills beyond music, such as speech perception in noise and more general cognitive abilities? Can musical training protect against some aging effects in these domains? Many studies have addressed these questions in recent years without reaching a common consensus. The purpose of this symposium is to bring together researchers who have been actively contributing to this debate, to assess current evidence and opinions, to seek common ground, and to chart a way forward to answer these important questions. The session will bring researchers bridging basic and clinical sciences from the fields of auditory and speech perception and neuroscience, musical training, hearing aids and cochlear implants, to explore together the potentials and limitations of effects of musical training throughout the lifespan. To our knowledge, ARO has not hosted such a session in recent years.

Musical Training and Hearing-In-Noise Perception
Emily Coffey, *Concordia University*

Musical training is a powerful and well-studied model of human neuroplasticity, which harnesses emotional and reward networks and auditory-motor feedback loops to sharpen and reinforce practised skills. It is thought to improve the quality of basic auditory encoding and higher-level functions such as selective attention and working memory. Both sound encoding quality and higher-level functions are important for perceptual skills outside of a musical context, such as speech-in-noise perception, which strongly influences health and well-being. Musical training is an attractive avenue for potentially enhancing poor or degraded perceptual skills; however, the specific nature of musical training benefits, particularly for speech-in-noise perception, is not yet clear. I will present our lab’s recent work using electroencephalography (EEG), magnetoencephalography (MEG), and functional magnetic resonance imaging (fMRI) to explore the relationship between musicianship, perceptual skills, and neurophysiology. I will also discuss the design of musical tasks for encouraging neuroplasticity, and present some of the Open Science tools we have developed to facilitate and encourage research progress in these areas.

Music Training and Nonmusical Abilities: The Role of Natural Musical Ability
E. Glenn Schellenberg, *ISCTE-IUL*

Music training is associated with many nonmusical abilities, including those involving listening to speech or other auditory stimuli, as well as general cognitive ability. Individuals with training often show an advantage on tasks that measure these skills, and such advantages are assumed to be a consequence of music training. In fact, music training is often claimed to be a good (even ideal) model for the study of changes in brain and behavior that occur as the result of experience (i.e., plasticity). In recent years, however, research from my laboratory raises doubts about these claims. We have documented that individuals with music training differ systematically from other individuals in terms of demographics, personality, cognition, and natural musical ability (aptitude or talent), which are unlikely to be the consequence of any intervention or experiential

factor. Moreover, evidence from twin studies points to a genetic component to musicality and music achievement, which raises further doubts about the proposed causal effect of music training, as do findings showing that transfer effects are rare. Rather, individuals from high-SES families who also have good cognitive abilities, high levels of the personality trait called openness-to-experience, and a facility for music are more likely than other individuals to take music lessons, particularly for years on end. Results in line with this view show that: (1) speech perception and grammatical ability have a stronger association with musical ability than with music training, (2) the association between music training and reading ability disappears when general cognitive ability is held constant, (3) the link between music training and cognitive ability disappears when musical ability is held constant, and (4) non-musical variables that predict music training also predict musical ability. In a five-year longitudinal study, musical ability at 8 years of age predicted the amount of music training taken subsequently. By contrast, at 13 years of age, links between music training and musical ability disappeared when musical ability at age 8 was held constant. These results, as well as those from independent laboratories, ensure that music training is a particularly poor model for the study of plasticity.

Neural Correlates of Auditory Processing in Individuals Engaged in Music Training – A Perspective Across the Lifespan

Assal Habibi, *Univeristy of Southern California*

Playing a musical instrument is a complex multisensory experience requiring several skills including reading and translating abstract musical notation to fine and coordinated motor movements in order to produce a sound. The mastering of this rich and demanding process requires regular and intense practice, often from a young age. While there is a growing body of evidence suggesting that music training benefits cognitive development, the associations between music training and health outcomes specifically in relation to improvement of auditory and language skills is not clear. This presentation will highlight a series of research studies on the role of music training and associated health outcomes across the lifespan. The results from the first two studies provide evidence that music training in children and adolescents leads to improvement of pitch and rhythm perception, speech in noise perception and neuroplastic functional changes in the associated auditory regions that may have long-term positive effects on language development and competency. Results from the third study provide evidence that participating in short-term musical activities leads to better speech in noise perception in older adults without prior music training – and adding to the accumulating evidence that engaging in enrichment activities, such as choir singing provide a cost-effective and sustainable community-based intervention to improve auditory and communication abilities in aging adults. These findings together demonstrate that brain-to-behavior changes induced by music training can positive health and well-being outcomes specifically in relation to auditory abilities, language, and communication skills in individuals across the lifespan.

Association of Musical Training With Auditory and Speech Neural Coding and Perception

Kelly Whiteford, *University of Minnesota, Department of Psychology*

Numerous studies have reported a link between engagement in musical training and enhanced neural processing and perception of sound, ranging from fine-grained pitch discrimination to the perception of speech in noise, with training-related neural changes emerging as early in the auditory pathways as the brainstem or even the cochlea. Such findings suggest a role for experience-dependent plasticity in the early auditory system, which may have meaningful perceptual consequences. However, the generalizability of the musician advantage remains unclear. For example, small-sized samples often represent extreme ends of the musical spectrum; the nature and magnitudes of the musician advantage are sometimes small or inconsistent; and methodological differences and varying analytical techniques complicate comparisons between studies. This multi-site study aims to examine the robustness of the musician advantage across the adult lifespan by replicating and extending eight key experiments involving both perception and neural coding across a large sample of listeners ($n > 300$) at six universities in the US and Canada (Boston University, Carnegie Mellon University, Purdue University, University of Minnesota, University of Rochester, and University of Western Ontario). All participants were tested on all 8 experiments in a laboratory setting, including pitch discrimination, behavioral estimates of frequency tuning, speech and non-speech informational masking, speech perception in noise and babble, and two physiological measures of F0 encoding of speech sounds using electroencephalography. Data collection is ongoing, but so far data from over 240 participants have

been collected. Participants also completed additional measures to control for potential confounding factors, including an objective measure of musical aptitude (melody discrimination from the Mini Profile of Music Perception Skills), a cognitive assessment (Ravens Advanced Progressive Matrices), a measure of extended high-frequency hearing, and survey questions related to personality and socio-economic status. Formal statistical analyses were preregistered on the Open Science Framework and will be conducted once data collection is complete. The results will provide in-depth and high-powered insight on the nature and robustness of the musician advantage across the adult lifespan. [Supported by NSF-BCS grant 1840818.]

What Benefits Can We Derive From Music Training for Cochlear-Implanted individuals?
Eleanor Harding, *University of Groningen, University Medical Center Groningen, Department of Otorhinolaryngology/Head and Neck Surgery*

Cochlear implant users report difficulties perceiving music and certain aspects of speech, such as vocal cues and emotions, and 'cocktail-party' speech in multi-talker noise. While evidence exists supporting music as a training tool to improve music and speech perception, results are not unanimous across studies and the precise mechanisms that may contribute to both near- (music domain) and far- (speech domain) transfer training effects are not well understood. Previous research, including from our group, has indicated that musically trained individuals show advantages in perceptual tasks that involve music and pitch. We have also observed beneficial effects for perception of speech in the presence of a speech masker, but not for other maskers (such as steady noise), and also not in all populations (adults vs. adolescents). In some situations, such as perception of voice gender, we have observed musician and non-musician groups weighting voice cues differently than each other, but with no clear benefit from being a musician. Taken together, this evidence suggests that musical training may be a beneficial tool for improving these areas of auditory perception in cochlear implant users. However, perhaps the overall picture is more complex than we can easily characterize: the influence of musical training on psychoacoustic tasks varies across the literature, moreover, musical training is reported to variably affect cognitive processes such as auditory working memory and neural tracking of the acoustic envelope of music and speech. These cognitive processes may influence performance on certain tasks, such as speech-on-speech perception, via top-down processes. In this talk, we will cover our previous and current work with musicians, on potential musician and music training effects and their mechanistic origins, and how we try to utilize this knowledge in developing new ways of using music as an auditory training tool.

Effects of Singing and Musical Training to Speech Perception and Language Skills of Children With Hearing Impairments
Ritva Torppa, *Psychology and Logopedics, Cognitive Brain Unit (CBRU), Faculty of Medicine, University of Helsinki, Finland*

There is growing body of evidence from follow-up studies and randomized controlled trials on the positive effects of musical training to perception of pitch, music and speech, to cognitive and language skills, and to associated brain networks in adults and children with normal hearing (NH). Musical training is expected to lead to similar effects for children with hearing impairments (HI) since they enjoy music and singing similarly to children with NH.

Even though the field of HI is still lacking large-scale randomized controlled trials, the current evidence shows that musical training and even informal singing at home can lead to similar positive effects in children with HI as found in children with NH. For instance, a longitudinal study showed that the attention-related P3a brain responses to changes in pitch and timbre were larger and developed more in children with CIs who sang regularly at home than in their peers with CIs who did not sing. In another study, the children with CIs who participated in musical hobbies were better in perception of pitch and prosodic stress and in expressive language skills (word finding, verbal IQ and phonological awareness measured with rhyming) compared to musically non-active children with CIs. Moreover, a longitudinal study showed that perception of speech in noise, timbre, question/statement prosody and spectral resolution improved only in the children with HI participating in musical training while not in the passive control group. The preliminary results from our new cross-over design study imply that musical intervention, which focused on singing, improved expressive language skills (e.g., semantic word fluency) of 2-6 years old children with HI. Regarding to

pitch perception, the development during intervention varied in a sample of 3-6 years old children with HI, some of them showing clear development.

This presentation will show why music can improve speech and music processing and perception as well as language skills of children with HI. The current evidence suggests that it would be beneficial for children with HI to sing at home and take part in musical hobbies and training.

To Understand the Benefits of Musical Training on Speech Perception We Need to Focus on the Assessment of Musical Skills and the Initiation of Adequately Powered Intervention Studies
Frank Russo, *Toronto Metropolitan University*

The evidence for the benefits of music training on speech processing and perception have been difficult to pin down. While correlation and cross-sectional studies abound, the evidence has been mixed and enthusiasm about positive findings are clouded by legitimate questions regarding confounding variables. The mixed evidence is not exactly surprising. The types of musical skills that a musician will possess as there is no accepted canon for musical training (e.g., learning percussion vs. violin; playing by ear or notation), and individuals will come to training with varying predispositions. Thus, I will argue that it is generally not satisfactory to simply count the number of years of musical training or to create a binary split between musicians and non-musicians when accounting for effects of music training. Correlational studies would be better served by modelling speech perception skills on the basis of specific musical skills that are assessed through experiment. The studies that have initiated a musical training intervention are more informative as they control the manor of training across participants and are able to take individual baselines into account. However, these studies have by-and-large been hindered by small and heterogenous samples. Moreover, the control conditions are often passive, leaving open the possibility that benefits accrue simply from adding a new activity or even worse, demand characteristics. In my own lab, we have completed three studies involving music training interventions, all in participants with some level of hearing loss. None of these studies is perfect and all suffer from relatively small sample sizes. Nevertheless, we have observed some positive outcomes. Our study on children who use cochlear implants showed benefits on perception of speech emotion. Our study on older adults with hearing loss showed benefits on speech perception in noise. Our study on older adults with hearing loss who use hearing aids, showed no benefits for speech perception in noise or emotion. However, in examining the baseline data we did observe correlations between musical skills (e.g., rhythm perception) and speech perception in noise. My current view is that musical training may benefit speech processing and perception but the effects are highly variable. Future correlational studies should assess musical skills and speech perception in large cohorts of participants. In addition, multi-site training intervention studies should be undertaken allowing for larger sample sizes and the multi-level modelling of group- and individual-level predictors.

Symposium #21 - New Frontiers in Genetic Testing for Hearing Loss

2:00 p.m. - 4:00 p.m.
Crystal Ballroom D-E

New Frontiers in Genetic Testing for Hearing Loss

Chair: Eliot Shearer, *Harvard Medical School*

Genetic Testing for Hearing Loss: Early Diagnosis Critical for Best Outcomes
Eliot Shearer, *Harvard Medical School*

Genetic testing for hearing loss is critical for etiologic diagnosis and has become the standard of care in evaluation of children with hearing loss. Hearing loss is the most common disorder identified on the newborn screen in the United States and earlier diagnosis leads to the best speech, language, and quality of life outcomes. Genetic newborn hearing screening is therefore the next frontier in reducing time to genetic diagnosis of hearing loss. This type of hearing screening has been developed, and is currently implemented, at some sites around the world. Early data from these genetic newborn hearing screening programs using targeted panels, exome sequencing, and genome sequencing will be presented. We will discuss progress and barriers towards widespread implementation of genetic newborn hearing screening.

Racial and Ethnic Disparities in Genetic Testing for Hearing Loss
Dylan Chan, *University of California, San Francisco*

Hearing loss is the most common congenital sensory impairment, with 1:500 children born deaf or hard-of-hearing (D/HH). Hearing-loss gene-panel testing (HL-GPT) is the most impactful and effective etiologic test for childhood sensorineural hearing loss (SNHL). Genetic diagnosis for hearing loss informs prognosis, provides early identification of syndromic associations, enables family counseling, and is the foundation for impending gene therapy. Racial/ethnic disparities in hearing-loss genetic testing access and outcomes are enormous: Latino and Black individuals with SNHL are 5 times less likely to receive a genetic diagnosis upon standard clinical HL-GPT compared with Whites and Asians. This difference is connected with a tremendous disparity among the populations that have been included in published hearing-loss genetic studies, with individuals of African or indigenous American genetic ancestry vastly underrepresented compared with their European and Asian counterparts. In this presentation, we will review the impact and extent of racial and ethnic disparities in hearing-loss genetics and genetic testing, and discuss the importance of greater inclusion of underrepresented minority groups in both clinical and research efforts going forward.

Non-Syndromic Hearing Loss Mimics: Beyond Usher and Pendred Syndrome
Shelby Redfield, *Boston Children's Hospital*

Syndromic hearing loss (SHL) accounts for approximately 30% of genetic diagnoses for pediatric hearing loss (HL) and has important implications for patients and families. Current clinical algorithms for genetic testing do not include all HL phenotypes, such as unilateral and asymmetric HL. Some recent studies suggest that a larger number of SHL genes than was previously appreciated can mimic a nonsyndromic HL phenotype, especially in early childhood. This raises the possibility of underdiagnosis of SHL. We identified cases of nonsyndromic HL mimics with genetic diagnoses of SHL in our pediatric patient population via retrospective chart review. While many SHL patients who presented with apparently isolated HL had Usher or Pendred syndrome, 12 patients with isolated HL received SHL diagnoses for conditions typically associated with additional features in early childhood. Pathogenic variants were identified in 8 genes (MITF, PAX3, SOX10, FGFR3, EYA1, LARS2, KMT2C, and TFAP2A). In some cases, subtle syndromic features were appreciated after genetic diagnosis. A majority of these 12 patients had unilateral or asymmetric HL. Of the 12 diagnoses, 6 had Waardenburg syndrome, 2 subjects had Muenke syndrome, and 1 subject had Kleefstra syndrome type 2, Perrault syndrome type 4, or branchiootorenal syndrome. One patient had isolated unilateral HL and a dual diagnosis of branchiooculofacial syndrome and mosaic chromosome 5p15.33p13.3 gain. Pediatric otolaryngologists are often the first specialist to see a child with subtle SHL after a newborn hearing screen referral. Increased access to comprehensive genetic testing for patients with any hearing loss phenotype within an otolaryngology pracitce will improve SHL diagnosis and facilitate early referral to appropriate specialists, tailored intervention, and improved prognostic and recurrence information for families.

In or Out: The Role of RNA-Splicing in Hereditary Hearing Loss
Kevin Booth, *Indiana School of Medicine, Dept. Medical and Molecular Genetics*

RNA-splicing is a highly complex and tightly regulated process. RNA-binding proteins (spliceosome proteins) recognize key nucleotide motifs, which allows for an orchestrated removal of intronic sequences and the formation of messenger RNA (mRNA). DNA variants which alter the highly conserved nucleotide motifs where the spliceosome protein members bind can result in mis-regulation of RNA-splicing and consequently the formation of mutant mRNA. Historically, only variants impacting the highly conserved donor or acceptor splice-sites (+/- 2 base pairs into the intron) have been considered as splice-altering variants. More recently, exonic coding variants and intronic variants (outside the canonical splice-sites), have been implicated in impacting RNA-splicing. Additionally, growing evidence suggests a large number exonic disease-causing variants attenuate wildtype RNA-splicing. Interestingly, not all coding variants that result in mis-splicing, have the same effect and often there are many mutant mRNA transcripts created. Outside the analysis of patient RNA, mini-gene in vitro splicing assays have become the cornerstone of understanding a variants impact on RNA splicing. Understanding a variants effect, is critical to providing an accurate genetic diagnosis. Furthermore, understanding a variants impact on RNA-splicing has important

therapeutic implications as defects in RNA-splicing can be corrected using Antisense Oligonucleotides. In this podium session, I highlight the in silco and in vitro workflows of analyzing a variants impact on RNA-splicing and highlight the importance of investigating coding variants impact on RNA-splicing using case studies.

Implications of Structural Variations for Hearing Loss Detected in Newborn Genome Sequencing Data
Matthew Hoi Kin Chau, *Baylor College of Medicine*

Genetic screening in congenitally deaf and hard-of-hearing (DHH) newborns at birth allows for early intervention and diagnosis which leads to improved speech and language outcomes, depending on the DHH etiology. Recently, compelling evidence has shown that genetic testing in newborn hearing screening (NBHS) positively impacted clinical outcomes. SEQaBOO (SEQuencing a Baby for an Optimal Outcome) is a program at Harvard Medical School evaluating genome sequencing and parental attitudes towards genomic medicine in DHH newborns following a positive NBHS or confirmatory diagnostic audiometry (1 month). SEQaBOO also incorporated optional reporting of ACMG secondary findings v3.0 for parents. While single nucleotide variants and small insertions and deletions (indels) in genes associated with nonsyndromic and syndromic DHH have been reported previously, a collaboration with the Chinese University of Hong Kong then investigated additional chromosomal abnormalities in the genome sequence data. Genome-wide copy number variations were detected from genome sequencing data of SEQaBOO participants using a read-depth-based approach. Structural rearrangements were detected by discordant read pairs. Absence of heterozygosity (AOH) was assessed by the rates of heterozygous genotypes. The additional value and clinical significance of structural variants (SVs) detected were investigated. Among SEQaBOO babies ultimately diagnosed as DHH (n=31), a causative genetic etiology (SNV and indels) was reported previously in 6 babies. The SV detection protocol in 81 families revealed incidental findings in 9 babies. These included one case of XYY syndrome and four cases of inherited heterozygous deletions of STRC and CATSPER2. Biallelic pathogenic variants or contiguous gene deletions across this region are associated with deafness-infertility syndrome. In one family, both parents carried heterozygous deletions of STRC and CATSPER2 and are at risk (1/4) of having affected offspring. Balanced chromosomal translocations were detected in two families who had conceived by IVF following multiple miscarriages. GS results fine mapped the breakpoints of these translocations to the nucleotide level, of which they disrupted three genes including CDK19, DYM, and PHF21B. These findings have implications for subsequent pregnancy management. Extended regions of absence of heterozygosity (AOH) on two chromosomes were detected in two probands, suggestive of uniparental disomy or remnants of identity by descent, warranting further follow up. SV detection in the genome sequencing data of the SEQaBOO study revealed additional clinically relevant findings. These data and others show the effectiveness of genome sequencing for SV detection in newborns with hearing loss.

Women, Gender, Minorities and Allies in Science Symposium
4:00 p.m. - 6:00 p.m.
Crystal C

Women, Gender Minorities, and Allies in Science
Chair: Tanvi Thakkar, *University of Wisconsin-La Crosse*
Co-Chair: Kelly N. Jahn, *University of Texas at Dallas*

Women are categorically underrepresented in Science, Technology, Engineering, and Math (STEM) careers. Underrepresentation is particularly problematic for early career women scientists who must navigate career advancement while facing gender-related stereotypes, unconscious biases, and social pressures that their male counterparts do not experience. The Association for Research in Otolaryngology (ARO) community currently lacks resources for women and gender minority members to form a united group. This symposium aims to provide a safe space for women, gender minorities, and male allies to come together to learn about systemic gender-related barriers in science and academia. The symposium will also serve as a platform for early career women in the otolaryngology field to showcase their research programs. As such, the goal of this event will be two-fold: (1) to inform the ARO community about gender-based biases in the sciences and

(2) to elevate the work of a few inspiring early-career women in our field. The symposium will host two keynote speakers from psychology, Dr. Grace Deason and Dr. Erica Srinivasan, who will provide their expert perspectives on women in science. They will be joined by four early-career women from the otolaryngology field who will showcase their own research programs, Dr. Kirupa Suthakar, Dr. Melissa Polonenko, Dr. Mishaela DiNino, and Dr. Maureen Shader. The symposium broadly appeals to the ARO community by fostering inclusivity, diversity, and intersectionality as it relates to women, gender minorities, and male allies in science and academia.

From Barriers to Opportunities: How Stereotypes Shape Women's Experiences in Science
Grace Deason, *Psychology Department, University of Wisconsin – La Crosse*

Women are stereotypically emotional and communal, whereas scientific careers are associated with objectivity and isolation. Psychological research demonstrates that this mismatch between a group stereotype and a social role leads to prejudice and can undermine women's interest and motivation. Barriers for Black and Latina women in science are greater than those faced by their white and Asian counterparts. Fortunately, studies have also begun to explore methods for minimizing the mismatch and mitigating bias. This talk will review psychological research on the effects of gender stereotypes in science and go beyond individual-level remedies to articulate creative institutional strategies for change.

Fine Tuning Auditory Processing at the Periphery: What Do We Gain From Feedback Circuitry?
Kirupa Suthakar, *NIH/NIDCD*

The ability to distinguish signal from noise is a fundamental feature of sensory processing. Our auditory systems are constantly bombarded with sounds of varying meaning and relevance. An accurate perception of our environment thus depends on the ability to correctly identify and contextually filter incoming sounds, resulting in an appropriate response, both perceptually and behaviorally. My research focuses on the mechanisms underlying gain control in normal hearing, congenital hearing loss and noise induced cochlear synaptopathy, broadly focusing on the two-way stream of information between the brain and ear via the primary afferent neurons and olivocochlear efferent pathways.

Auditory Processing and Perception Across the Lifespan
Mishaela DiNino, *University at Buffalo*

Auditory system physiology and function change across the lifespan, influencing perception of sound from birth to older adulthood. My program of research examines how those natural lifespan alterations interact with hearing loss and use of auditory prostheses to further affect auditory perception. I primarily focus on how these mechanisms contribute to speech perception - a process that is the cornerstone of human verbal communication but that is greatly impacted by variations in auditory system function. In this talk, I will describe investigations of the relationship between normal lifespan changes and processes important for speech understanding.

Assessing Auditory Neurodevelopment in Children With Normal Hearing and Hearing Loss
Melissa Polonenko, *University of Minnesota*

The human brain remarkably and flexibly uses a wide range of sensory inputs. While this flexibility enables a person to adapt to the environment, it also leaves them vulnerable to sensory loss, particularly during sensitive developmental periods. My research program investigates how young children with normal and impaired hearing learn to communicate and navigate within their world. This translational work includes developing new ways to assess multi-sensory function and to evaluate outcomes with hearing aids and cochlear implants. This talk will highlight recent and upcoming work that uses a variety of stimuli and techniques to promote hearing health in children.

Naturalistic Speech Processing in the Adult Auditory Cortex Immediately After Cochlear Implantation
Maureen Shader, *Purdue University*

In the human brain, functional changes can occur due to sensory deprivation. These changes could impair sensory processing following the reintroduction of sensory input, as in the case of cochlear implantation. This talk will present a study using a light-based neuroimaging method to investigate brain activity to naturalistic speech stimuli immediately after cochlear-implant activation. Methodological and analysis considerations will also be summarized which aim to improve the reliability of optical neuroimaging measurements on the individual-subject level. By maximizing its reliability, neuroimaging experiments have the potential to reveal individual differences in cortical processing that contribute to the variability in cochlear-implant outcomes.

Gender Discrimination and Gender Stereotypes: Exploring the Impact on Women in Academia
Erica Srinivasan, *University of Wisconsin – La Crosse*

Historically, women have faced major gender discrimination in academia, resulting in structural inequities such as underrepresentation in the sciences, gender disparities in high-status positions, gaps in attaining tenure and promotion, salary gaps, invisible labor, and unequal division of service work. The pandemic further exacerbated these issues, particularly among women caregivers. In this session, we will discuss some steps taken in an academic institution during the pandemic to address structural inequities. Additionally, we'll explore internalized gender stereotypes, ways in which they might impact both student and faculty experiences, and ways to build support.

gEAR Workshop - Explore and Customize II: Customizing Dataset Collections

5:00 p.m. - 5:45 p.m.
Biscayne 1

A primary feature of the portal is visualizing expression from multiple datasets side-by-side. This workshop teaches you how to find datasets in the gEAR and group them into your own collections, viewing them together on the search pages. This workshop includes a hands-on component, bring your laptop along.

Ronna Hertzano, *University of Maryland School of Medicine*
Joshua Orvis, *Institute for Genome Sciences*

Tuesday, February 14, 2023

Podium #22 - Illuminating Auditory Function and Dysfunction Through Genetics

8:00 a.m. - 10:00 a.m.
Ocean's Ballroom 5-12

Moderators: Karen Avraham and Cynthia Morton

A Novel Missense Variant in the GJB6 Gene Causes Autosomal Dominant Hearing Loss in Both Human and Mouse

Wenxue TANG*[1], Hongen XU[1], Sen ZHANG[1], Sang HU[1], Manman WU[1]
[1]*Precision Medicine Center, The Academy of Medical Sciences, Zhengzhou University*
Background: Hereditary deafness is the most common monogenic disease affecting all ages, ethnicities, and genders, and its diagnosis and pathogenesis-based treatment are challenging in the clinic. We found a five-generation postlingual hearing loss family from a large-scale diagnosis of hearing loss families in China.
Methods: Whole-genome SNP genotyping and exome sequencing were applied to detect genetic causes of HL in the family. Sanger sequencing was used to verify the segregation of the candidate variant. In order to confirm the pathogenicity of the variant GJB6 R75W, we successfully established a mouse model capturing the phenotype of the family by using gene-editing technology. We used the mouse model to determine how the R75W variant causes hearing loss by affecting the function of hemichannels and gap junctions.
Results: Whole-genome linkage analysis identified a region 13q11-13q14.13 with a LOD value of 3.02. Whole-exome sequencing of selected patients and normal individuals found a novel heterozygous missense

variant, c.223C>T (p.Arg75Trp) in the GJB6 gene was segregated with hearing loss phenotype. The Gjb6 R75W heterozygous mice were validated further by the autosomal dominant hearing loss displayed in all the animals (n=30), ABRs of mice measured at postnatal day 35 (P35) were significantly elevated across a frequency range of 4–32 kHz. The hearing loss in mice gradually worsened in the next few months. In contrast, the littermate-controlled pups without the Gjb6 R75W had normal hearing at P21, P30, and P60 across a frequency range of 4–32 kHz. Immunolabeling data and Western blot quantification in Gjb6 R75W mice all showed significantly reduced Cx26 protein levels in comparison to the littermate-controlled pups group. We subsequently investigated the time course and pattern of cell degeneration in the Gjb6 R75W mice and found that the gross morphology of the organ of Corti in the Gjb6 R75W mice was similar to that of WT mice. Degeneration of HCs in the Gjb6 R75W mice was much slower, and most OHCs in all cochlear turns were degenerated six months or longer after birth.
Conclusions: R75W variant in the GJB6 gene causes autosomal dominant hearing loss in both humans and mice.

A Novel Candidate ARHL Gene Detected in a UK Biobank Cohort GWAS Causes Progressive Hearing Loss in the Orthologous Mouse Knockout

Benjamin Silver*[1], Carlos Aguilar[1], Anwen Bullen[1], Michael Bowl*[1], Sally Dawson*[1]
[1]UCL Ear Institute
Background: In previous work, using a genome wide association study (GWAS) approach in the UK Biobank Cohort, we identified 44 genes associated with self-reported hearing loss in participants aged between 40 and 69 years (Wells et al. 2019). Thus, providing a candidate gene list for age-related hearing loss (ARHL) genes, which included 34 novel genes not previously linked to auditory function. As part of a post-GWAS evaluation of these novel candidate genes, that combined in-silico, in-vitro and in-vivo methods, we have identified an orthologous gene knockout mouse that displays a progressive hearing loss phenotype.
Methods: Auditory function was assessed in homozygote, heterozygote and wildtype mice using ABR and DPOAE recordings at different ages. Characterisation of the anatomy and morphology of the inner ear of these mice was assessed using wholemount preparations and vibratome sections, with immunostaining for cell-specific markers, including Myo7a and Sox2.
Results: Mice homozygous for the knockout allele demonstrate an early-onset progressive hearing loss. At 1-month of age, hearing loss is more pronounced at higher frequencies, with thresholds of 70 dB SPL observed at 32kHz compared with thresholds of <50 dB SPL recorded at 8kHz. By 2-months of age the hearing loss has progressed with mice exhibiting ~75 dB SPL thresholds across all frequencies tested (8, 16 and 32kHz). By 5-months, hearing thresholds are further elevated to 80 dB SPL across all frequencies. DPOAE recordings at this age show reduced emissions in mutant mice suggesting outer hair cell (OHC) dysfunction. Analysis of cochlear wholemount preparations show that hair cell number and organisation appear normal at P2-P4, whereas after the onset of hearing at P19-P21 a significant reduction in the number of basal OHCs is observed in homozygous knockout mice ($p<0.001$). By P58-P60 the number of OHCs present in homozygous mutants is significantly reduced throughout the cochlea from base to apex compared to wildtype ($p<0.001$), consistent with the progression of hearing loss to all frequencies. Inner hair cell numbers for mutant mice are not significantly different from wildtype mice. Examination of single cell RNA-seq data from the gEAR portal (https://umgear.org/) suggests that transcripts for this gene are highly enriched in hair cells. Ongoing work is focused on revealing the functional deficit underlying the progressive hearing loss through further in-vivo characterisation of the mutant mice and in-vitro studies involving transfection of tagged expression constructs in cell lines.
Conclusions: Characterization of a knockout mouse model for a novel candidate GWAS association detected in the UK Biobank Cohort identifies a role for this gene in auditory maintenance and/or protection and provides validation of the original GWAS approach using self-reported hearing data.
*Joint senior authors

Elucidation of Semidominant Inheritance of the Auditory Phenotype Related to p.R1939Q of OTOF

Yehree Kim*[1], Kyu Hee Han[2], Kwon-Woo Kang[3], Min Young Kim[1], Eunyoung Yi[3], Byung Yoon Choi[1]
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Background: Alterations in OTOF (DFNB9) explain 91% of radiologically intact auditory neuropathy spectrum disorder in the Korean pediatric deaf population. The most common mutant allele is p.Arg1939Gln accounting for 40% of the total mutant DFNB9 alleles in Koreans. The exon where the p.R1939Q variant resides is translated exclusively in the cochlea, suggesting that there could be potentially more deleterious effect of the p.R1939Q variant on the cochlea compared with other variants. We aimed to elucidate the dosage effect, if any, of the p.R1939Q allele on the cochlear ribbon synapses and auditory function both in humans and rodents.
Methods: Otof p.R1934Q knock-in(KI) mouse model modeling the p.R1939Q allele from humans was generated. In the rodent model, auditory brainstem responses, mRNA level, Otof protein level, synaptic components and spiral ganglion neuron population were measured and compared between wildtype, heterozygous and homozygous mice. In human experiment, gap detection and frequency discrimination tests were performed on heterozygous carriers of p.R1939Q allele and compared to age-matched controls.
Results: First, homozygous p.R1934Q KI mice did not show any ABR responses while OAEs were preserved, recapitulating the human DFNB9 phenotype. Significantly reduced Otoferlin protein levels were observed in homozygous mice despite no difference in mRNA levels. Interestingly, ABR wave I amplitudes of heterozygous R1934Q KI mice were significantly reduced over several suprathreshold intensities compared with wildtype controls. In conjunction with this, decreased synaptic components and SGN population were observed in the heterozygous mice, albeit to a lesser degree than in the homozygous mice. In parallel with these findings, gap detection and frequency discrimination abilities were decreased in the human heterozygous carriers of OTOF R1939Q despite normal hearing thresholds.
Conclusions: We first show the pilot data suggesting the presence of dosage effect of the p.R1939Q of OTOF on the cochlear synapse both in humans and rodents. This result may serve as a good example for elucidating the existence of semi-dominant inheritance in many autosomal recessive hearing loss disorders.

Early-Onset Hearing Loss and Increased Embryonic Lethality Due to Genetic Interaction With the Strain-Specific Cdh23ahl Allele

Sherylanne Newton[1], Walter Marcotti[2], Steve Brown[3], Michael Bowl*[4]
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Background: Cadherin 23 (CDH23) is an integral component of tip links, which are essential for mechanical gating of the transducer channels in response to sound-induced deflection of the stereocilia bundle. In humans, mutations in CDH23 are the cause of DFNB12 and USH1D. In mouse, many Cdh23 alleles have been reported, including the strain-specific Cdh23ahl allele that predisposes carrier strains to progressive hearing loss beginning at high-frequency from 3- to 6-months of age. This includes the two commonly used C57BL/6J and C57BL/6N strains.
Recently, we demonstrated that the neural cell adhesion molecule Neuroplastin genetically interacts with the Cdh23ahl allele through the previously well-described association between CDH23 and the Plasma Membrane Calcium ATPase (PMCA) proteins. Here we investigate if Embigin, a member of the same protein family as Neuroplastin, is also required for hearing and whether it genetically interacts with the Cdh23ahl allele.
Methods: To elaborate upon the genetic architecture of Cdh23ahl-associated hearing loss and investigate for pleiotropic effects, we have undertaken phenotypic characterization of an Embigin knockout mouse mutant (Embtm1b) on both a standard C57BL/6NTac background (C57BL/6NTac-Cdh23ahl) and a coisogenic background, where we have repaired the Cdh23ahl allele (C57BL/6NTac-Cdh23753A>G).
Results: Embtm1b/tm1b mice on the standard C57BL/6NTac background exhibit progressive high-frequency hearing loss beginning at 3-6 weeks of age, which is accompanied by a corresponding reduction in DPOAE output at 6-weeks of age. This loss of sensitivity occurred in the absence of: hair cell loss; stereocilia dysmorphology; ribbon synapse loss; or, any other gross cochlear defect. Furthermore, absence of Embigin resulted in a perinatal sub-viability, with just 12.8% of weaning-age mice genotyped as Embtm1b/tm1b. Whole body microCT showed that a large proportion of E18.5 Embtm1b/tm1b embryos had notable defects, including: narrowing of the brain ventricles; interventricular septum defects; and, cochlear malformations.
In stark contrast, Embtm1b/tm1b mice on the repaired C57BL/6NTac-Cdh23753A>G background display normal ABR and DPOAE thresholds up to 32-weeks of age, and are present at the expected Mendelian ratio.

Interestingly, unlike Neuroplastin, we could find no evidence of a physical interaction between Embigin and PMCAs using co-immunoprecipitation of cochlear lysates.
Conclusions: We demonstrate that Embigin interacts with the Cdh23ahl allele, causing high-frequency hearing deficits from as early as 1-month of age. Unlike in the case of Neuroplastin, the cause of hearing impairment in the Embigin mutant is not due to an interaction with PMCAs. In addition to these auditory deficits, we also find that co-inheritance of the Cdh23ahl allele with the Embtm1b allele also results in embryonic cardiac and brain abnormalities, associated with perinatal sub-viability. These findings are the first to show a role for Cdh23ahl in pathologies outside of the cochleae.
Our findings have important implications for everyone studying mutant alleles maintained on strains harbouring the Cdh23ahl allele.

A New Mouse Model for Wolfram Syndrome Illuminates the Fundamental Role of WFS1 in Endocochlear Potential Production

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Background: Wolfram syndrome is a neurodegenerative rare disease encompassing diabetes mellitus, diabetes insipidus, optic atrophy, hearing loss and neurological symptoms.
Variants in WFS1, a gene coding for a protein called Wolframin, account for 99% of WS cases, out of which 60 % will present with hearing loss. In addition to this autosomal recessive genetic disorder, WFS1 variants have been associated, in a dominant fashion, with non-syndromic low frequency sensorineural hearing loss (LFSNHL) as well as syndromic progressive hearing loss, diabetes mellitus, and optic atrophy, commonly denominated as Wolfram-like syndrome diseases.
Multiple models, carrying a variant or deletion of one exon of Wfs1 gene, have been developed. So far, none of the animal models (zebrafish, mouse, rat) of the pathology are presenting with an early onset hearing loss.
Methods: To understand the role of WFS1 in the auditory pathway, we generated a knock-in mice reproducing a human mutation leading to severe hearing loss in all affected individuals.
We analyzed the auditory and vestibular functions of our transgenic line, using ABR, DPOAEs and behavioral tests. Scanning electron microscopy, transmission electron microscopy and immunofluorescent labeling were used to characterize the cellular deficits. The study was completed with endocochlear potential measurements and stria vascularis characterization.
Results: The homozygous mice develop a progressive hearing loss as early as P23 and are profoundly deaf by P29, at all tested frequencies. The hearing loss is correlated with neurosensory hair cell loss but the spiral ganglion neurons do not seem affected by the mutation. Interestingly, deafness is concomitant with a severe vestibular syndrome, assessed by a panel of behavioral tests. This alteration is associated with a fusion of the stereocilia and the degeneration of the vestibular hair cells, recapitulating the phenotype seen in the organ of Corti.
In addition, we showed that the endocochlear potential of the mutant mice is greatly decreased compared to their wild-type littermates. Progressive vacuolization and alteration of the stria vascularis were determined with transmission electron microscopy analyses. More specifically, vacuoles are initially observed in intermediate cells, increasing rapidly in size, leading to the degeneration of both intermediate and marginal cells, as shown by immunofluorescent labeling. At P31, only the basal cells remain intact in the stria vascularis of the mutant mice.
Conclusions: In summary, we described a new mouse model of Wolfram syndrome presenting with a profound post-natal hearing loss, associated with a strong vestibular syndrome. Taken together, our data suggest an important role of WFS1 in the maintenance of the endocochlear potential and the stria vascularis, as well as the hair cell survival.

Cross-Species Transcriptome Analysis of Sensory Hair Cells

Mahashweta Basu*[1], Seth Ament[2], John Brigande[2], Alain Dabdoub[2], Albert Edge[2], Lisa Goodrich[2], Andrew Groves[2], Stefan Heller[2], Ronna Hertzano[2], Tatjana Piotrowski[2], David Raible[2], Yeohash Raphael[2], Neil *Segil[2], Jennifer Stone[2], Litao Tao[2], Mark Warchol[2]*

[1]*Institute for Genome Sciences, University of Maryland School of Medicine,* [2]*Hearing Restoration Project*
Category: Genetics B: General
Background: Hair cells (HCs) -- the mechanoreceptors of the inner ear -- exhibit considerable diversity in their morphology and function within and across species. Furthermore, and of clinical significance, cochlear HCs regenerate robustly in many non-mammalian vertebrate species, but not in humans and other mammals. Recently, single-cell genomics has emerged as a powerful technology to describe the molecular basis for cell-type diversity. Here, we undertook a comprehensive meta-analysis to describe the shared and unique features of auditory, vestibular, and lateral line HCs in four mammalian and non-mammalian species.
Methods: We performed an integrative analysis of single-cell transcriptomic data from human utricle; mouse utricle (P2, P7), cochlea (P1, P2, P7, P15), and cristae (P3, P7); chicken utricle (P7) and basilar papilla (P7); and zebrafish lateral line (5dpf). For harmonization across all 12 datasets, we linked genes to their orthologs in other species and then calculated the Area Under the receiver operating characteristic Curve (AUC) score as a measure of that gene's ability to distinguish HCs from supporting cells in each dataset. Finally, to determine commonalities and differences across the landscape of HC types in all species and organ types surveyed, we performed an unsupervised clustering analysis of genes with enrichment in at least one HC type. Clusters were independently validated using two independent datasets from adult zebrafish inner ear, one from mouse cochlea (P1), and from mouse utricle (P1).
Results: We identified 4977 genes enriched in at least one type of HC, as defined by an AUC score $\geq$0.7. Unsupervised clustering generated 21 clusters of genes with similar expression patterns across datasets. Patterns in these clusters suggest that species differences are more prominent than those between auditory and vestibular subtypes within a species. In addition to species-specific clusters, 4 clusters contained candidate pan-HC genes, with high average AUC scores in most HC types; these genes also showed higher AUC scores in four independent datasets used for validation. Among these putative pan-HC gene clusters, one cluster contained 110 genes, including 15 deafness genes and 9 known HC markers. Further, among 54 genes in second cluster, 42 were independently identified as enriched in HCs, based on the literature and analysis of datasets in the gEAR (umgear.org). This analysis revealed several novel HC-enriched genes.
Conclusions: These results provide a platform to compare HC responses to damage in different species, identify new deafness genes, gain insights into universal HC functions such as mechanotransduction, and assay the outcome of efforts to regenerate HCs from supporting cells in organoids or in vivo. A comprehensive profile allowing direct comparison of gene expression across species has been built in the gEAR (umgear.org) to facilitate data sharing.
Supported by the HRP: https://hearinghealthfoundation.org/hearing-restoration-project

Podium #23 - Psychoacoustics

8:00 a.m. - 10:00 a.m.
Oceans Ballroom 1-4

Moderators: Vibha Viswanathan and Antje Ihlefeld

A Musicians' Advantage for Interaural-Level-Difference Discrimination Only Among Highly Trained Musicians Who Started Training Early and Continued to Play

Beverly A. Wright*[1], Huanping Dai[2]
[1]*Northwestern University,* [2]*University of Arizona*
Background: Musicians outperform non-musicians on some auditory tasks. This musicians' advantage has been observed for tasks that are closely related to musical elements such as pitch (frequency discrimination) and rhythm (temporal-interval discrimination). What remains largely unknown is the extent to which this advantage extends to basic auditory abilities not closely related to music. To help fill this gap, we compared the performance of musicians and non-musicians on two auditory skills associated with sound localization that are not typically emphasized in musical training: the discrimination of interaural-level-differences (ILDs) and the discrimination of interaural-time-differences (ITDs).
Methods: We tested two separate groups of young adults on ILD discrimination at 4 kHz (n=246) and ITD discrimination at 0.5 kHz (n=137). We recruited the participants without regard to their musical-training histories, but each participant completed a questionnaire from which we determined his/her years of musical training, starting age of training, and years since training ceased.

Results: Initial analyses of the entire data set based solely on the number of years of musical training revealed no apparent musicians' advantage for either ILD or ITD discrimination. However, subsequent comparisons of more selected groups—non-musicians (<2 years of training) and extreme musicians (10 or more years of training, started training at or before age 7, still playing)—revealed a musicians' advantage for ILD discrimination. This advantage appeared to depend on both starting training early (at or before age 7) and still playing, as revealed by comparisons of ILD-discrimination thresholds across the extreme musicians (n=22) and three other groups of highly trained musicians (10 or more years of training) who: (1) started training late (after age 7), but were still playing (n=11); (2) started training early (at or before age 7), but had ceased playing (n=40); or (3) started training late and had ceased playing (n=11). There was no apparent musicians' advantage for any of the parallel analyses for ITD discrimination.
Conclusions: The present observations of a musicians' advantage for ILD discrimination, but not for ITD discrimination lead to three main conclusions. First, the set of restricted circumstances under which the musicians' advantage for ILD discrimination was observed—namely, extensive musical training, starting training at an early age, and still playing--suggests that nurture contributes to the musicians' advantage on this task. Second, the apparent absence of a musicians' advantage for ITD discrimination, in contrast to ILD discrimination, suggests that ILD processing is more malleable than ITD processing. This conjecture is consistent with previous reports of sex differences and learning for ILD discrimination but not for ITD discrimination. Finally, the observation of a musicians' advantage for ILD discrimination suggests that the musicians' advantage can extend to at least some basic auditory abilities that are not central to music.

Perceptual Benefits From Long-Term Exposure to Naturalistic Sound Patterns

Bruno Mesquita*[1], Björn Herrmann[2], Casey Roark[3], Ingrid Johnsrude[4]
[1]University of Western Ontario, [2]Rotman Research Institute, [3]University of Pittsburgh, [4]The Brain and Mind Institute, Western University
Background: Our brains are proficient in capturing regularities - that is, recurring structure - in the environment to optimize perceptual inferences based on relevant information in stochastic input. Sensory information is multi-dimensional, and the relationship between sound dimensions may be, in itself, a source of information.

Many sounds in our environment covary dynamically. For example, higher-pitched music or speech tends to have a faster tempo (Broze and Huron 2013, Topbas et al 2012). Such patterns may be associated with illusory effects on perception where music and speech also tend to be perceived as faster when only pitch is increased (Boltz, 1998; Bond and Feldstein, 1982). These covariances and their perceptual effects may be learned through life experience. Covariance in stimulus dimensions may aid perceptual decisions and perceptual organization by providing redundancy, especially in noisy and ambiguous listening conditions. Here, we investigate how long and short-term learning of statistical regularities in sounds may shape our ability to categorize them and to perceptually segregate maskers from target speech.
Methods: Two online experiments were conducted on native English speakers aged 18 to 35 years. Auditory stimuli consisted of 3-s sinusoidally amplitude-modulated (AM) inharmonic tone complexes for which the AM rate, and the frequency of all individual components, either increased or decreased linearly over the duration of the sound. AM rates, starting frequency, and rate of frequency change were random, but stimuli always had positive (pCO) or negative (nCO) covariance across the two dimensions of AM rate and frequency change. In Experiment 1, participants grouped stimuli into categories defined by the relationship between the two dimensions. Because of long-term prior knowledge, we predicted that people would learn to categorize pCO sounds (both dimensions increasing or decreasing) faster than nCO sounds. In Experiment 2, participants were first familiarized with either pCO or nCO exemplars, and then reported words they heard from target speech (using the BUG corpus; Kidd, Best, and Mason, 2008) masked by novel pCO and nCO exemplars. We predicted that people would be more successful at reporting words masked by sounds with a familiar covariance structure.
Results: Preliminary results of Experiment 1 indicate better categorization of pCO compared to nCO sounds, perhaps reflecting lifetime experience with pCO sounds. In Experiment 2, familiarity with the masker did not benefit target identification.
Conclusions: Previous work has shown that long-term priors related to regularities in our environment influence behavior in categorization paradigms that rely on associations in static acoustic dimensions (Roark and Holt, 2022, Roark and Holt, 2019b). Here, we demonstrate that this effect extends to dimensions that

change over time. However, our results indicate that these effects either do not extend to benefits for sound segregation, or such benefits are not captured by our experimental design.

Probing Sensitivity to Statistical Structure of Rapid Sound Sequences Using Deviant Detection Tasks

Alice Milne*[1], Christopher Conway[2], Maria Chait[3]
[1]University College London, [2]Boys Town Research Hospital, [3]UCL Ear Institute

Background: The brain is highly sensitive to auditory regularities. We exploit the predictable order of sounds in many scenarios, from auditory scene analysis to language acquisition. To navigate the world, we must be able to predict upcoming events and rapidly detect unexpected occurrences.
In rapidly unfolding auditory stimuli, the context in which we encounter an unexpected event will impact its saliency: the brain shows a stronger deviance response when an unexpected tone frequency occurs in a sequence that has a regular (deterministic) repeating pattern, compared to a randomly ordered set of tones (Southwell and Chait,2018, Cortex). This suggests that our representation of ongoing sensory information shapes the processing of incoming information.
Methods: Over a series of experiments, we presented sequences of rapid 50ms tone pips that were either arranged randomly or followed an underlying statistical structure. We studied how the context (sequence type) would affect detection of a deviant tone that occurred outside the frequency range of the main sequence. Notably the speed of the stimuli prevented conscious identification of the statistical structure.
Results: Behavioral experiments tested how two different types of statistical structure would impact deviance detection rates and reaction times. The first experiment manipulated Transitional Probabilities (TPs) by presenting triplets of tones in a random order (Saffran et al., 1996, Science), a common paradigm in the field of statistical learning. The same methodology was then used to look at sequences generated from a "community" structure that cannot be recognised through simple TPs. Consistently we found that the deviant tone was easier to detect when there was a predictable structure, exhibited through either better detection rates, faster reaction times or both. These results demonstrate that deviant detection tasks provide a useful way to measure learning as it unfolds and provides evidence for how different types of statistical structures affect learning.

We also inspected how the neural response to the deviant tone would be affected by the context. In an EEG study we presented random, probabilistic (Saffran-based) and also deterministic sequences that contained an outlying frequency as a deviant. During this paradigm participants were distracted and not actively attending to the deviants. Using a cluster-based permutation analysis we identified ROIs in time-channel space that corresponded with the N1-P2-N2 waveform typically seen to a change in an ongoing sound. The amplitude of these peaks was modified based on the context of the deviant tone in terms of both the sequence type in which it occurred and the predictability of the sequence at the moment of deviation.
Conclusions: In sum, these results suggest that the brain rapidly forms predictions about upcoming sounds and modifies its response to deviations based on the reliability of that information. The findings are discussed in relation to the predictive coding framework.

Statistical Regularities Across Task-Irrelevant Dimensions Impact Auditory Decisions

Austin Luor[1], Sahil Luthra[1], Barbara Shinn-Cunningham[1], Adam Tierney[2], Frederic Dick[3], Lori Holt*[1]
[1]Carnegie Mellon University, [2]Birkbeck College, University of London, [3]University College London

Background: Listeners build up statistically-driven expectations of what they will hear. However, there is no consensus on how input regularities influence perception, attention, and behavior. Here, we examine how statistical learning across the global probability of sound events influences dimension-selective attention, an important form of auditory attention that is understudied compared to the classic "cocktail party" scenarios involving selective attention to one sound source competing with another.
Methods: Building from classic psychophysics, we manipulate the global probability of tones' acoustic frequency across two tasks: suprathreshold duration identification and near-threshold tone-detection-in-noise. In each, frequency is ostensibly task-irrelevant. For duration decisions, participants simply report whether a tone duration is short or long (50 vs. 90 ms; duration decision). For tone-in-noise detection, participants report which of two intervals of continuous noise contained a near-threshold tone. In each task, we manipulate the global probability of tone frequency across trials. Together, the tasks afford an

opportunity to understand how listeners accumulate regularities across a dimension (frequency) and how this statistical learning might influence dimension-selective attention to frequency and infrequent events.
Results: We find that the global probability of tone frequency influences each task. Duration decisions are faster and detection decisions are more accurate for high-probability tones compared to low-probability tones. The influence of global probability builds quickly and switches rapidly with changes in probability. Even a seemingly 'neutral' equiprobable distribution influences behavior in the context of a switch to statistics that bias probability prior to or after experiencing the 'neutral' statistics. Moreover, a bimodal distribution of frequency probabilities speeds duration decisions and improves tone-in-noise detection accuracy at each of the two higher-probability modes. Yet behavior is not strictly tied to experienced input regularities. Among low-probability tones, those nearer to a more-probable frequency are detected more accurately in noise and faster in duration decisions than more distant frequencies, convergent with expectations of an attentional filter. Compellingly, there is high convergence of results across the distinct tasks. Examination of the tone-in-noise detection task, for which there is a 'baseline' for threshold detection, suggest that both facilitation for high-probability sounds and suppression of low-probability sounds play a role in eliciting these patterns of results.
Conclusions: Overall, the reported experiments underscore that statistical structure naturally shapes the features of sound to which a listener attends. Moreover, this dimension-selective attention is automatic and obligatory, thereby helping listeners to focus on sound features that reliably encode information in a particular statistical setting. At the broadest level, examination of statistical learning of task-irrelevant dimensions offers a productive approach to determining how statistical learning evolves across active behavior, provides a strong foundation for dissociating competing theoretical accounts, and offers a link to animal neurobiological models of frequency-selective attention.

Cooling Visual Cortex Differentially Impacts Multisensory Responses Across Regions of Ferret Auditory Cortex

Rebecca Norris*[1], Stephen Town[1], Katherine Wood[2], Jennifer Bizley[1]
[1]UCL, [2]University of Pennsylvania
Background: Multisensory integration is evident across many contexts and species, with temporal coherence proposed as a key mechanism facilitating the formation of cross-modal objects. Integration of multisensory stimuli at the neural level has been demonstrated across cortical and subcortical areas. In the ferret, some neurons in auditory cortex (AC) respond to visual stimuli, and in others, responses to sound can be modulated by visual stimuli. AC receives input from several potential sources of visual information, including visual cortex. Here, we investigated the role of a sub-region of visual cortex (the suprasylvian cortex, SSY, and adjacent area 21) in multisensory integration. SSY and area 21 send dense projections to AC, particularly to the anterior ectosylvian gyrus (AEG).
Methods: To assess the functional relevance of these connections, we recorded the responses of AC neurons to auditory, visual and concurrent audiovisual stimuli before, during and after transient inactivation of SSY via cortical cooling in ferrets under ketamine-medetomidine anaesthesia.
Results: We identified 473 stimulus responsive units, 337 (71%) of which were responsive to broadband noise, and 167 (35%) of which responded to a white light flash. 68 units (14%) responded to both sound and light, and 264 showed interaction effects when auditory and visual stimuli were concurrent. Cooling SSY impacted 41% (70/169) of visually responsive units, most of which exhibited decreased responses. However, we also recorded 3/70 units in which responses emerged or increased during cooling. We found that a higher proportion of units recorded from AEG were visually responsive or multisensory and impacted by cooling, as compared to primary or posterior regions of AC. Visual responses were evident across all cortical layers, with infragranular layers exhibiting the greatest proportion of visually responsive units.
Conclusions: Our findings support a functional role for both excitatory and inhibitory effects of visual cortex on audiovisual integration in AC, while also implicating the involvement of additional pathways.

Cortical Neural Processing Under Auditory-Cognitive Load Exceeds Response Time

Brilliant Brilliant*[1], Yifat Yaar-Soffer[2], Yael Henkin[2], Andrej Kral[1]
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Background: Most communication in everyday life takes place in adverse listening conditions. The impact of sensory-perceptual load (S-P) (e.g., background noise, competing talkers) and auditory-cognitive (A-C) load (i.e., task demands related to cognitive functions, such as working memory and attention) on these underlying neural processes requires further investigation. Moreover, the duration of the underlying neural processes is unknown. The objectives in this study were to identify biomarkers related to cognitive load and identify brain processes related to these biomarkers.
Methods: In this study electroencephalographic recordings (64 channels) have been obtained from young normal hearing adult listeners (n = 20). Two auditory tasks, a vowel identification task and an auditory Stroop task, were presented in two listening conditions, quiet and noise. Time-frequency representations using wavelet analysis were derived and both evoked and induced responses were analyzed. The time frequency representation of the vowel identification task was compared to the Stroop task (congruent condition) to identify the A-C load effect, while a comparison between the two listening conditions, in quiet and in noise, revealed the S-P load effect.
Results: Stimuli in both vowel identification- and Stroop task evoked responses mainly in delta-theta bands right after the stimulus onset (~0 – 300 ms from stimulus onset). Alpha desynchronization and theta and beta synchronizations were observed in the induced domain, indicating extensive post-response processing seconds after the behavioral response (~300 – 2000 ms). Comparison between the impact of S-P and A-C load revealed that higher S-P load (noise vs. quiet) decreased the evoked theta power, increased the induced activations in alpha-beta frequency band, and further increased the induced theta power after the response. Higher A-C load (Stroop task vs. vowel identification task) resulted in an increased, long-lasting theta activity (0 – 2000 ms).
Conclusions: The present methodology exposed biomarkers of high-level auditory processing during adverse listening conditions. The time frequency representation shows that neural activities covered the entire observed time, including seconds after the behavioral response. These activities differed between S-P and A-C loads, suggesting distinct underlying neural processes.
*Brilliant and Yaar-Soffer contributed equally to the study

Studying the Effect of the Efferent System on Auditory Overshoot Using a Computational Subcortical Auditory Model

Afagh Farhadi*[1], Laurel H. Carney[2]
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Background: Physiological studies show that listeners' performance in detecting a brief tone in a simultaneous wideband noise improves when there is a long delay between the onset of the tone and the masker. This phenomenon is referred to as auditory overshoot. A model for overshoot based on medial olivocochlear (MOC) efferent activity was studied here, because the efferent system's time constant is consistent with the dynamics of auditory overshoot.
Methods: The model used here was an updated version of the efferent model proposed in Farhadi, et. al. (ICASSP 2021 pp. 291-295) which includes the MOC efferent system that dynamically controls cochlear gain. The MOC controls cochlear gain based on the ascending input from the wide-dynamic-range cells in the brainstem and the auditory-nerve (AN) fluctuation information in the descending feedback from midbrain cells. Model perceptual thresholds were estimated based on the discharge rate of model band-enhanced neurons in the inferior colliculus (IC); these neurons are excited by fluctuations on their inputs. Thresholds were found using simulations of the method of constant stimuli, using a two-interval task with a 500-ms silence between intervals and a criterion of 70.7% correct. The decision variable was set to find the maximum IC rate in a short time window around the onset of the brief target tonal signal. The tone duration was 10 ms and the frequency of the tone was 4 KHz. The delays between the onset of the noise and the onset of the tone for short and long delay conditions were 2 ms and 200 ms, respectively. The difference in the threshold for the two delay conditions was measured as the overshoot effect for a range of tone levels (40-90 dB SPL).
Results: Similar to the psychoacoustic thresholds, the thresholds of the model with efferents were improved for the longer delay condition compared to the shorter one. In contrast, the thresholds for a model without efferents showed the opposite trend: thresholds worsened with increasing delay between the noise onset and the target tone. The thresholds for the models with and without efferent were similar for the short delay

condition. This finding is consistent with our understanding of the dynamics of the efferent system; 2 ms is too short for this system to change the cochlear gain.
Conclusions: The simulated responses of a subcortical auditory model with and without MOC efferent gain control were used to study the overshoot phenomenon. The simulation results support the hypothesis that the efferent system is a potential mechanism underlying the overshoot phenomenon.

Podium #24 - Inner Ear: Anatomy and Physiology

8:00 a.m. - 10:00 a.m.
Crystal Ballroom D-E

Moderators: George Burwood and Clark Elliott Strimbu

Cholesterol Metabolism and Trafficking in the Organ of Corti

Yuna Werchner*[1], Roos Voorn[2], Christian Vogl[3], Tobias Moser[2], Lina Maria Jaime Tobon[1]
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Background: Cholesterol is a crucial component of animal cells, where it stabilizes and stiffens the membrane and modulates ion channel function. These two functions are important for synaptogenesis and synaptic transmission, which rely on active zones with proper localization, spatial organization and correct function of their molecular players such as voltage-gated Ca2+ channels. Such properties might be particularly crucial at high-throughput synapses – such as the ones between cochlear inner hair cells (IHCs) and afferent spiral ganglion neurons.
IHCs are the sensory cells that transduce sound into electrochemical signals to trigger afferent activity in the auditory pathway and thus make hearing possible. IHCs are found in the organ of Corti, a highly organized sensory epithelium that consists of IHCs, outer hair cells (OHCs) and a variety of supporting cells (SCs). It has previously been shown that disruption of cholesterol homeostasis within the organ of Corti leads to hearing impairment. On a cellular level, cholesterol disruption results in dysfunction of OHC electromotility and IHC potassium channels as well as in a structural collapse of hair bundles.
To date, physiological cholesterol homeostasis and its key players within the organ of Corti remain poorly understood. We hypothesize that IHCs require high amounts of cholesterol to mature synapses and maintain their high and sustained synaptic activity. We further hypothesize that IHCs rely on exogenous cholesterol provided by SCs to satisfy this demand and that the level of de novo cholesterol synthesis declines with advancing age. Our aim for this study was to characterize the synthesis, uptake and trafficking of cholesterol within the organ of Corti.
Methods: To study the synthesis of cholesterol, we performed RNAscope, single cell RNAseq and single cell RT-qPCR to compare the expression levels of enzymes involved in cholesterol synthesis among IHCs, OHCs and SCs. In order to study the uptake and trafficking of cholesterol, we performed live cell imaging of organotypically-cultured organs of Corti incubated in fluorescent cholesterol analogs.
Results: Our results show that IHCs and OHCs take up cholesterol from the extracellular medium into vesicular organelles located at the cellular apex. In comparison, the surrounding SCs display less cholesterol uptake. To show that this uptake is a regulated mechanism that depends on cellularly available cholesterol, we disrupted cholesterol homeostasis by depleting cholesterol from the membrane or inhibiting its intracellular trafficking via the endolysosomal pathway. Both manipulations significantly increase the uptake of cholesterol in the organ of Corti.
Conclusions: Our study indicates that IHCs and OHCs can take up cholesterol from the extracellular medium via the endolysosomal pathway and in quantitative relation to their cellular cholesterol needs. This supports our hypothesis of an active, regulated cholesterol homeostasis in the organ of Corti.

Intrinsic Mechanical Sensitivity of Mammalian Auditory Neurons Contributes to Sound-Driven Neural Activity

Maria C Perez-Flores*[1], Eric Verschooten[2], Jeong Han Lee[1], Hyo Jeong Kim[1], Philip X Joris[2], Ebenezer N Yamoah[1]
[1]Physiology and Cell Biology, University of Nevada, Reno, [2]Laboratory of Auditory Neurophysiology, Medical School, Campus Gasthuisberg, University of Leuven, Leuven, Belgium

Background: Mechanosensation is the basis for hearing, balance, and touch sensory systems. Mechanosensation is unmatched in speed and its diverse range of sensitivities, reaching its highest temporal limits with the sense of hearing; however, hair cells (HCs) and the auditory nerve (AN) serve as obligatory bottlenecks for sounds to engage the brain. Like other sensory neurons, auditory neurons use the canonical pathway for neurotransmission and millisecond-duration action potentials (APs). The auditory system utilizes relatively slow transmission mechanisms to achieve ultrafast speed and high audio-frequency hearing. Here, we address this paradox and report that the mouse, and chinchilla, AN are mechanically sensitive, and minute mechanical displacement profoundly affects its response properties. Sound-mimicking sinusoidal mechanical and electrical current stimuli affect phase-locked responses. In a phase-dependent manner, the two stimuli can also evoke suppressive responses.
Methods: In vitro experiments were performed using mice and chinchillas. Spiral ganglion neurons (SGNs) were enzymatically and mechanically isolated and maintained in culture for 2-5 days. In vivo experiments were achieved on both sexes' adult wild-type chinchilla (Chinchilla lanigera), free from a middle ear infection and weighing between 200 and 400 g.
Results: Using in vitro simultaneous whole-cell recordings and mechanical stimulation, we showed that adult SGNs are mechanically sensitive. Mechanical stimulation of the cell body elicited an inward current, which reverses at ~0 mV. Mechanically activated inward currents and membrane voltage responses were sensitive to GsMTx4 peptide, a mechanosensitive channel blocker—simultaneous sinusoidal stimulation with current injection and mechanical displacement altered firing rate and temporal coding. In vivo, single-unit recordings from AN fibers demonstrated that 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline (NBQX) and Ca2+ channel blockers, potent inhibitors of synaptic transmission, suppress spontaneous APs, but sound-evoked APs persisted at high intensities. These findings suggest that AN fibers' intrinsic mechanical sensitivity contributes to sound-evoked activity and ill-understood features such as multi-component AN tuning curves and peak-splitting.
Conclusions: We propose that mechanical sensitivity interacts with synaptic responses to shape responses in the AN, including frequency tuning and temporal phase locking. Combining neurotransmission and mechanical sensation to control spike patterns gives the mammalian AN a secondary receptor role, an emerging theme in primary neuronal functions. Furthermore, the sensitivity of AN fibers to displacements or pressure differences suggests new treatment strategies for the hearing impaired and that a mechanical interface can provide an additional activation mode to supplement current strategy.
Grants to ENY supported this work from the National Institutes of Health (DC016099, DC015252, DC015135, AG060504, AG051443). PXJ was supported by grants from KU Leuven (BOF, OT-14-118) and Fonds Wetenschappelijk Onderzoek (Research Foundation Flanders) G0B2917N.

Knockout of the Kv1.8 Channel Subunit Affects Vestibular Type I and II Hair Cell Receptor Potentials and Vestibulomotor Functions

Hannah Martin*[1], Omar Lopez-Ramirez[1], Dana Silvian[1], Emily Scott[1], Anna Lysakowski[2], Ruth Anne Eatock[1]
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Background: Distinct basolateral voltage-gated K+ conductances (g) distinguish type I and II vestibular hair cells (HCs). Additionally, large calyx synapses envelop type I HCs while smaller bouton terminals contact type II HCs. We have found that Kv1.8 subunits are necessary for both the low-voltage-activated g-K,L of type I HCs and the rapidly activating, rapidly inactivating g-A of type II HCs. Constitutive knockout of Kv1.8 (Lee et al. Hear Res 300:1, 2013) greatly increased the membrane time constant in both HC types. We expect slower membrane charging times to have the strongest impact on HC responses to relatively fast head motions above 10 Hz, towards the upper range of natural head motions (Carriot et al. 2017 J Physiol 595:2751). Here, we present results on how absence of Kv1.8 affects early and late stages in vestibular function: HC receptor potentials and certain vestibulomotor behaviors. Any effects likely reflect Kv1.8 absence from the inner ear, given the low expression of Kv1.8 in other tissues (Lee et al., above).
Methods: We recorded from semi-intact utricles excised from Kv1.8-null mice and wildtype and heterozygous littermates (postnatal days 10-60). In vivo, inertial forces during horizontal acceleration or head tilt deflect utricular hair bundles; we used stiff probes to displace hair bundles with step or sinusoidal waveforms, and whole-cell patch clamp to record HC current and voltage responses. In Kv1.8-null and heterozygous littermates (2-6 months), we tested various activities that involve vestibular input: swim, open field, balance beam, and rotarod.

Results: Absence of Kv1.8 increased response gain but slowed it. Maximal receptor potentials increased from 5-10 mV to ~40 mV in type I HCs and from ~40 mV to ~50 mV in type II HCs. Receptor potential lowpass corner frequencies fell from >100 Hz to ~20 Hz in type I HCs and from ~70 Hz to ~25 Hz in type II HCs. For stimuli >10 Hz, absence of Kv1.8 significantly increased phase (timing) lag of the receptor potential in both hair cell types.
The battery of vestibular function tests revealed motor and balance abnormalities in Kv1.8-null mice. Kv1.8-null mice did not maintain horizontal swimming posture, were less likely to rear and jump, and showed subtle deficits while crossing a narrow beam, such as frequently wrapping their tails around the beam to increase stability. No difference was seen on the rotarod in the light.
Conclusions: Absence of Kv1.8 augmented but slowed the receptor potentials of both type I and II hair cells to stimuli in the frequency range of natural head motions. These large changes may contribute to abnormal performance of Kv1.8-null mice on some challenging balance tasks.
Supported by R01 DC012347 (RAE and AL) and NSF GRF (HRM). We thank S. Jones and T. Friedman for sharing Kv1.8 mutant mice.

Novel Pathophysiological Mechanisms in Age-Related Hearing Loss

Sonal Prasad*[1], Marja Pitkanen[1], Anders Fridberger[1]
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Background: There are four different types of Presbycusis / Age-related hearing loss (ARHL), sensory, neural, mechanical and metabolic. The metabolic type is closely linked to stria vascularis degeneration and loud sound exposure. Ca2+ dependent proteins are implicated to be related to strial ion transport. We established that changes in Tectorial membrane (TM) Ca2+ and temporary hearing loss after loud sound exposure is related. As TM is an acellular structure that lacks active mechanisms for Ca2+ regulation which implies that Ca2+ pumps located elsewhere in the inner ear are physiologically important. The stria vascularis, TM attachment to the modilus, and the stereocilia of sensory cells are known to express Ca2+ transporters such as NKCC, TRPV5 and 6, PMCA1 pumps.
There has been no clear evidence that how extracellular Ca2+ modulates the function of organ of Corti and hence Ca2+ transporters and ion channels could be physiologically relevant. We hypothesize that how sustained Ca2+ changes in both TM and extracellular fluids contributes to the development of metabolic ARHL. To test our hypothesis, infusions of Furosemide was used which interferes with adenylate cyclase and Na+/K+-ATPase and inhibits NKCC transporter in the stria vascularis and spiral ligament.
Methods: We investigated our novel hypothesis by a combined in vivo and in vitro approach using guinea pigs. In guinea pigs, cochleostomy was performed where osmotic mini pumps were used to deliver Furosemide (5 mg/ml) at the rate of 0.5 µl/hr to the cochlea through a basal opening. Hearing and organ of Corti functionality was assessed after 7 days using in vivo physiological recordings, ex vivo cochlear mechanics recordings, time-resolved confocal microscopy, fluorescence correlation spectroscopy and high-resolution confocal imaging.
Results: We found profound reduction in sound-evoked responses originated by different cell types in the cochlea shown through ABR, DPOAEs, CAPs, 3-tone suppression and decline in cochlear mechanics displayed through CM, SP, and EP with depressed bundle deflection. Morphological damages in the hearing organ (OHCs, SCs, IHCs, TM, RM, Stria) were observed. Using FCS, we determined higher Ca2+ level in TM for operated reference animals while in TM and endolymph for Furosemide treated indicated lower Ca2+ level.
Conclusions: Collectively, the results demonstrates that how functional changes in the organ of Corti leads to sustained changes in the extracellular Calcium level contributing to metabolic ARHL.

The Role of MYO7A Isoforms in Tuning Hair Cell Function

Sihan Li*[1], Andrew Mecca[2], Jeewoo Kim[1], Guisy Caprara[3], Jinho Park[4], Elizabeth Wagner[1], Jonathan Bird[4], Anthony Peng[2], Jung-Bum Shin[1]
[1]University of Virginia, [2]University of Colorado Anschutz Medical Campus, [3]University of Colorado, [4]University of Florida

Background: In hair cells, tip-link tension is essential for the sensitivity of the mechano-electrical transduction (MET) process. Our previous study provided evidence that the unconventional Myosin VIIa (MYO7A) is the molecular motor that tensions the MET complex. We further established that MYO7A isoforms with unique N-terminal extensions are differentially expressed in inner and outer hair cells (IHCs

and OHCs), correlating with reported differences in tip-link tension. The goal of the present study was to explore the hypothesis that the differential expression of functionally distinct MYO7A isoforms directly affects hair cell physiology such as tip-link tension and resting open probability, and hearing sensitivities across different frequencies. We also sought to identify cis- and trans factors that regulate Myo7a expression.
Methods: 5' RACE was performed to identify MYO7A isoforms expressed in hair cells. Isoform-specific MYO7A deletion or affinity tagged mouse lines were generated by using CRISPR/Cas9. MET currents were recorded in response to fluid jet stimulations, and hair bundle motion was monitored by a high-speed camera. SEM and immunofluorescence microscopy were used to investigate hair bundle morphology. ABRs and DPOAEs were measured to test hearing. Vestibular functions will be analyzed using animal pose estimation algorithms based on deep learning.
Results: We identified multiple isoforms of Myo7a in the inner ear. Two isoforms were identified by using 5' RACE: a widely studied canonical isoform (MYO7A-C) and an unreported novel isoform (MYO7A-N). To study the expression and functional differences of MYO7A isoforms, we generated mouse lines in which MYO7A isoforms are genetically deleted or tagged. Immunofluorescence analyses of these mice indicated that IHCs predominantly express MYO7A-C, and much lower levels of MYO7A-N. In OHCs, MYO7A-C and MYO7A-N are expressed in opposing gradients along the tonotopic axis. Moreover, simultaneous deletion of both MYO7A-C and MYO7A-N results in nearly abolished MYO7A immunoreactivity and disorganized hair bundle. Thus, we conclude that MYO7A-C and MYO7A-N are the major isoforms in the auditory system. Our patch-clamp experiments on Myo7a-ΔC and hair cells showed a significant reduction of resting open probability in IHCs, consistent with the proposed role of MYO7A in generating tip-link tension. MET in Myo7a-ΔN OHCs was also altered. We have plans to test whether MYO7A isoforms differ in their intrinsic motor properties in vitro.
Finally, we also report the discovery of a novel enhancer element and a transcription factor candidate involved in regulating Myo7a expression in a tonotopic manner.
Conclusions: Our studies reveal an unexpected isoform diversity of MYO7A in the cochlea and highlight their essential roles in tensioning the MET complex. The differential expression of MYO7A isoforms with distinct motor properties might contribute to the tonotopic gradient of tip-link tension in OHCs, with potential importance for establishing the remarkable frequency range of mammalian hearing.

The Time Course of Monocytes Infiltration After Acoustic Overstimulation

Seong Hoon Bae*[1], Jinsei Jung[1], Seung Ho Shin[1], Haeng Ran Park[1], Sang Hyun Kwak[2]
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Background: Cochlea macrophages regulate cochlea inflammation and may harbors the potentials to protect hearing function from injury, including acoustic overstimulation. Cochlea macrophage numbers increase at 3–7 days after acoustic stimulation. However, the exact timing of macrophage infiltration and maturation from inflammatory monocytes is unclear. Furthermore, neutrophils may also be involved in this process. Therefore, in this study, we investigated time-dependent immune cell infiltration, macrophage transformation, and neutrophil involvement following acoustic stimulation.
Methods: Flow cytometry and immunofluorescence were conducted in C-X3-C motif chemokine receptor 1 (CX3CR1)C=GFP mice after acoustic overstimulation (at baseline and at 1, 2, 3, and 5 days after exposure to 120 dB for 1 h) to identify inflammatory monocytes in the cochlea. RNA-sequencing and quantitative polymerase chain reaction were performed to identify differentially expressed genes.
Results: Inflammatory monocytes infiltrated into the lower portion of the lateral wall within 2 days after acoustic overstimulation (dpn), followed by transformation into macrophages at 3–5 dpn via CX3CR1 upregulation and Ly6C downregulation. In addition, inflammatory monocytes were aggregated inside the collecting venule only at 1 dpn. Neutrophils were not a major type of phagocyte during this response. The gene encoding C-C motif chemokine ligand 2 gene was significantly upregulated as early as 3 h after acoustic overstimulation.
Conclusions: Given these results, treatment to control immune response after a noise-induced hearing loss should be applied as soon as possible.

Tonotopic Differences in Stereocilia Remodeling Confirm Its Role in Maintaining Resting Tension in the Mechanotransducer of Mammalian Auditory Hair Cells

Abigail Dragich*[1], Sara Gonzalez[1], Isabel Aristizabal[1], A. Catalina Velez-Ortega[1], Gregory Frolenkov[1]

[1]*University of Kentucky*
Background: In order to detect soft sounds, auditory hair cells tension their mechano-electrical transduction (MET) machinery at rest. A classical model developed for non-mammalian hair cells postulates that myosin motors at the upper end of the tip links climb along the actin cores of stereocilia, applying constant upward tension. However, some studies suggest that proteins associated with the upper end of the tip link may have limited mobility, at least in mammalian auditory hair cells. Therefore, we explored an alternative mechanism of tip link tensioning based on recent findings that transducing stereocilia in the hair bundle retract or elongate when resting current through the MET channels is decreased or re-established, correspondingly. We argued that MET-dependent retraction of stereocilia may either: i) not affect resting MET current if the upper end of the tip link is freely moved by myosin motors or, ii) increase resting MET current if it is somehow locked to the stereocilium actin core.
Methods: We performed whole-cell patch clamp recordings of MET currents in young postnatal outer hair cells (OHCs) using fluid-jet deflections of stereocilia while initiating MET-dependent stereocilia retraction in four ways: blocking MET channels with either i) tubocurarine or ii) prolonged negative hair bundle deflection, and inhibiting Ca2+ entry into stereocilia with iii) prolonged hair cell depolarization, or iv) intracellular calcium chelation with BAPTA. Intracellular drugs preventing actin polymerization were used to test the role of actin cytoskeleton remodeling in the observed changes of resting MET current after presumable stereocilia retraction. These changes of resting MET current were compared with the extent of MET-dependent stereocilia retraction assessed by scanning electron microscopy (SEM).
Results: In all whole-cell patch clamp experiments, presumable stereocilia retraction caused a prominent post-stimulus increases in resting MET currents which persisted for several seconds. Drugs disrupting actin polymerization inhibited recovery of these MET current "overshoots". These data suggest that: i) actin polymerization is indeed involved in regulation of the resting tension within MET machinery; and ii) the upper end of the tip link is likely to be locked to the stereocilia actin core. Interestingly, our new experiments showed that these MET current "overshoots" have a prominent apex-to-base gradient. Inducible MET-current "overshoots" are significantly larger in OHCs at the apex of the cochlea than at the base. Consistently, SEM revealed significantly larger remodeling of transducing stereocilia in apical OHCs compared to basal OHCs.
Conclusions: The observed tonotopic differences further support the role of MET-dependent stereocilia remodeling in self-adjusting the resting tension within the MET machinery of mammalian auditory hair cells.

Live Imaging of Pre-Synaptic Ribbons in the Neonatal Mouse Cochlea

Noura Ismail*[1], Peu Santra[1], Yesai Park[1], Ian Matthews[1], Dylan Chan[1]
[1]*University of California, San Francisco*
Background: Moderate noise exposure results in loss of synapses between inner hair cells (IHCs) and spiral ganglion neurons (SGNs). This noise-induced cochlear synaptopathy is thought to underlie physiologic changes in auditory brainstem response (ABR) waveforms and perceptual hearing changes known as "hidden hearing loss". Changes in synapses after acoustic overstimulation include loss or dissociation of pre- and post-synaptic terminals, migration of synapses along the basolateral membrane of IHCs, and terminal swelling. However, the mechanisms underlying these synaptic changes are uncertain. In this study, we describe a live synaptic imaging model to directly observe the behavior of presynaptic ribbons in the neonatal cochlea.
Methods: RIBEYE-tagRFP mice were a gift of RW. These mice constitutively overexpress RIBEYE conjugated to tagRFP, and have typical age-related hearing loss seen in the background C57Bl6 strain. P4-5 neonatal cochleae from RIBEYE-tagRFP mice were dissected and cultured overnight with preservation of the modiolus, SGNs, and IHC-SGN synapses. Cultures were loaded with FM1-43 for delineation of hair cells. Cochleae were treated for 1 hour with 0.4 mM kainic acid (KA) as a model of glutamate excitotoxicity, or with media as control. Confocal (Nikon A1R upright with 60x water immersion objective and stagetop incubator (OKOlab)) z-stacks across the mid-portion of the cochlea were obtained at regular intervals and pre-synaptic ribbon size, location, and number were tracked (Imaris). Pre-synaptic ribbons at the basolateral membranes of both inner and outer hair cells (IHCs and OHCs) were observed. Additional antibody staining for pre-synaptic ribbons (anti-CTBP2), post-synaptic terminals (anti-GluR2), and SGNs (anti-neurofilament, NF) was additionally performed after tissue fixation. Auditory brainstem response

(ABR) recording was performed in response to 8-32 kHz pure-tone pips on 4-month-old RIBEYE-tagRFP mice and wild-type age-matched controls.
Results: RIBEYE-tagRFP mice had comparable hearing and ABR waveforms compared with wild-type age-matched controls. Pre-synaptic ribbons were readily visualized in live neonatal cochlear cultures from RIBEYE-tagRFP mice. Co-labelling with anti-CTBP2, anti-GluR2, and anti-NF confirmed that RIBEYE-tagRFP signal co-localized completely with anti-CTBP2 staining and was detected adjacent to anti-GluR2 and NF labeling to enable visualization of paired synapses with associated neurites. Treatment with kainic acid induced an increase in RIBEYE-tagRFP puncta size but no change in number, consistent with previous reports. Multi-hour live imaging of RIBEYE-tagRFP puncta with and without kainic acid treatment allowed direct visualization of changes in number, location, and size of puncta associated with inner and outer hair cells.
Conclusions: Neonatal cochlear cultures from RIBEYE-tagRFP mice are an effective tool for live imaging of pre-synaptic ribbons. The endogenous, genetically encoded signal accurately replicates traditional immunohistochemical staining. This model will be useful to directly visualize the effect of glutamate excitotoxicity and other ototoxic stimuli on synapse location, number, and size, in order to better understand mechanisms and treatments for cochlear synaptopathy.

Podium #25 - Vestibular Potentials, Blast Injury and Diagnostics

10:30 a.m. - 12:30 p.m.
Ocean's Ballroom 5-12

Moderators: John Lee and Courtney Stewart

Sound-Evoked Vestibular Myogenic Potentials in Rattus Norvegicus

Federica M. Raciti*[1], Yasniary Morales[1], Hillary Snapp[2], Suhrud Rajguru[3]
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Background: Over the years, vestibular evoked myogenic potentials (VEMPs) have become an essential component in the diagnostic vestibular test battery as they represent an objective measure of the integrity of the vestibulothalamic pathway. VEMPs are vestibular-dependent reflexes elicited via air-conducted sound stimuli (ACS) or bone-conducted vibration (BCV) from tonically contracted cervical muscles (cVEMPs) and extraocular muscles (oVEMPs). The anatomical projections of otolith afferents to neck and eye muscles make these techniques a valid diagnostic tool for primarily assessing saccular and utricular function. Given the increased popularity of VEMPs in the clinical domain, it is essential to establish standard methods and protocols, examine the neural basis of the responses, and validate relevant features for interpretation and use.
Methods: This study presents an extensive dataset of cVEMPs measured at multiple intensities and frequencies in naïve Brown Norway rats. SmartEP evoked potentials system (IHS, USA) was used to record sound-evoked VEMPs from the sternomastoid muscle in anesthetized female and male adult rats. The head was turned to one side at a 90° angle to maintain muscle tension and held in place via a custom-made band. Pure tone bursts from 1 to 16 kHz (50-100 dB SPL) were delivered via the ER3A insert earphone. The myogenic signals were amplified by 100k and band-passed (30-1000 Hz). Each recording consisted of 256 sweeps, acquisition rate=5/s with an alternating phase, and sampling rate of 200µs. cVEMPs were characterized by the presence of a positive (P1)-negative (N1) waveform at 3-5 ms from the stimulus onset.
Results: Within the cohort, reliable and replicable cVEMP responses were obtained at all frequencies down to 50 dB SPL. A cubic fit was performed on cVEMPs' wave I (P1-N1) amplitudes (R2=0.96) and P1 latencies (R2=0.98) at 90 dB SPL across frequencies (1-16 kHz). For the following analysis, the cVEMP responses were grouped based on the stimulus tone. Amplitude and latencies of myogenic potentials were averaged in low (1-4 kHz), mid (6-10 kHz), and high frequency (12-16 kHz) ranges. The responses evoked by stimuli in the mid-frequency range were significantly larger (7.9±0.3 uV, $p<0.01$) and faster (3.4±0.04 ms, $p<0.001$) than the ones obtained at lower frequencies. However, no significant difference was observed when both amplitude and latency of mid-frequency responses were compared with cVEMPs from the 12-16 kHz frequency range. Mean P1 amplitudes were 6.3±0.3 uV for 1-4 kHz and 7.2±0.3 uV for the 12-16 kHz range. Mean P1 latencies were 3.7±0.03 ms for 1-4 kHz and 3.5±0.03 ms for 12-16 kHz. No significant sex differences were observed in any of the analyses performed.

Conclusions: This work details the characteristics of ACS-evoked myogenic potentials in a pre-clinical rat model. The results will guide the development of optimal cVEMP test protocols to elucidate pathophysiological characteristics of vestibular dysfunctions further.

Temporal Modulation Transfer Functions of Amplitude-Modulated Ocular Vestibular Evoked Myogenic Potentials

Christopher Clinard*[1], Raghav Jha[1], Erin Piker[1]
[1]*James Madison University*

Background: Ocular vestibular evoked myogenic potentials (oVEMPs) are commonly used to evaluate utricular function and are recorded from the extraocular inferior oblique muscle. Almost all previous oVEMP studies have used transient stimuli to elicit onset responses. Several studies have reported using long-duration, amplitude-modulated tones to elicit cervical VEMPs, reflecting saccular function and recorded from the sternocleidomastoid muscle. However, there appears to be no previous report of amplitude-modulated oVEMPs (AMoVEMPs) in the literature. Here, we used amplitude-modulated sounds to examine sustained, phase-locked oVEMP activity. The purposes of this study were to 1) define TMTFs of the AMoVEMP using multiple analysis approaches, and 2) to determine whether AMoVEMPs reflect nonlinear distortion products.
Methods: Participants were young adults (ages 19-21, n=15) with no history of vestibular lesions or middle-ear pathologies. Stimuli were AM tones with carrier frequency of 500 Hz and modulation frequencies ranging from 11 to 397 Hz. Stimuli had duration of 1.024 sec and were delivered at 65 dB HL using a B81 bone vibrator. Participants maintained a fixed, upward eye gaze of 30 degrees during the recordings. FFT-based analyses included amplitude, signal-to-noise ratio, phase coherence, and modulation gain. Transient oVEMPs were also elicited using a 500 Hz toneburst (2-0-2 ms).
Results: All participants had present responses. TMTFs were defined for each analysis type, and the shape of the TMTF varied across analysis types. The amplitude TMTF had a peaked shape with maximal amplitude at approximately 100 Hz. TMTFs for SNR, phase coherence, and modulation gain had broader shapes with robust responses over a range of modulation frequencies (37-113 Hz). Phase coherence analyses and polar histograms demonstrated robust synchrony to the temporal envelope (e.g., phase coherence > 0.8). AMoVEMPs were present at modulation frequencies as high as 263 Hz. Within individuals, the spectra of transient oVEMPs had substantial overlap with the shape of the AMoVEMP amplitude TMTF. Harmonic distortion products of the modulation frequency, reflecting nonlinear vestibular processing, will also be reported.
Conclusions: AMoVEMPs can encode amplitude modulation across a range of modulation frequencies. In addition, overlap between transient oVEMP spectra and AMoVEMP amplitude TMTFs suggest that the amplitude TMTF is influenced by the spectra of the motor unit action potentials of the inferior oblique muscle. AMoVEMP TMTFs have shapes that differ from those of amplitude-modulated cervical VEMPs. Nonlinear distortion products were present in AMoVEMPs, consistent with nonlinear vestibular processing. This novel approach to ocular VEMPs may be used to ask new questions about human vestibular physiology in healthy and pathological systems.

Vestibular Functional and Histological Changes in a Blast-Induced mTBI Rat Model

Melissa Ghulam-Smith*[1], Federica M. Raciti[2], Jianzhong Liu[2], Laura Gomezllanos[2], Suhrud Rajguru[3], Michael Hoffer[4]
[1]*University of Miami/Miami VAMC,* [2]*University of Miami,* [3]*Department of Otolaryngology and Biomedical Engineering, University of Miami,* [4]*University of Miami School of Medicine, University of Miami Ear Institute*

Background: Mild traumatic brain injury (mTBI) affects approximately 100 to 300 per 100,000 persons worldwide each year. Blast-induced trauma, particularly among military personnel and increasingly among civilians, is a common mechanism of injury leading to mTBI. Neurosensory deficits such as hearing loss, dizziness, vertigo or loss of balance are common debilitating symptoms post-blast and represent potential damage to the auditory and vestibular peripheral end organs. Existing studies have characterized the mechanisms underlying hearing loss in mTBI, however damage to the vestibular end organs and the mechanisms for functional and structural recovery remain poorly characterized.
Methods: We have established an ecologically valid preclinical model of mTBI using a blast tube to produce injuries that can account for the clinical features and complexity of human TBI. Baseline cervical

vestibular evoked myogenic potentials (cVEMPs) and ABR are obtained from a group of rats using frequency tones of 1kHz and 8kHz at various intensities. The animals are exposed to a blast between the intensities of 9-12 psi. cVEMPS and ABR post-blast are obtained serially on days 1, 3, 7, 14 and 28. Latency and amplitudes are analyzed and compared to baseline to assess recovery of function. Post-mortem histology allows assessment of hair cell loss and damage to the inner ear end organs. Mixed multi-modal statistical analysis with a post-hoc analysis separating blasted animals into a low- vs high-intensity subgroup based on exposure and hearing impairment (ABR) post-blast are used.
Results: Post-blast functional analysis (cVEMPs) revealed significant increases in cVEMP thresholds that began to recover on day 3 and fully recovered by day 7 for the 1 kHz stimulus. Additionally, we observed reductions in amplitude and latency at both 1 and 8 kHz with recovery by day 7. Anatomical assessment include hair cell and synapse counts of the otolith organs.
Conclusions: Preliminary results demonstrate an ecologically valid model of mTBI with vestibular hypofunction on day 1 with recovery over time post-blast. cVEMP protocol established in the lab allows for characterization of the effects of blast intensity on the degree of hypofunction observed. Additionally, this model will allow us to investigate the potential role of vestibular synaptopathy in the long-term vestibular deficits observed in mTBI. Results highlight a model that may aid development of sensitive diagnostic tests and therapeutic strategies to mitigate vestibular dysfunction post-blast.

The Impact of Blast Overpressure on Vestibular Pathway Function

Syed Naqvi*[1], Rod Braun[1], Avril Genene Holt[1]
[1]*Wayne State University School of Medicine*

Background: The increasing wide spectrum of blast exposure is a common source for auditory dysfunction among military personnel, resulting in symptoms such as hearing loss, tinnitus, and tympanic membrane perforations. People exposed to blast at mild to moderate intensities can develop mild traumatic brain injury sequelae with symptoms of dizziness, vertigo, and nystagmus, implicating disruption of vestibular pathways. However, the specific nuclei within the vestibular pathway being affected by blast are not well delineated. Therefore, we used vestibular short-latency evoked potentials (VsEPs) to assess the impact of striolar hair cell activation on activity in irregular afferent fibers and neurons in the vestibular nuclear complex (VNC) both before and after blast exposure. Additionally, auditory brainstem responses (ABRs) were used to assess auditory function.
Methods: Male Sprague-Dawley rats (n=12) were divided into two groups, blast-exposed (n=6) and sham (n=6) animals. Isoflurane was vaporized (Precision Vaporizer) and delivered into a plexiglass chamber containing the animal and anesthesia was induced using 4% (oxygen carrier) and was maintained with 2% isoflurane. The average blast overpressure was 20 psi (8 ms). VsEP and ABR testing were conducted before, one day, and 20 days after blast or sham exposure. VsEP waveforms were collected and analyzed (P1 and P2) by delivering jerk stimulation (linear acceleration) in the naso-occipital plane at intensities of 500, 2500, and 4200 g/s. Stimulation consisted of 200 jerk pair presented in each of eight trials. Amplitudes and latencies of the VsEPs were analyzed using custom MATLAB scripts with ANOVAs and T-tests used as appropriate. Wave I ABR thresholds were evaluated at 4, 20, and 32 kHz.
Results: While P1 VsEP amplitude did not change in the blast group when compared to controls, the lowest jerk intensity (500 g/s) showed an increased latency of ~0.35 msec after one day. Although, there was a decrease in P1 amplitude at moderate intensities (2500 and 4200 g/s), latencies in blast animals remained unchanged compared to controls. A similar trend was observed for P2 amplitude and latency. Furthermore, blast animals had a significantly higher ABR wave I threshold than sham animals one day after exposure at 4, 20, and 32 kHz ($p<0.05$). No significant difference in ABR thresholds were observed between blast and sham groups after 20 days.
Conclusions: Irregular fiber activity (P1) and neuronal responses in the VNC (P2) decrease at early and late time points following blast exposure. These results suggest long-term effects of blast on both peripheral and central vestibular function. Response times for irregular fibers and VNC neurons revealed reduced sensitivity to low intensity vestibular stimulation. Although these vestibular pathologies were present at later time points, hearing thresholds had already recovered during the same time period.

The Automated Vestibular System

Eshita Singh*[1], Erin Williams[2], Devin Kennedy[2], Jerry Bieszczad[3], Lindsay Allen[3], Odile Clavier[3], Michael Hoffer[4]

[1]University of Miami/Jackson Memorial Hospital, [2]University of Miami Miller School of Medicine, [3]Creare LLC, [4]University of Miami School of Medicine, University of Miami Ear Institute
Background: Dizziness is a common complaint, especially after concussion or traumatic brain injury, with about one third of the population experiencing dizziness at some point in their life. Unfortunately, vestibular rehabilitation therapy requires specialized physical therapists who may not easily be accessible to service members or general population living in remote areas, delaying the onset of therapy. The Automated Vestibular Rehabilitation System (AVRS) is an automated computer system that administers basic vestibular rehabilitation without the need for a specialized physical therapist present. AVRS uses eye tracking and gait monitoring to confirm the patient is performing exercises with the right form and technique. The goal of the study is to compare the performance of patients when given vestibular therapy instructions by a live physical therapist versus instructions via the automated system.
Methods: Patients were recruited from University of Miami clinics on a voluntary basis and underwent a prescreening process. Medical records were not reviewed. Each individual performed both an instructed and an automated trial of VORX1 and VORX2 exercises with the order randomized. A team member blinded to the participant assignment rated each individual's eye and head tracking performance with the following grading scale: 0-2 for eye tracking (0-performed incorrectly, 1-performed correctly during second half of exercise, or 2-performed correctly throughout), 0-2 for head tracking (similar scale as eye tracking), and 0-4 for overall VOR performance (sum of eye and head tracking). Descriptive statistics were utilized for demographics. Mean grading score was compared using pairwise t-test between automated group vs live instruction group on both VORX1 and VORX2 exercise.
Results: There were 22 total participants including 3 vestibular patients and 19 balance-normal controls. Of the 19 balance-normal controls, 6 received automated instructions first and 13 received live instructions first. The VORX1 mean performance was 2.8 [SD 1.33] for the automated instruction group and 4 [SD 0] ($p=0.004$) for the live instruction group. For VORX2, the mean performance of the automated instruction group was 3.2 [SD 0.98] and 3.3 [SD 0.9] ($p=0.823$) for the live group.
Conclusions: This is a small proof of concept study for a new automated vestibular system. Our preliminary results show there was no difference between the automated vs live group in VORX2 exercises, indicating the automated system provided guidance comparable to a live therapist. Significant difference in performance in VORX1 exercise suggests the automated instructions can be improved to obtain better results. Future work in this study will involve refining instructions, obtaining more vestibular patients, and conducting efficacy studies to compare automated vs standard rehabilitation practices with a trained in-person physical therapist.

Comparison of v-HIT Test Results of Two Models in Patients With Vestibular Dysfunction

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Background: The video-Head Impulse Test (v-HIT) is a simple yet powerful test of all semicircular canal functions. Currently, several companies offer devices to perform v-HIT. However, there is a concern that the results of the vertical semicircular canal test may differ because of the different testing methods used in each device.
In this study, vHIT was performed on a group of patients with vestibular dysfunction using two devices, Eye See Cam (ESC) and ICS Impulse (ICS), and the test results on the affected side were compared.
Methods: On the same day, the same examiner performed the vHIT test on the two models. The order of the vHIT tests was randomized. vHIT test as well as the caloric test, c-VEMP, and o-VEMP tests were also performed.
Subjects: 16 patients with vestibular dysfunction (male:female 4:12, mean age 58.5 years)
Statistics: Paired T Test and Peason`s correlation coefficient test.
Results: Results:
① Lateral semicircular canal: Gain was 0.41±0.20 for ESC and 0.44±0.17 for ICS, no significant difference ($p=0.43$); Catch-up saccades (CUS) were 100% for both models; significant correlation between the two models ($r=0.81, p<0.01$).

② Anterior semicircular canal: Gain was 0.77±0.32 for ESC and 0.51±0.28 for ICS (p=0.014). 25% of CUS was detected for ESC and 18.8% for ICS. No significant correlation was found between the two models (r=0.17,p=0.532).
③ Posterior semicircular canal: Gain was 0.63±0.35 for ESC and 0.47±0.26 for ICS, with no significant difference (p=0.107). 69% of CUS was detected in ESC and 50% in ICS, with no significant correlation between the two models. (r=0.40,p=0.13)
Conclusions: The lateral semicircular canal, there were no differences in methods between the models, and the results were similar, suggesting that the devices were equally capable. However, for the vertical semicircular canal, the two models did not agree. This was thought to be due to differences in the vertical semicircular canal testing methods. Since objective evaluation of vertical semicircular canal function is difficult to achieve other than with the vHIT, further research is needed to accumulate more cases and to determine which machine is the correct vertical semicircular canal test.

Improving the Diagnostic Differentiation of Meniere's Disease and Vestibular Migraine Using Vestibular Perceptual Thresholds and Imaging

Cameron Fattahi*[1], Amsal Madhani[1], Susan King[1], Andreas Eckhard[1], Amy Juliano[1], Steven Rauch[1], Faisal Karmali[1], Richard Lewis[1], Divya Chari[1]
[1]Massachusetts Eye and Ear Infirmary, Harvard Medical School
Background: Patients presenting with recurrent episodic vertigo and dizziness such as Meniere's disease (MD) and vestibular migraine (VM) can present a diagnostic challenge as they can both produce similar symptoms of recurrent vertigo, tinnitus, motion intolerance, and hearing loss. Current diagnostic criteria are based on patient history with little contribution from objective measures. Vestibular perceptual thresholds, which refer to the smallest appreciable stimulus detected by the participant in various translations and rotations of body movement, have been shown to have the potential to differentiate different types of vestibular dysfunction. In addition, recent evidence suggests that delayed gadolinium (Gd)-enhanced high-resolution magnetic resonance imaging (MRI) of the inner ear may enable visualization of vestibular and cochlear endolymphatic hydrops (EH). Herein, we compare vestibular perceptual thresholds and imaging of the inner ear in patients with definite MD, patients with definite VM, and normal control (NC) subjects.
Methods: Nine patients with MD, 11 patients with VM, and 11 normal control (NC) subjects underwent perceptual threshold testing. Five perceptual thresholds were compared among subjects: three linear motion translation at 1.0 Hz (inter-aural (y-translation), naso-occipital (x-translation), and earth-vertical (z-translation)), one rotatory motion at 1.0 Hz (yaw rotation about an earth-vertical axis), and roll tilt at 0.2 Hz. MD subjects underwent clinical vestibular testing (cVEMP and vHIT) and 4-hr delayed intravenous Gd-enhanced MRI. All subjects completed a dizziness handicap inventory (DHI).
Results: MD subjects had significantly elevated z-translation thresholds compared to VM and NC subjects (t=2.134, p=0.047 and t=2.134, p=0.048, respectively). VM patients demonstrated significantly reduced roll tilt thresholds compared to MD and NC subjects (t=2.078, p=0.050 and t=2.667, p=0.011, respectively). Perceptual thresholds from all other motions were not significantly different among the groups (p>0.05). Saccular dilation of the affected ear positively correlated with increased z-translation thresholds (r2=0.457), while utricular dilation correlated with increased y-translation thresholds (r2=0.584). Clinical vestibular tests (cervical vestibular evoked myogenic potential (cVEMP), video head impulse test (vHIT)) and subjective DHI scores did not correlate with perceptual thresholds in any motion or imaging findings (p>0.05).
Conclusions: Perceptual threshold testing may provide insight into the underlying pathophysiology of the disease process (e.g. elevated z-translation thresholds in Meniere's disease may suggest saccular dysfunction and reduced roll tilt thresholds in VM patients may suggest central integration deficits of otolith-canal signals). Future avenues for research include determining whether the combination of perceptual threshold testing and delayed Gd-enhanced inner ear MRI scans improve the diagnostic differentiation of MD and VM better than currently available clinical audiovestibular testing.

Biomarkers of Postural Control in Vestibulopathy

Julie Corre*[1], Jean-Francois Cugnot[1], Anissa Boutabla[1], Samuel Cavuscens[1], Maurizio Ranieri[1], Robert Peterka[2], Nils Guinand[1], Angelica Perez Fornos[1]
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Background: Sensory information from the visual, somatosensory, and vestibular systems have to be tightly regulated to maintain a controlled, upright posture. Analysis of body sway following balance perturbations

allow to determine the relative contribution of each sensory system to postural control by calculating "sensory weights" (Wsomatosensory, Wvisual, and Wvestibular). The sum of each sensory weight contributing to postural control is 1. Investigating the role of the vestibular system in postural control is crucial to determine the functional impact of vestibular loss and design intervention to alleviate the chronic instability and prevent falls.
Methods: This study was conducted in 19 patients with unilateral vestibulopathy (UV) and 17 patients with bilateral vestibulopathy (BV). Results were compared to 20 sex-age matched healthy subjects (HS). Postural assessments were performed using a modified SMART EquiTest CRS device (Natus Medical Inc, Seattle WA), which delivers continuous surface or visual surround rotations that evoke antero-posterior body sway in subjects. The test started with a 4 min warm-up in order to familiarize the subject with the environment. Then subjects underwent 4 min tests in three conditions: (1) surface-tilt stimuli with eyes open and visual surround fixed, (2) surface-tilt stimuli with eyes closed, and (3) visual surround tilt with fixed surface and with all using 2° peak-to-peak stimulus amplitudes. Patients were also equipped with a 3-DOF Head Tracker that continuously records head movements in the Yaw, Pitch, and Roll planes.
Results: The main parameter of interest was the vestibular sensory weight derived from Condition 2, where balance relies on somatosensory (e.g., proprioception) and vestibular cues. We found that BV subjects yield a somatosensory weight of 0.97 indicating nearly zero vestibular contribution to balance (since Wvestibular = 1 – Wsomatosensory). This sensory weight was significantly different from UV (0.62) and HS (0.34). Secondary parameters included somatosensory weights from Conditions 1 and 3. We found that BV subjects consistently yield a heavier weight, which significantly differed between UV and HS (Condition 1: respectively 0.43; 0.37 and 0.34; Condition 3: respectively 0.27; 0.12 and 0.008) indicating that BV and UV subjects adapt to their vestibular deficit by altering how they use visual and somatosensory cues. In all three conditions, there were no significant difference between HS and UV, likely due to central compensation mechanism following unilateral vestibular loss. Further analyses are underway to determine if other metrics (system time delay and head movements) are relevant and have a good discriminative capacity.
Conclusions: We designed a test allowing to quantify performance during postural assessments but also reliably distinguishing between HS, UV and BV patients. This closer-to-reality test could be a useful diagnostic and rehabilitation tool as it reflects the severity of vestibular-induced functional impairment and can also measure the efficacy of rehabilitation interventions.

Podium #26 - Cisplatin Ototoxicity

10:30 a.m. - 12:30 p.m.
Oceans Ballroom 1-4

Moderators: Peizhe Wu and Katharine Fernandez

The Ototoxic Drug Cisplatin Localises to Stress Granules Altering Their Dynamics and Composition

Jack Martin*[1], Stephen Terry[1], Jonathan Gale#[1], Sally Dawson#[1]
[1]UCL Ear Institute
Background: Cisplatin is a highly effective, but ototoxic, platinum-based chemotherapeutic. The hearing loss that develops in up to 80% of adults and 90% of children (Frisina et al., 2016; van As et al., 2016) undergoing cisplatin chemotherapy is bilateral, sensorineural, progressive and irreversible. The mechanisms underlying cisplatin ototoxicity are not fully understood but there are multiple proteins that have been shown to interact with cisplatin, several of which are involved in the formation and regulation of stress granules (SGs), condensations of mRNA and RNA-binding proteins. We previously identified a role for SGs in protecting against aminoglycoside antibiotic-induced ototoxicity (Goncalves et al., 2019). Furthermore, recent work has shown that Caprin1, an RNA binding protein and key SG component and regulator is necessary for maintenance of auditory function (Nolan et al., 2022).
Methods: We examined the effects of cisplatin on SG dynamics and composition in two cell lines derived from the mouse cochlea and human retinal pigment epithelium, UB-OC2 and RPE1 cells respectively. Additionally, cisplatin was localised using live cell imaging of Texas Red conjugated cisplatin (cis-TR) in cell lines stably expressing Caprin1-mEmerald.
Results: Cisplatin-induced SGs are significantly ($p<0.001$) diminished in size and quantity compared to typical arsenite-induced SGs and they are persistent after 24 hours recovery. Cisplatin-induced SGs also had

significant reductions in the sequestration of eIF4G and signalling proteins RACK1 and DDX3X. Sequestration of DDX3X in SGs prevents the assembly of the NLRP3 inflammasome reducing the production of inflammatory cytokines like IL-1β. Recruitment of RACK1 to SGs represses pro-apoptotic MAPK signalling. Live cell imaging of cis-TR revealed its localisation to SGs and retention for at least 24 hours. In separate experiments, cisplatin pre-treatment rendered cells unable to form a typical SG response to a subsequent arsenite stress.
Conclusions: Taken together, our study has revealed that cisplatin localises to SGs, causes impaired assembly, increased persistence and altered composition of what could be termed 'non canonical SGs'. Furthermore, cells that have been exposed to cisplatin are unable to form SGs in response to additional stress. If this scenario was replicated in the cochlea, it could sensitise cochlear cells to further insults by compromising their protective function. Whether the aberrant/non-canonical SGs induced by cisplatin underlie the susceptibility of cochlear cells to cisplatin remains to be determined. Localisation to persistent SGs may also contribute to the retention of cisplatin in the cochlea. These findings suggest several novel targets for the prevention of cisplatin ototoxicity and may also have wider implications for cisplatin resistant tumours by increasing our understanding of non-DNA based effects of cisplatin treatment.
Joint senior authors

Tamiflu (Oseltamivir Phosphate) Protects from Cisplatin-Induced Hearing Loss in an in Vivo Translational Model

Richard Lutze*[1], Matthew Ingersoll[1], Regina Kelmann[1], Daniel Kresock[1], Tal Teitz[1]
[1]*Department of Pharmacology and Neuroscience, Creighton University School of Medicine*
Background: Cisplatin is a commonly used and effective chemotherapy agent for a variety of solid tumors, but 40-60% of people treated with cisplatin have significant hearing loss. There are currently no FDA-approved drugs to prevent cisplatin ototoxicity creating a need to identify compounds that prevent this detrimental side effect. Oseltamivir phosphate (commonly known as Tamiflu) is an FDA-approved drug that is well tolerated in patients for the treatment of influenza. Tamiflu is a top hit that protects from cisplatin-induced cell death in a high throughput screen and cochlear explants. Tamiflu did not interfere with cisplatin killing efficacy in tumor cell lines. Additionally, Tamiflu protects from cisplatin ototoxicity in mice when injected with a single, high dose of cisplatin. Chemotherapy patients receive multiple, lower dose cisplatin injections over many cycles, so this study investigates the efficacy of Tamiflu in a more clinically relevant mouse model (Fernandez K. et al., Hear Res 2019).
Methods: Baseline auditory brainstem response (ABR) were performed on adult CBA/CaJ mice. Mice were treated with 3 mg/kg cisplatin in the morning and Tamiflu (50 or 10 mg/kg) in the morning and evening. Each cycle consisted of 4 days of drug treatment with a 10-day recovery period. Tamiflu was given for 1 extra day, for a total of 5 days per cycle because previous studies show that the 24 hours post cisplatin treatment is a critical treatment window. Three total cycles were performed and after the recovery period of cycle 3, ABRs were performed again to measure threshold shifts. After hearing tests, the cochleae were dissected, fixed, and stained with myosin VI to quantify the number of outer hair cells surviving the 42-day multi cycle period.
Results: Mice treated with Tamiflu had significantly lower ABR threshold shifts compared to cisplatin alone treated mice. 50 mg/kg had protection at the 8, 16, and 32 kHz frequencies while 10 mg/kg had protection at the 16 and 32 kHz frequencies. Both 50 and 10 mg/kg had 20 dB lower ABR threshold shifts at 32 kHz compared to the cisplatin alone group. Both Tamiflu doses also protected from cisplatin-induced outer hair cells death at the 8 and 16 kHz region. A dose of 10 mg/kg of Tamiflu administered twice daily in mice is 65% of the human equivalent daily dose that is currently prescribed for influenza treatment.
Conclusions: This study shows that Tamiflu protects from cisplatin ototoxicity in a clinically relevant mouse model. Tamiflu treated mice have significantly lower ABR threshold shifts and Tamiflu protects from cisplatin-induced outer hair cell death. Future studies will focus on testing Tamiflu's protection in mice at 4 months after the 42-day multi-cycle experiment to test if the hearing protection is maintained for longer periods of time.

Cisplatin Drives Mitochondrial Dysregulation in Zebrafish Lateral-Line Hair Cells

David Lee*[1], Emily Bell[1], Angela Schrader[1], Mark Warchol[1], Lavinia Sheets[1]
[1]*Washington University in St. Louis*

Background: Cisplatin is a commonly used chemotherapy that causes irreversible hearing loss. Cisplatin drives excessive reactive oxygen species (ROS) formation in cochlear hair cells, yet the cellular mechanisms of pathologic ROS production remain undefined. Mitochondrial dysfunction has been implicated in cisplatin-induced ROS production. Zebrafish may offer insights to the impact of cisplatin on mitochondria as their lateral-line organs contain optically accessible hair cells, permitting live imaging of dynamic cellular processes. We therefore investigated the effect of cisplatin on hair cell mitochondrial bioenergetics over time within zebrafish lateral-line organs.
Methods: In vivo time-lapsed imaging of 6-day-old zebrafish with genetically encoded biosensors of hair cell mitochondrial calcium, mitochondrial oxidative stress, and cytosolic calcium were used to investigate mitochondrial dynamics after cisplatin exposure. In the first set of experiments, zebrafish were treated with 1 mM cisplatin for two hours followed by a two-hour recovery period. Time-lapsed images were acquired at baseline and every 10 minutes thereafter for the entire four-hour treatment. Changes in fluorescence relative to baseline of individual hair cells in the presence or absence of cisplatin treatment were measured. In the next set of experiments, transgenic zebrafish expressing a ratiometric indicator of cumulative mitochondrial redox activity in hair cells were used to explore whether cumulative mitochondrial activity affects susceptibility to cisplatin. Baseline ratios of red:green fluorescence of living and dying hair cells that underwent treatment with cisplatin as above were compared.
Results: Time-lapsed imaging of transgenic zebrafish demonstrated a slow rise in hair cell mitochondrial calcium, mitochondrial oxidative stress, and cytosolic calcium followed by a rapid elevation immediately prior to death. The maximum change in fluorescence of dying hair cells exposed to cisplatin were 3.49-fold (95%CI=3.19-3.70), 1.66-fold (95%CI=1.39-1.87), and 1.34-fold (95%CI=1.29-1.41) greater than non-exposed hair cells, respectively. Notably, these spikes in dysregulated cellular bioenergetics in response to cisplatin occurred in a delayed and progressive fashion. The time to half maximum fluorescence prior to hair cell death for mitochondrial calcium, mitochondrial oxidative stress, and cytosolic calcium were 36.4 (95%CI=32.8-40.0), 24.9 (95%CI=22.5-27.4), and 25.4 (95%CI=23.6-27.2) minutes, respectively, yet the median time to hair cell death after cisplatin exposure was 140 (95%CI=130-140) minutes. These results suggest a potential therapeutic window after cisplatin but before mitochondrial dysregulation that may prevent hair cell death. In addition, zebrafish with a genetically encoded biosensor of cumulative mitochondrial activity demonstrated that the median redox history was 1.67-fold (95%CI=1.55-1.80) higher in dying hair cells than living hair cells, suggesting cumulative mitochondrial activity influences vulnerability to cisplatin.
Conclusions: Hair cell mitochondrial dysregulation following cisplatin is an early cellular event that culminates immediately prior to hair cell death. Future studies should explore whether intervention after cisplatin exposure but prior to terminal peaks in mitochondrial dysfunction may prevent hair cell death.

CSF1R Inhibition Induces Macrophage Ablation and Protects Against Cisplatin-Induced Hearing Loss in Mice

Cathy Yea Won Sung*[1], Mark Warchol[2], Lisa Cunningham[3]
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Background: Cisplatin is a widely used platinum-based anti-cancer drug that is toxic to mechanosensory hair cells in the inner ear and results in significant and permanent hearing loss in pediatric and adult cancer patients. Macrophages, the major resident immune cells in the cochlea that become activated in response to tissue injury, are important drivers of both inflammatory and tissue repair responses. Here we use a clinically relevant mouse model of cisplatin-induced ototoxicity to examine whether macrophages play a role in hearing loss and outer hair cell loss (OHC) in response to cisplatin.
Methods: Mice underwent three cycles of cisplatin treatment, each cycle consisting of a once-daily cisplatin injection for 4 days followed by a 10-day recovery period. Selective macrophage ablation was achieved by treating subsets of mice with PLX3397 seven days prior to and throughout the cisplatin treatment, which effectively ablated >96% of all cochlear macrophages. PLX3397 is an inhibitor of the colony-stimulating factor 1 receptor (CSF1R), which is required for survival of microglia and cochlear resident macrophages. The four experimental groups employed in this study were Saline+Vehicle, Saline+PLX3397, Cisplatin+Vehicle, and Cisplatin+PLX3397. Hearing sensitivity was assessed prior to PLX3397/cisplatin administration and at the end of the third cycle of the cisplatin treatment protocol using auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE), which indirectly measure OHC

function. Furthermore, platinum levels were measured by inductively coupled plasma mass spectrometry (ICP-MS) on microdissected cochlear tissues.
Results: Our data indicate that macrophage ablation was protective against cisplatin-induced hearing loss and OHC death. ABR threshold shifts (indicative of hearing loss) were significantly reduced and DPOAE amplitudes were significantly increased in mice treated with both cisplatin and PLX3397 compared to mice treated with cisplatin alone, suggesting that macrophage ablation protected against cisplatin-induced hearing loss and OHC dysfunction. Quantification of cochlear hair cells suggests that the protective effect of macrophage ablation by PLX3397 treatment was due to the protection of OHCs against cisplatin-induced hair cell loss. To address the mechanisms by which macrophage ablation reduces cisplatin ototoxicity, we investigated whether macrophage ablation reduced cisplatin accumulation in the inner ear. We observed reduced platinum levels in inner ear tissues from all PLX3397-treated mice, suggesting that macrophages contributed to cisplatin entry into the cochlea.
Conclusions: Our data suggest that macrophage ablation was protective against cisplatin-induced hearing loss and OHC death. Additionally, the protective effect of macrophage ablation against cisplatin ototoxicity correlated with reduced platinum levels in the inner ear, indicating that macrophages contributed to cisplatin entry into the cochlea. As PLX3397 is presently in clinical trials as an anti-cancer drug, we plan to fully characterize its protective effect against cisplatin-induced hearing loss in mice and explore the potential for its use in combination with cisplatin.

Dabrafenib Protects against Cisplatin Ototoxicity in a Clinically Relevant Mouse Model

Matthew Ingersoll*[1], Richard Lutze[1], Chithra Pushpan[1], Regina Kelmann[1], William Hunter[2], Huizhan Liu[3], David He[3], Tal Teitz[1]
[1]Department of Pharmacology and Neuroscience, Creighton University School of Medicine, [2]Department of Pathology, Creighton University School of Medicine, [3]Department of Biomedical Sciences, Creighton University School of Medicine
Background: Cisplatin is commonly used for effective treatment of many malignant tumors, however, treatment leads to hearing loss in 40-60% of patients, severely impacting patients' overall quality of life. There is currently no FDA-approved compound available for treatment of cisplatin ototoxicity, creating a clear need for further study of potential therapeutic agents. Recently, when administered at clinically relevant doses, BRAF inhibitor dabrafenib was found to protect from both noise- and cisplatin-induced hearing loss in vivo. Dabrafenib is FDA-approved for treatment of multiple cancers and may be repurposed to preserve patient hearing during cisplatin treatment. In addition, dabrafenib does not interfere with cisplatin's tumor killing ability in cell lines. Initial cisplatin ototoxic studies employed a single high-dose protocol, however, to improve clinical relevance this investigation tests dabrafenib using a more robust multi-cycle cisplatin regimen to mimic multiple rounds of patient chemotherapy.
Methods: Baseline auditory brainstem response (ABR), distortion product otoacoustic emissions (DPOAE), and endocochlear potential (EP) measurements are recorded prior to treatment initiation. During each treatment cycle, mice are IP-injected with previously optimized (Fernandez K. et al, Hear Res 2019) 3 mg/kg cisplatin once-daily for 4 days, followed by a 10-day recovery period. During cisplatin treatment, mice are treated with dabrafenib (15, 3, or 0.6 mg/kg) or carrier-alone twice daily by oral gavage, with the first daily treatment administered 45 minutes prior to cisplatin injection. This treatment cycle is repeated three times, after which ABR, DPOAE, and EP measurements are recorded immediately and 4-months post-treatment to assess hearing loss. Mouse cochleae were fixed and stained with myosin VI to determine loss of outer hair cells (OHCs) at the basal, middle, and apical regions. In addition, mouse kidneys were stained with hematoxylin and Periodic acid-Schiff and graded by a pathologist to assess renal damage.
Results: All doses of dabrafenib protect against cisplatin-induced hearing loss as measured by ABR and DPOAE threshold shift, with 15 and 3 mg/kg exhibiting similar protection immediately after treatment concluded and 3 mg/kg most effective 4-months post-treatment. Dabrafenib at 0.6 mg/kg conferred less significant hearing protection. Additionally, 15 and 3 mg/kg dabrafenib significantly reduced OHC loss at the middle and basal regions. Moreover, cisplatin did not cause significant renal toxicity or changes in EP. Dabrafenib did not cause renal toxicity when combined with cisplatin and significantly reduced cisplatin-associated weight loss.
Conclusions: This data confirms dabrafenib protects from cisplatin-induced hearing loss using a clinically relevant mouse model, with 3 mg/kg dabrafenib being most effective at one tenth the prescribed human

equivalent dose. The combination of dabrafenib with cisplatin did not increase systemic toxicity, and mitigated weight loss in mice compared to cisplatin treatment alone. The study indicates dabrafenib is a promising candidate for future clinical trials.

Development of DB-020, a Locally Administered Product for Protection Against Cisplatin-Induced Ototoxicity

Heather Wolff[1], Tera Quigley[1], Shane Raines[2], Habeeb Oseni[1], John Soglia[3], John Keilty[3], Qi-Ying Hu[3], Fuxin Shi[3], Brendan Arsenault[3], Jonathon Whitton[1], John Lee*[1]
[1]Decibel Therapeutics, [2]Decibel consultant, [3]Decibel Therapeutics (formerly)

Background: Cisplatin, one of the most commonly used chemotherapeutic agents, has severe dose-limiting side effects, including ototoxicity, which leads to permanent hearing loss in many patients.
Cisplatin has been shown to preferentially distribute to the cochlea, resulting in the death of sensory cells in the cochlea and hearing loss in in multiple animal models. A number of different mechanistic approaches have been explored for protection against cisplatin-induced ototoxicity, including delivery of agents such as sodium thiosulfate (STS) to the cochlea at the time of cisplatin administration to inactivate cisplatin and protect sensory cells and hearing loss. There are no approved therapies for the prevention or treatment of cisplatin-induced ototoxicity for adults.
Methods: DB-020 is a proprietary formulation of STS designed for local, transtympanic injection to the ear to protect against hearing loss in cancer patients receiving cisplatin chemotherapy, while minimizing systemic STS exposure.
Preclinical studies conducted in rodent animal models have demonstrated sustained levels of STS in perilymph with minimal systemic exposure that results in protection of sensory cells and hearing loss from cisplatin ototoxicity when DB-020 was administered at the time of cisplatin. These data, along with additional preclinical studies supported a Phase 1 clinical study in healthy volunteers which showed unilateral and bi-lateral administration of DB-020 was safe and well tolerated across a range of doses. The study also showed that systemic STS exposure following DB-020 was minimal and at levels lower than would be expected to have impact on cisplatin.
Based on these data, the safety and efficacy of DB-020 is being evaluated in a double-blind, placebo controlled Phase 1b clinical trial in oncology patients who are receiving cisplatin in the US and AUS. Patients enrolled in the Phase 1b clinical trial were randomized to receive one of two doses of DB-020 in one ear along with receiving placebo in the contralateral ear, enabling each patient to serve as their own control.
Results: Consistent with the results of a Phase 1 clinical trial of DB-020 previously completed in healthy volunteers, data from the topline interim analysis demonstrated that DB-020 was well tolerated, with adverse events generally mild to moderate. 88% of patients experienced ototoxicity in their placebo-treated ear, and of these patients, 87% were partially or completely protected from ototoxicity in their DB-020-treated ears.
Conclusions: Positive topline safety and efficacy data from the interim analysis of the ongoing Phase 1b study supports continued development of DB-020 as a potential therapy to protect against hearing loss in patients receiving cisplatin chemotherapy for cancer.

Sequestration of Ototoxins in Cisplatin-Induced Ototoxicity by Chelate-Functionalized Magnetic Micelles

Pan Liao*[1], Kang Du[2], Ivan Dmochowski[2], Daqing Li[1]
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Background: Cisplatin (cis-diamminedichloroplatinum, Pt(NH3)2Cl2) has been widely used to treat head and neck squamous cell carcinoma. However, cisplatin-induced ototoxicity significantly decreases the quality of life, causing an important challenge to patient care.
Methods: In this work, we reported two magnetic micelles that effectively and efficiently facilitate sequestration of ototoxins in cisplatin-induced ototoxicity. These micelles were composed of biocompatible amphiphilic block copolymers with regions of hydrophobic polycaprolactone (PCL) and hydrophilic polyethylene glycol (PEG) groups. The surface-exposed hydrophilic terminus was functionalized with two different chelating groups including glutathione (GSH) and dithiocarbamate (DTC) to capture cisplatin. Superparamagnetic iron oxide nanoparticle (SPION) encapsulated in the hydrophobic core made it possible to magnetically maneuver the micelles, which can be applied to the inner ear detoxification.

Results: The dynamic light scattering (DLS) results revealed that the micelles made from PCL-PEG-GSH and PCL-PEG-DTC were monodisperse in aqueous solutions with hydrodynamic diameters of 106 and 116 nm, respectively. ICP-OES measurements demonstrated that the cisplatin sequestration effectiveness can be successfully achieved in 95% and 73% after 96 hours of reaction in 37 °C with GSH- and DTC-micelle solutions of 150 μM and 10 μM cisplatin, respectively. The elemental distribution of a single cisplatin-saturated DTC-micelle demonstrated tightly binding between the Pt and micelles by scanning transmission electron microscopy (STEM). In addition, the cochlear organotypic culture studies showed no morphology changes or damages in cochlear hair cells in the presence of the GSH-micelles at the highest tested concentration of 150 μM.
Conclusions: The present study suggests that both magnetic micelles can effectively bind to cisplatin whereas GSH-micelles may have a better cisplatin binding efficiency, and DTC-micelles may be more effective in magnetically maneuvering. These biocompatible chelate-functionalized SPION micelles may have great potential to be used to sequester ototoxins in the inner ear to alleviate cisplatin-induced ototoxicity.

Targeting CXCL1 Chemokine Signaling for Treating Cisplatin Ototoxicity

Raheem Al Aameri*[1], Entkhab Alanisi[2], Oluwatosin Adu[3], Dheyaa Al Sallami[4], Ian Alberts[5], Sri Patel[5], Leonard Rybak[6], Vickram Ramkumar[3]
[1]SIU School of Medicine, [2]Mustansiriyah University/Department of Biology., [3]SIU Medicine/Pharmacology, [4]SIU Medicine/MMICB, [5]SIU Medicine, [6]SIU Medicine/Department of Surgery
Background: Cisplatin is chemotherapy used for solid tumor treatment like lung, bladder, head and neck, ovarian and testicular cancers. However, cisplatin-induced ototoxicity limits the utility of this agent in cancer patients, especially when dose escalations are needed. Ototoxicity is associated with cochlear cell death through DNA damage, the generation of reactive oxygen species (ROS) and the consequent activation of caspase, glutamate excitotoxicity, inflammation, apoptosis and/or necrosis. Previous studies have demonstrated a role of CXC chemokines in cisplatin ototoxicity. In this study, we investigated the role of CXCL1 in mediating cisplatin-induced hearing loss.
Methods: Adult male Wistar rats (200-250g) were treated with SB225002 (1.4 nmoles/ear) trans-tympanically, followed by cisplatin (11mg/kg, i.p.). Auditory brainstem responses (ABRs) and cochlear whole mount were performed to assess hearing loss, OHC morphology and ribbon synapse loss. Immunohistochemical studies evaluated the levels of CXCL1, CD45 and CD68 in cochlea. Organ of Corti-derived cells (UB/OC-1) treated with cisplatin in the presence and absence of the antagonist SB225002 to determine the effects on CXCL1/CXCR2 interaction.
Results: CXCL1 is expressed in the cochlea and its expression is increased after cisplatin treatment in a time-dependent manner. This was associated with a progressive increase in CD45, IBA1 and CD68-positive immune cells especially in the stria vascularis, spiral ligament and spiral ganglion neurons. Moreover, cisplatin treatment produced significant loss in outer hair cells, loss of inner hair cell ribbon synapses and reductions in Wave 1 amplitudes. Trans-tympanic administration of SB225002, a chemical inhibitor of CXCR2 (receptor target for CXCL1) reduced immune cell migration and protected against hearing loss, loss of outer hair cells and loss of inner hair cell ribbon synapses. Similarly, knockdown of CXCR2 by trans-tympanic administration of CXCR2 siRNA protected against hearing loss and loss of outer hair cells and reduced ribbon synapses. One underlying mechanism underlying protection by these agents involves suppression of the cochlear inflammatory responses as indicated by reductions in the expression of CXCL1, NOX3, iNOS, TNF-α, IL-6 and COX-2. We also show that SB225002 reduced the migration of CD68 and CD45 inflammatory cells into the cochlea. These results implicate the CXCL1 chemokine as an early player in cisplatin ototoxicity, possibly by initiating the immune cascade to promote entry of early immune cells into the cochlea. Thus, CXCR2 could serve as a druggable target for treating cisplatin ototoxicity.
Conclusions: These results implicate that CXCL1 chemokine as an early player in cisplatin ototoxicity, possibly by initiating the immune cascade, and indicate its target (CXCR2) as a relevant target for treating cisplatin ototoxicity.

Symposium #27 - Online Experimentation in Audition: Recent Advances and Future Directions

10:30 a.m. - 12:30 p.m.
Crystal Ballroom D-E

Online Experimentation in Audition: Recent Advances and Future Directions

Chair: Nori Jacoby, *Max Planck Institut for Empirical Aesthetics*
Co-Chair: Malinda McPherson, *University of California, San Diego*

During COVID-19 lockdowns, many auditory scientists were forced to begin or expand their use of online data collection platforms. Three years later, what have we learned? How can psychoacoustics, and rigorous experimentation more broadly, be conducted online? What are the upsides and downsides of web-based data collection? Moving forward, what kinds of new opportunities are enabled by online experimentation? The goal of this symposium is to disseminate recent advances using online data collection, share methods for online research, and review potential pitfalls of online experimentation. Presenters will discuss different ways online experiments can be conducted, including one-on-one interactions, asynchronous data collection, and active behavioral measurement paradigms. By bringing together perspectives from clinical and basic scientists, we hope to explore how online experimentation can be used to augment a broad range of research programs for years to come.

Target Audience: We believe this symposium will be of interest to all auditory scientists and clinicians working with human participants, including those who work with clinical populations, and those who do basic research and psychophysics.

Breaking Auditory Psychophysics Out of the Laboratory

Lori Holt, *Carnegie Mellon University*

The methodologies of auditory psychophysics tend to rely on strongly sound-attenuated environments, finely calibrated equipment, and small numbers of expert or highly trained listeners who are motivated and compliant with task demands. This high level of 'auditory hygiene' is important: seemingly minute differences in stimulus delivery and timing, background noise levels, or participant engagement during an arduous task can dramatically affect experimental results. Laboratory disruptions due to COVID-19 inspired unexpectedly successful innovations in porting auditory research from well-controlled laboratories to participants' home offices and living rooms, using only the internet bandwidth, computers, and headphones at participants' immediate disposal. We will share three success stories in online auditory psychophysics, and convey lessons that can carry forward to facilitate future post-pandemic research. The first is that robust and efficient human auditory psychophysics is possible with inexpert online listeners using only their home equipment, even for tasks that would seem to demand high control. We demonstrate that online measures are effective even for highly finicky paradigms like tone-in-noise threshold estimation, and frequency-selective attention measured using the probe-signal tone detection in noise paradigm. The second lesson is that studying samples of convenience who tend to be 'WEIRD people' from Western, educated, industrialized, rich and democratic backgrounds sometimes leads us to the wrong conclusions about auditory processing. We share examples from our research for which some effects replicate cleanly across in-laboratory university students versus online citizens of the world, and – interestingly – some do not. The third lesson is that gamifying online testing can be highly useful in engaging special populations, or in testing targeted research questions, but that even more typical (dull) psychophysics tasks can succeed online with attention to participant engagement. Finally, we conclude with opportunities and challenges for carrying online testing forward to hasten and democratize future auditory psychophysics research, even beyond the COVID-19 pandemic.

An Online, Reaction-Time Based, Longitudinal Study on Long-Term Auditory Memory

Roberta Bianco, *Istituto Italiano di Tecnologia*

Longitudinal studies are fundamental for understanding auditory memory and plasticity. One major challenge of in-lab approaches is that participants must commit to coming to the lab on a regular basis, often for only brief memory recall sessions. This could be even more problematic for populations with reduced mobility (e.g., ageing or clinical). Online testing can overcome this limitation allowing one to test diverse large samples remotely and with efficiency. We demonstrate the feasibility of online approaches with an auditory memory paradigm based on a reaction times (RTs), tested on young and old participants.

Auditory memory for tone patterns was tested online with a paradigm previously used with young participants in the lab. This test requires participants to listen to rapid tone-sequences and to quickly respond to regularly repeating patterns (REG) emerging from random sequences. Unbeknownst to them, a few different patterns reoccur every ~3 minutes (REGr). RTs to novel REG are taken as a measure of the amount of information held in short-term memory until the pattern is detected; RTs to REGr are expected to decrease with exposure indicating long-term memory formation of previously heard patterns. Old and young adults (N = 191; aged 60-70 and 20-30 years) were recruited online and performed the test on day 1 (20 min) and a recall test 8 days later (5 min, 1 dropout). Participants were excluded based on an initial headphone check (Milne et al., 2020) and attention checks interspersed in the main task (i.e., absent or slow responses to simple tone changes) (final N =132). RTs to simple tone changes were further used as a measure of individuals' RTs to simple changes to distil the computation time required to detect the patterns. The results from the online young sample replicated the pattern of RTs to REG and REGr conditions observed in lab. Furthermore, we found age-related impairments in both short- and long-term memory measures, but preserved long-term memory in both groups as assessed on day 8. Short- and long-term memory effects were not linked with visual-spatial memory or processing speed measures.

Overall, there is an exciting promise of online longitudinal studies for tracking how auditory memory changes over long-time periods in populations which would be difficult to repetitively bring to the laboratory. Sensitive measures such as RTs can be reliably collected online, but at the cost of excluding a large number (~30%) of participants failing audio-equipment and attention checks.

Online Auditory Psychophysics Enables New Psychoacoustic Paradigms
Malinda McPherson, *University of California, San Diego*

Recent work has illustrated how traditional psychoacoustic experiments can often be implemented successfully using online crowdsourcing. While online data collection sacrifices precise control over sound quality and participant environment, it enables experiments when in-person activities are limited, and thus gained converts during the pandemic. However, online experiments are more than a fallback. In particular, they facilitate data collection on a scale that is difficult to attain in the laboratory. In this talk I will describe several results from experiments that were only feasible because of online recruitment. For example, we have measured individual differences in pitch discrimination judgments across hundreds of participants. Individual sessions in these experiments lasted up to two hours, analogous to typical in-person experiment durations, and the measured pitch discrimination judgments were comparable to those obtained in tightly controlled laboratory conditions. However, online recruitment enabled us to enroll the large numbers required to assess, and to replicate, individual differences (>700 participants) – sample sizes that would have been impractical to obtain in the lab. I will also describe several experiments whose design required participants to complete only a single trial per condition. Such experiments again require very large samples to achieve adequate power, making them impractical for the lab. But such approaches can yield new insights and are readily possible when implemented online. We will argue that even outside of extreme circumstances such as a pandemic, the ability to recruit large numbers of participants make online experiments an attractive tool, and expands the range of psychoacoustic paradigms.

Comparing the Reliability of Virtual and In-Person Post-Stroke Neuropsychological Assessment With Speech and Language Tasks
Julie Fiez, *University of Pittsburgh*

Neuropsychological testing is essential for both clinical and basic stroke research; however, the in-person nature of this testing is a limitation. Virtual testing overcomes the hurdles of geographic location, mobility issues, and permits social distancing, yet its validity has received relatively little investigation, particularly in comparison to in-person testing. We present results assessing virtual versus in-person administration of language and communication tasks with 48 left-hemisphere stroke patients (21F, 27 M; mean age = 63.4 ± 12; mean years of education = 15.3 ± 3.5) in a quasi-test-retest paradigm. Each participant completed two testing sessions: one in their home and one in the research lab. Participants were assigned to one of eight groups, with the testing condition (fully in-person, partially virtual), order of home session (1st , 2nd), and technology (iPad, Windows tablet) varied across groups. Across six speech-language tasks that utilized

varying response modalities and interfaces, we found no significant difference in performance between virtual and in-person testing. However, our results reveal key considerations for successful virtual administration of neuropsychological tests, including technology complications and disparities in Internet access.

Online Auditory Experimentation in Ageing and Clinical Disorders
Meher Lad, *Newcastle University*

Online research has allowed scientists to greatly expand the scope of questions they ask and the range of participants they include in their studies. However, this comes with its costs. Online experiments can create different contextual effects, there are limitations on the kind of experiment one can perform and the type of participant one includes in their study. These are increasingly important to consider with older participants and those with cognitive impairments.

In this talk, I will present some successful studies that we have performed, in older participants with and without cognitive impairment, studying auditory perception and range of auditory cognitive processes from scene analysis to auditory memory. I will discuss some of the challenges we faced and some efforts we made to overcome these. Finally, I will present scenarios that we are yet to overcome with patient populations.

Running Online Auditory Experiments in Complex Production Modalities
Manuel Anglada-Tort, *Max Planck Institute for Empirical Aesthetics*

Online experiments using recruitment services (such as Prolific or Amazon Mechanical Turk) are becoming increasingly important in cognitive science. However, conducting online research in the auditory domain is particularly challenging: it requires participants to use certain hardware (headphones or microphone), be in a quiet environment, and provide complex behavioral responses, such as subjective ratings or reaction times with high millisecond-level precision. In this talk, I examine key challenges and recommendations when conducting online research in complex auditory modalities, such as recording participants' responses (tapping or singing) through the web browser with high temporal fidelity. I will then discuss how these challenges can be addressed by combining several useful techniques, such as economic pre-screen tasks, data quality monitoring online, motivational incentives, and feedback based on performance. Finally, I will show that by applying these recommendations researchers can now conduct large-scale online experiments that would be nearly impossible in the laboratory, reducing experimental costs while massively increasing the efficiency, scalability, and diversity of auditory research.

Extending the Possibilities of Auditory Psychophysics With Massive Online Experiments
Nori Jacoby, *Max Planck Institut for Empirical Aesthetics*

Experiments conducted online can significantly increase the scale and scope of experimental research. Here I introduce PsyNet (https://www.psynet.dev/), a new Python package for developing online behavioral experiments. PsyNet streamlines the development of highly complex experiment paradigms, ranging from adaptive psychophysics to iterated learning to cultural evolution over social networks. It also streamlines experiment deployment, taking care of server provisioning, participant recruitment, data-quality monitoring, and participant payment. As a result, every experiment can be replicated by using only one terminal command. This presentation illustrates how PsyNet can be used to study classical questions in auditory perception such as pitch and consonance perception, and how we can apply it to significantly increase the number of stimuli, participants, and control experiments in a single study, as well as the diversity of the participants in auditory psychology.

gEAR Workshop - Intro and Analyze scRNA-seq Data: The Single-Cell Workbench
12:30 p.m. - 2:00 p.m.
Biscayne 1

Introduction (30 min)

This will be a walk-through of the basic features of the portal, giving the background needed for the more tool-specific sessions. Topics include account creation, basic gene expression searches, data sharing, site navigation and overall feature discussion. We will also overview some of the new features of gEAR and point users to online webinars where they can learn more about them.

Analyze scRNA-seq data: The Single-cell workbench
Learn how to use the workbench without any programming experience, walking through all the steps of a scRNA pipeline using a point and click interface. This workshop includes a hands-on component, bring your laptop along.

Ronna Hertzano*, University of Maryland School of Medicine*
Joshua Orvis*, Institute for Genome Sciences*

Behind the Scenes With Publication!

12:45 p.m. - 1:45 p.m.
Merritt 2

An expert panel of editors from the journals dedicated to advancing your scientific careers has been convened to discuss important issues associated with their journals, and additionally you will learn of initiatives through which you can exercise scientific leadership through reviewing, new features, or organizing focused collections

Larry Hoffman*, Geffen School of Medicine at UCLA*

EDI in Publishing
Ruth Litovsky*, University of Wisconsin*

The Impact Factors and Bibliometrics
Dan Sanes*, New York University*

The Innovations in Peer Review
Barbara Shinn-Cunningham*, Carnegie Mellon University*

The Leadership Opportunities in Publishing
Benjamin Crane*, University of Rochester*

spARO Mentoring Session: Careers in Academia

12:45 p.m. - 1:45 p.m.
Canaveral 1-2

Establishing yourself as a principal investigator with a new lab in an academic environment can be an exciting yet bumpy road to navigate. The panelists in this session will share their observations and experiences in establishing and funding independent research laboratories, discuss the keys for successfully applying for research support, and suggest avenues available to new or mid-career faculty members to facilitate the success of their lab. This session will consist of short presentations from a panel of leading scientists in an interactive forum for questions and discussion

Lisa Goodrich*, Harvard University*
Brandon Cox*, Southern Illinois University School of Medicine*

Podium #28 - Hair Cells: Function and Mechanotransduction

2:00 p.m. - 4:00 p.m.
Ocean's Ballroom 5-12

Moderators: Hong-Bo Zhao and Haruna Suzuki-Kerr

The Mechanotransduction Channel Complex of Hair cells: A Sophisticated Molecular Machine

Ulrich Mueller*[1], Xufeng Qiu[1], Christopher Cunningham[2], Xiaoping Liang[1], Bo Zhao[3]
[1]Johns Hopkins University, [2]University of Pittsburgh, [3]Indiana University School of Medicine

Background: Organisms of all phyla express mechanosensitive ion channels with a wide range of physiological functions. In recent years, several classes of mechanically gated ion channels have been identified. Some of these ion channels are intrinsically mechanosensitive. Others depend on accessory proteins to regulate their response to mechanical force. The mechanotransduction machinery of cochlear hair cells provides a particularly striking example of a complex force-sensing machine.
Methods: Using genetic mouse models combined with electrophysiology and biochemistry, we have here functionally characterized the role of TMC1, TMIE, CIB2 and LHFPL5 for mechanotransduction by cochlear hair cells.
Results: We show that TMC1 and TMIE are obligatory ion channel subunits that are required to form a functional mechanotransduction channel. CIB2 is a regulatory channel subunit that regulates channel transport and function. LHFPL5 mediates interactions between tip-links and mechanotransduction channels and is critical to establish maximal force-sensitivity of the mechanotransduction channel.
Conclusions: Our findings thus define functions for individual components of the mechanotranduction channel complex and reveal mechanisms of channel gating.

The Calcium and Integrin-Binding Protein 2 (CIB2) Transmits the Force to the Mechanotransducer of Mammalian Auditory Hair Cells

Isabel Aristizabal*[1], Arnaud Giese[2], Abigail Dragich[1], Sofia Zuluaga-Osorio[1], Shadan Hadi[1], Saima Riazuddin[3], Zubair Ahmed[3], Gregory I Frolenkov[1]
[1]University of Kentucky, [2]SENSORION SA, [3]University of Maryland School of Medicine

Background: Calcium and Integrin-Binding protein 2 (CIB2) is essential for hearing and mechano-electrical transduction (MET) in the auditory hair cells (Riazzudin et al., 2012, Giese et al., 2017, Liang et al., 2021). CIB2 interacts with pore-forming subunits of the MET channel, TMC1/2 (Giese et al., 2017. Liang et al., 2021). In addition, CIB2 is required for the transport and/or retention of TMCs at stereocilia tips (Liang et al., 2021). CIB2 also interacts with whirlin (Riazzudin et al., 2012), which is indirectly attached to the cytoskeleton via myosin XVa. Thus, CIB2 may provide a mechanical link between the MET channel and the cytoskeleton. Unfortunately, investigations of the exact role of CIB2 in mechanotransduction are complicated by the fact that deafness-associated variants with disrupted CIB2-TMC1/2 interaction do not have any measurable MET currents in the auditory hair cells. Therefore, we generated a new mouse strain with p.R186W mutation in Cib2 that preserves CIB2-TMC1/2 interaction.
Methods: We recorded MET currents elicited by deflection of the stereocilia bundle in freshly isolated Organ of Corti explants from P4-P8 mice. To resolve the speed of force transmission to the MET channels, we designed a custom piezo-driven stiff probe that is able to produce ~1 μm deflection in less than 10 μs, which was determined by video recordings with a high-speed camera at 90,000 fps. The MET currents produced by stereocilia deflections with this probe were used to calculate the time constants of channel activation and adaptation. The activation of MET current was slower than the stimulus and best fitted to the double Boltzmann curve, confirming our ability to resolve the activation of the MET channels. To determine the effects of CIB2 deficiency on hair bundle stiffness, we also video recorded and measured (at 3,000 fps) the hair bundle deflections produced by conventional fluid-jet. Finally, we examined the effects of p.R186W mutation on the stereocilia ultrastructure using focused ion beam serial sectioning and backscattered electron microscopy (FIB-SEM).
Results: As expected, both inner and outer hair cells of Cib2R186W/R186W mice exhibited decreased but detectable MET currents, consistently with a recent report (Liang et al., 2021). However, MET currents in Cib2R186W/R186W hair cells showed no fast adaptation and the time constant of their activation was significantly slower compared to wildtype littermates, suggesting the deficiency in the force transmission to the MET channels. Consistently, the lower tip-link density was disrupted in Cib2R186W/R186W stereocilia and hair bundle stiffness was decreased. Interestingly, heterozygous Cib2R186W/+ hair cells had apparently normal MET currents with a “wildtype” speed of activation.
Conclusions: We concluded that the p.R186W mutation in CIB2 disrupts force transmission to the MET channels in homozygous but not heterozygous mice.
Supported by NIH (R01DC012564 and S10OD025130)

LOXHD1 is Required for TMC1 Localization at the Lower Tip-Link Insertion Area

Pei Wang[1], Katharine K Miller[1], Enqi He[1], Siddhant S Dhawan[1], Christopher L Cunningham[2], Nicolas Grillet*[1]
[1]Stanford University, [2]University of Pittsburgh

Background: We are investigating the molecular function of the LOXHD1 gene, which we previously linked to an autosomal-recessive form of hearing loss in mice and humans (DFNB77) (Grillet N., AJHG, 2009). LOXHD1 is selectively expressed by hair cells and is required for their function, as assessed by ABR and DPOAE. LOXHD1 encodes a protein comprising 15 polycystin/lipoxygenase/alpha-toxin (PLAT) domains, known in other proteins to bind lipids and proteins. Previously, we found that mice carrying mutations in the 10th PLAT repeat have affected inner hair cell (IHC) mechanotransducer (MET) currents, with a sudden 95% decrease in current amplitude between postnatal days (P)7 and P11. This decrease was not due to loss of tip links, loss of the upper tip-link protein USH1C, or loss of the lower tip-link protein LHFPL5 (Trouillet et al., 2021). How LOXHD1 affects MET currents remains unknown. Due to the existence of alternative splicing isoforms, an earlier or additional function of LOXHD1 may have been masked in existing mouse models. For similar reasons, the localization of LOXHD1 in the hair bundle has remained uncertain.
Methods: To further understand LOXHD1's function in hair cells, we produced a large deletion (160 kb), eliminating all LOXHD1 PLAT domain-coding sequences in mice. We phenotyped this Loxhd1Delta allele at the auditory and electrophysiology levels. We used scanning electron microscopy (SEM) to assess hair bundle defects. Finally, we monitored the expression of MET channel components TMC1 and TMC2 using tagged knock-in alleles (Cunningham et al., 2020) with immunofluorescence and SUB-Immunogold-SEM (Miller et al., in preparation). Additionally, we produced a tagged knock-in mouse allele to localize LOXHD1.
Results: Using our tagged knock-in mouse, we localized LOXHD1 at the lower tip-link insertion area. With our Loxhd1Delta mice, we revealed that, unlike other Loxhd1 mutant alleles which develop recessive hearing loss, Loxhd1Delta showed elevated ABR and DPOAE thresholds in both homozygous and, to a lesser degree, heterozygous animals. This dominant phenotype indicates that LOXHD1 isoforms can partially compensate for point mutations affecting PLAT10. We found that IHCs lose MET between P7 and P11, suggesting that LOXHD1 is dispensable for earlier MET. Because the time frame of the MET phenotype corresponds to the molecular switch of the MET channel complex from a "TMC1/TMC2" to a "TMC1 only" situation, we tracked TMC1 in the hair bundle. We could still detect TMC1 in the hair bundle, but its localization at the lower tip-link area was significantly reduced in IHCs at P11 and P21.
Conclusions: The deafness protein LOXHD1 localizes at the tip of the transducing stereocilia and maintains TMC1 at this location. The function of LOXHD1 differs from that of TMIE and CIB2, which prevent TMC1 from being localized to the hair bundle when inactivated. LOXHD1 represents a new element in mature MET machinery.

The Tetraspan LHFPL5 Establishes Force Sensitivity of the Mechanotransduction Channel of Cochlear Hair Cells

Xufeng Qiu*[1], Xiaoping Liang[1], Jose P. Llongueras[1], Christopher Cunningham[1], Ulrich Müller[2]
[1]Johns Hopkins University, [2]University of Pittsburgh

Background: Mechano-electrical transduction (MET) at cochlear hair cells converts sound-induced mechanical stimuli into electrical signals, which is critical for sound perception. Hair cells are the sensory cells with F-actin based stereocilia protruding from the apical surface. The MET channels are located in the shorter stereocilia of hair cells near tip links, the fine extracellular filaments that are thought to gate the MET channel. TMC1/2, TMIE, LHFPL5, and CIB2 are essential components of the MET channel complex. TMC1/2 and TMIE bind to each other to form a functional ion channel complex and mutations in each of these proteins affect channel pore properties. CIB2 binds to TMC1/2 and is an auxiliary subunit that regulates MET channel localization and function. LHFPL5 has been proven to bind with TMC1, TMIE, and PCDH15, and is essential for regulating mechanotransduction and channel properties. However, the molecular mechanisms by which LHFPL5 regulates MET remains elusive.
Methods: Co-Immunoprecipitation was applied to detect protein-protein interactions in HEK293 cell lines. Whole-mount immunostaining in either acute dissected cochlea or cultured explants was applied to study the localization of TMC, PCDH15, and LHFPL5 in hair cells. CRISPR/CAS9 technique was used to generate

LHFPL5 mutant mouse lines. MET currents were recorded by whole-cell patch clamping on hair cells with a stiff probe or fluid jet stimuli applied to the hair bundle. Rescue experiments were performed by injectoporation of cultured cochlear explants.
Results: Taking advantage of structure-based predictions and Chimeric proteins between LHFPL5 and its homology, we characterized functional domains in LHFPL5 that mediate interactions with MET channel and tip links. These domains are critical to regulating the response of the MET channel to mechanical force, including the regulation of optimal channel activation and maximal force sensitivity. Using mutational analysis, we further identified a small N-terminal domain in LHFPL5 that is critical for the function of LHFPL5 in regulating MET channel activity and force sensitivity, without affecting hair bundle morphology or the localization of tip link protein and channel components. In addition, we mechanistically studied the N-terminal domain of LHFPL5 and found it interacts with TMC1 to regulate MET.
Conclusions: Our studies thus provide insights into the gating mechanisms of the MET channel in hair cells and define a crucial role for LHFPL5 in establishing the channel's force sensitivity.

TMEM63B Functions as a Monomeric High-Threshold Mechanosensitive Ion Channel in Outer Hair Cells

Wang Zheng*[1], Shaun Rawson[2], Jeffrey Holt[1]
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Background: TMEM63/OSCAs proteins form the largest family of mechanically-activated ion channels identified to date and are evolutionarily related to TMEM16/anoctamin and TMC families. OSCAs are found in plants while TMEM63 proteins exist in animals. TMEM63B is expressed in mouse outer hair cells where it was suggested to act as an osmosensor (Du et al, Cell Rep. 2020). Knock-out of TMEM63B in mice leads to outer hair cell death and hearing loss. How TMEM63B is gated by mechanical stimuli and contributes to outer hair cell homeostasis remains unclear.
Methods: TMEM63 protein structures were resolved with cryo-electron microscopy single-particle analysis using Titan Krios microscope. Images were taken at 300 kV and processed using cryoSPARC. Oligomeric states of TMEM63 proteins were determined by fluorescence size-exclusion chromatography and cross-linking assays. TMEM63-evoked mechanically-activated currents were induced by applying negative pressures in the recording pipette using a high-speed pressure clamp system. TMEM63 proteins were either overexpressed in heterologous cells or reconstituted in proteoliposomes before functional measurement. Chimera proteins and point mutant proteins were generated using NEBuilder or site-directed mutagenesis.
Results: We first revealed an unexpected monomeric conformation of TMEM63B and closely-related TMEM63A via single-particle cryo-electron microscopy, which contrasts with dimeric OSCA, TMEM16 and TMC1 channels. The monomeric state was confirmed by multiple lines of biochemical evidence. TMEM63s possess 11 transmembrane (TM) domains and a large intracellular loop (IL2) between TM2 and TM3. IL2 includes several evolutionary variations and was shown to mediate dimerization in OSCAs (Sebastian et al, eLife, 2018). Replacement of the IL2 of OSCA1.2 with that of TMEM63A or mutations in key variable residues in IL2 of OSCA1.2 resulted in monomeric OSCA proteins, revealing IL2 as a molecular determinant underlying the unique monomeric state of TMEM63s. Functionally, monomeric TMEM63s or OSCA1.2 derivatives evoked mechanically-activated currents with significantly higher thresholds, relative to dimeric OSCA1.2. Structural analyses unraveled substantial conformational differences in mechano-sensing domain IL2 and gating helix TM6 between monomeric TMEM63s and dimeric OSCA1.2, which render TMEM63s well-suited to act as high-threshold mechanosensitive ion channels.
Conclusions: TMEM63B and TMEM63A function as monomeric mechanosensitive ion channels with high thresholds. Functionally, this could allow TMEM63B channels to remain in the closed state during the normal function of electromotive outer hair cells and to open only when out hair cells experience severe osmotic stress. Our studies provide a framework for better understanding of the molecular function and gating of TMEM63, TMEM16 and TMC channels.

ESPNL Responds to Mechanotransduction and is Required to Maintain Mechanotransducing Stereocilia

Katelin Hawbaker*[1], Benjamin Perrin[1]
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Background: The stereocilia bundle is organized into 3 rows of increasing heights. Stereocilia lengths are precisely regulated to efficiently gate mechanotransduction channels located at the tips of stereocilia in the shorter rows in response to sound. ESPNL is an actin binding protein that contributes to stereocilia length regulation and localizes to mechanotransducing stereocilia tips. While most proteins that localize to stereocilia tips are found at consistent levels between neighboring stereocilia, ESPNL varies considerably between stereocilia on the same cell during early development. Since mechanotransduction influences stereocilia lengthening and widening during early development, we hypothesized that ESPNL could respond to mechanotransduction to refine stereocilia size.
Methods: We used CRISPR/Cas9 to generate a 20 bp deletion in mouse Espnl which was predicted to result in loss of function. Auditory function and stereocilia morphology Espnl +/+, Espnl +/- and Espnl-/- mice were assessed by auditory brainstem response and scanning electron microscopy at 4, 10, 16, and 24 weeks. Because mechanotransducing stereocilia were most affected by the Espnl mutation, we also assessed ESPNL localization during postnatal development when mechanotransduction activity shapes stereocilia morphology and in response to the mechanotransduction blocker tubocurarine.
Results: Espnl -/- mice exhibited progressive hearing loss. ABR thresholds above 22 kHz were elevated at 4 weeks of age and responses were undetectable above 16kHz by 24 weeks of age. Scanning electron microscopy revealed progressive degeneration of mechanotransducing rows of stereocilia. In the middle turn, the shortest row was largely lost by P11. The next shortest row also degenerated with age, with decreased and irregular lengths by 10 weeks of age. In 24-week-old Espnl-/- mice there were no hair cell bundles at the base and many bundles were missing in the middle turn. In early postnatal development, ESPNL localizes at high levels to some mechanotransducing stereocilia but not others and then gradually shifts to be evenly distributed at row 1 and 2 stereocilia tips by P7.5. The variable localization in early development suggests it could respond to local fluctuations in mechanotransduction activity. Correspondingly, ESPNL localization dramatically changed when explants were treated with the channel blocking drug tubocurarine. Within 2 hours, ESPNL shifted to be evenly localized at the tips of all stereocilia. After washout, ESPNL reverted to being localized at varying levels at mechanotransducing stereocilia tips and at low levels at the tips of stereocilia in the tallest row.
Conclusions: ESPNL localization responds to mechanotransduction activity in postnatal hair cell stereocilia and is required to maintain mechanotransducing stereocilia.

LHX3 is Required for the Maintenance and Maturation of the Inner Ear Hair Cell

Mei Xu*[1], Shuchun Li[2], Xiaoling Xie[2], Luming Guo[2], Lin Gan[2]
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Background: LHX3, a LIM-homeodomain transcription factor expressed in the nervous system and in the pituitary, is previously found to be essential for specifying of pituitary cell lineages, activating the expression of pituitary hormone genes, and determining subtype identities of motor neurons in the spinal cord. In humans, mutations in LHX3 cause complex hormone deficiency diseases referred to combined pituitary hormone deficiency type 3. Some of the extra-pituitary manifestations of LHX3 mutations in humans are sensorineural hearing loss. Nevertheless, it remains unknown whether LHX3 mutations directly affect the development and function of the cochlear hair cells.
Methods: In this study, we have generated Lhx3loxP conditional knockout mice and have used Pax2-Cre to delete Lhx3 specifically in the developing inner ear. Lhx3-null cochleae were analyzed and compared with the controls for the hair cell degeneration, synapse estimation by immunostaining, changes in the structure of stereocilia by SEM. RNA-Seq, CUT and Tag, TRAP-Seq, ISH and RNAScope ISH were used to identify the downstream gene network of Lhx3.
Results: Deletion of Lhx3 results in postnatal progressive degeneration of both inner and outer hair cells in the cochlea and led to hearing loss. Similar to other mutants causing age-related hearing loss, the accelerated hair cell loss in Lhx3-deficient mice followed a pattern from base to apex and in outer hair cells more than inner hair cells. The synaptic connections between inner hair cells and auditory fibers were decreased prior to hair cell degeneration. Additionally, the development of stereocilia in Lhx3-deficient hair cells was significantly disrupted with loss of staircase and V-shape organization. Furthermore, RNA-Seq, CUT and Tag and TRAP-Seq analyses revealed that Lhx3 regulated Barhl1 and Myo3a.
Conclusions: Lhx3 is indispensable for the maturation and survival of cochlear hair cells. Lhx3 regulates the gene regulatory network associated with hair cell survival and stereocilia development.

A Novel Gene Associated With Hearing Loss in Adult Humans is Necessary for Hearing in Mice

Alexandra Kaufman*[1], Alma Corona[1], Elika Fallah[1], Eva Morozko[1], Sarah Cancelarich[1], Kara Graham[1], Daniel Johnson[1], Roberto Donnianni[1], Kevin Bugge[1], Kavita Praveen[2], Giovanni Coppola[3], Meghan Drummond[1]

[1]Regeneron Pharmaceuticals, [2]Regeneron Genetics Center, Regeneron Pharmaceuticals Inc., [3]Regeneron Genetics Center

Background: The Regeneron Genetics Center recently conducted a genome wide association analysis to identify alleles associated with hearing loss in adults (Praveen et al., 2022). This analysis uncovered many known deafness genes in addition to several novel associations. We focused on genes in which predicted loss of function mutations increase risk for deafness in adults.

Methods: To evaluate the necessity of these genes for hearing, Regeneron generated knockout mice and we performed functional hearing assays. The mice have profound hearing loss at the onset of hearing and near complete hearing loss by 8 weeks. Histology data at 8 weeks show some hair cells are missing, and there is a visible supporting cell scar that is known to form when hair cells die, hair cell death rather than failure to develop. Hair cells that remain show abnormal morphology. Continuing work will characterize the time course of hair cell loss, beginning before the onset of hearing to assess the developmental requirement of this gene.

Results: We used RT-qPCR to evaluate body-wide gene expression and found that our gene of interest is expressed in cochlea of mice and other tissues of mice including brain, liver and testes. There was a slightly different expression pattern in humans, similarly high in the liver but lower in brain, for example. We also investigated the presence of alternatively spliced transcripts in cochlea and liver. RNA scope indicates that both putative isoforms are expressed exclusively in hair cells within the cochlea, including inner hair cells, outer hair cells, and vestibular hair cells.

Conclusions: Future work will explore the subcellular localization and binding partners of the gene, as well as cellular morphology and function of the mechanotransduction machinery.

Podium #29 - Factors Impacting Cochlear Implant Outcomes

2:00 p.m. - 4:00 p.m.
Oceans Ballroom 1-4

Moderators: Lina Reiss and Alexander Claussen

The Effects of Binaural Integration for Consonant and Vowel Identification in Bilateral Cochlear Implant Users

Matthew Ardis*[1], Raymond Goldsworthy[2]

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Background: With the preference of cochlear implantations shifting towards bilateral fittings, so too should the research to analyze the benefits or potential drawbacks (i.e., binaural interference) of bilateral fittings as opposed to single sided devices. A potential benefit to bilateral stimulation is the synergistic effect of two ears yielding a higher speech recognition score than their individual counterparts in a process called binaural integration. This binaural integration of phonemes is also thought to aid in speech recognition in background noise, which is currently a major struggling point for many cochlear implant users. Our lab ventures to explore this with a series of assessments completed online via our web-based portal (TeamHearing). Virtual testing has become a salient topic as of recent with the rise of COVID-19. Testing participants in this manor not only reduces the spread of COVID-19 but also allows for our tests to reach more participants. This will help more individuals with cochlear implants or hearing aids in their rehabilitation and gaining the most benefit from their respective devices.

Methods: The series of assessments include vowel identification, consonant identification, and sentence completion tasks in the presence of background noise (speech shaped noise). For vowel identification, participants completed each trial in a fixed SNR (quiet, 6dB, 0dB, -6dB, and -12dB) and were required to choose between vowel varying words (e.g., hawd, hod, hayd, etc.). The same was done for constant identification with changes being made to the target signal so that consonant varying words (e.g., ada, afa, aga, etc.) were played instead. Sentence completion in background noise was assessed using an adaptive

speech reception threshold procedure. Respondents heard a color and number within a carrier phrase and were asked to choose between predetermined options.
Results: Data will be analyzed to determine patterns of phonemic confusions (e.g., place/manor of articulation, etc.) and how identification is affected by background noise. More analysis will be done to consider if there is an effect of binaural integration/interference, particularly in noise, on word and phonemic recognition. Preliminary results will be presented.
Conclusions: Our primary hypothesis is the amount of benefit from binaural integration is affected by the disparity between ears (so that a larger disparity leads to a lesser benefit), etiology of hearing loss, and duration of deafness.

Effects of Peripheral Neural Health on Perception of Temporal Speech Cues in Cochlear Implant Recipients

Kara Leyzac*[1], Kelly Harris[1]
[1]*Medical University of South Carolina*
Background: Electrically-evoked compound action potentials (ECAPs) correlate with the density of surviving spiral ganglion neurons (SGNs) in cochlear implanted guinea pigs. In humans, these same ECAP measures sometimes correlate with speech recognition performance in cochlear implant (CI) users. However, it is not clear how higher SGN density promotes specific speech cues. We hypothesize that greater SGN density is important for auditory temporal encoding and that increasing carrier pulse rate may improve temporal coding, but only in channels with SGN densities sufficient to accurately encode faster rates. We tested these hypotheses using ECAPs, direct stimulation psychophysical gap detection, and direct stimulation cortical evoked potential gap detection, evoked by silent gaps in the pulse train.
Methods: Participants included adult post-lingually deafened (>18 years old) CI recipients who used Cochlear ™ implant systems and had at least 3 months' CI experience. ECAP amplitude-growth functions (AGFs) were measured using two interphase gaps (IPGs) of 7 and 30 μs. The difference in AGF slope between the two IPGs for each electrode was calculated ("IPG effect"). For each participant, we identified the electrodes estimated to stimulate the highest and lowest SGN survival rates, indicated by the highest and lowest IPG effects, respectively. Psychophysical gap detection thresholds were recorded on these two electrodes using a three alternative forced choice procedure. Auditory evoked potentials were recorded using direct stimulation and a 64-channel Neuroscan Quickcap connected to a SynAmps RT. The cortical acoustic change complex (ACC) was recorded to fixed duration silent gaps, using two carrier pulse rates (500 and 3500 pps) on two electrodes within each participant.
Results: Preliminary results suggest that, for loudness balanced stimuli, psychophysical gap detection is more precise for higher rather than lower carrier pulse rates. Cortical encoding of temporal gaps is more precise when using a higher carrier pulse rate, but this effect is modulated by the condition of the auditory nerve. Specifically, results thus far suggest that using higher pulse rates to an electrode estimated to excite a higher density of SGNs in the cochlea results in more precise encoding of temporal cues in the auditory cortex. However, a similar improvement in the encoding of temporal cues is not noted when higher pulse rates are delivered to electrodes estimated to excite a lower density of neurons.
Conclusions: Preliminary studies demonstrate the extent to which auditory nerve health drives cortical representation of temporal cues within individuals. These results may provide novel approaches to improving speech recognition in CI users

Perception of Speech in Competing Speech in Children With Hearing Aids or Cochlear Implants

Deniz Başkent*[1], Leanne Nagels[1], Pinar Ertürk[2], Gizem Babaoğlu[2], Başak Özkişi Yazgan[2], Deborah Vickers[3], Petra Hendriks[4], Gonca Sennaroğlu[2], Laura Rachman[1], Etienne Gaudrain[5]
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Background: Perception of speech masked by competing speech relies on various mechanisms, such as perceiving the relevant acoustic cues (i.e., voice cues), segregating the target and masker speech, inhibiting the masker speech, and inferring missing segments of the target speech. In hearing loss, acoustic cues could

be less accessible, and in children, perceptual cognitive mechanisms may still be developing. Further, while hearing aids and cochlear implants provide some compensation for hearing loss, the speech acoustic cues transmitted via these devices are often different than those perceived with normal acoustic hearing. We present two studies where we aimed to characterize perception of speech in competing speech in children with hearing aids or cochlear implants in comparison to children with normal hearing, attempting to tease apart auditory and developmental effects.
Methods: In a cross-sectional study, speech perception with a single-talker competing speech masker was measured in children with hearing aids (native Turkish speakers, age range 5-18yr) or cochlear implants (native Dutch speakers, age range 4-12yr), along with age- and language-matched normal hearing children. The same matrix test (Children Coordinate Response Measure), using colors and numbers as target words, was implemented for the two languages. Target-to-masker ratios and voice cue differences between the target and masker speech were manipulated, ranging from no voice difference to large voice differences.
Results: Results from normal-hearing children showed strong developmental effects in either test language. While many hearing-aid children performed similarly to their age-matched normal-hearing peers, a number of aided children performed lower. Implanted children also tended to perform lower than their age-matched normal-hearing peers, however, they seemed to perform better than most implanted adults (previously reported). All children showed improvements in performance with increasing target-to-masker ratios, and they also seemed to benefit from target-masker voice cue differences.
Conclusions: The findings show that both groups of children with hearing loss seem to benefit well from their hearing devices for understanding speech in competing speech. A small number of aided or implanted children perform lower than the normal-hearing children. In the case of cochlear implants, the implanted children seem to perform better than the implanted adults, in both overall performance and also in their effective use of voice cue differences between target and masker. Child participants have bilateral implants while the adults do not. On the other hand, while implanted children do not have the advantage of speech development via acoustic hearing, they also do not have to unlearn it, like adults have to do when acoustic hearing is replaced with implant electric hearing. Hence, it is possible that either the bilateral configuration or implantation during the critical period for neural plasticity help child implant users to better perceive the speech cues transmitted by their implants.

The Relationship Between Electrophysiological Measures of the Electrically Evoked Compound Action Potential and Speech Perception in Adult Cochlear Implant Users

Jeffrey Skidmore*[1], Jacob Oleson[2], Yi Yuan[3], Shuman He[3]
[1]The Ohio State University Wexner Medical Center, [2]University of Iowa, [3]The Ohio State University
Background: While most adult cochlear implant (CI) recipients have improved auditory perception with their device, there remains large variability in speech recognition performance among adult CI patients (Boisvert et al., 2020; Goudey et al., 2021). To expand our understanding of key factors accounting for the speech perception variability among adult CI users, this study assessed the relationship between electrophysiological measures of the electrically evoked compound action potential (eCAP) and speech perception scores measured in quiet and in noise in post-lingually deafened adult CI users. It also tested the hypothesis that how well the auditory nerve (AN) encodes electrical stimulation is important for speech perception with a CI in challenging listening conditions.
Methods: Study participants included 24 post-lingually deafened adult CI users. In each participant, eCAPs were measured at multiple electrode locations in response to single-pulse, paired-pulse, and pulse-train stimuli. Independent variables included six parameters calculated from the eCAP recordings: the electrode-neuron interface (ENI) index, the neural adaptation (NA) ratio, NA speed, the adaptation recovery (AR) ratio, AR speed, and the amplitude modulation ratio (Skidmore et al., 2021, Riggs et al., 2021; He et al., 2022). Dependent variables included speech perception scores measured using Consonant-Nucleus-Consonant (CNC) words and AzBio sentences presented in quiet, as well as in noise at signal-to-noise ratios (SNRs) of +10 and +5 dB. Linear regression models were created for each speech measure to identify eCAP parameters with meaningful predictive power.
Results: The ENI index was identified as the only independent variable that had unique predictive power for each of the speech test results. The amount of variance in speech perception scores (both CNC words and AzBio sentences) explained by the independent variables increased with increased difficulty in the listening condition. Over half of the variance in speech perception scores measured in +5 dB SNR noise (both CNC

words and AzBio sentences) was explained by a model with only three independent variables: the ENI index, NA speed, and AR speed.
Conclusions: The ENI index is a sensitive predictor of speech perception performance in post-lingually deafened adult CI users. Therefore, technologies and strategies that enhance the quality of the ENI should result in improved outcomes for CI patients. The responsiveness of the AN to electrical stimulation considerably impacts speech perception outcomes with a CI, especially in difficult listening conditions.

Effects of Manipulating Channel Interaction on Speech Perception in Adults With Cochlear Implants

Katelyn Berg*[1], Raymond Goldsworthy[2], Jack Noble[1], Benoit Dawant[1], Rene Gifford[1]
[1]Vanderbilt University, [2]University of Southern California
Background: Cochlear implants (CIs) enable sound awareness and spoken communication to a high majority of recipients (Holden et al., 2013). However, outcomes that rely on high-fidelity spectral encoding, such as understanding speech in noise remain challenging for CI recipients (e.g., Dornhoffer et al., 2021). Channel interaction contributes to CI outcome variability by interfering with electrode discrimination, tonotopicity, and independence (e.g., Oxenham, 2008), and is unavoidable in CIs because the electrodes rest in a highly conductive fluid and are relatively far from the neural interface (e.g., Jones et al., 2013; Won et al., 2014). A recently developed measure of channel interaction in CI recipients is spectral blurring which manipulates excitation patterns of CI stimulation. Spectral blurring experiments have shown a clear, inverse relationship between channel interaction and speech recognition (Goehring et al., 2020; 2021). However, the impact of electrode placement on this relationship is unknown. Image-based electrode selection is a promising approach for promoting channel independence by identifying electrodes causing extensive overlapping stimulation (i.e., channel interaction) using CT imaging and deactivating those electrodes (i.e., Noble et al., 2014). Therefore, the current study had two aims. Aim 1 characterized the effects of channel interaction using spectral blurring, with the hypothesis that performance would decline as the amount of spectral blurring increased. Aim 2 measured the degree of image-based electrode selection benefit, with the hypothesis that performance would improve, especially for CI recipients with poorer electrode placement.
Methods: Participants included six (anticipated N = 20) adults with at least one MED-EL CI. Performance was compared between six conditions, 1) baseline (no blurring), 2) all blurred, 3) apical blurred, 4) middle blurred, 5) basal blurred, and 6) image-based electrode selection. Electrode placement information (electrode-to-modiolus distance, angle of insertion depth, and scalar location) was calculated from post-insertion CT imaging (Noble et al., 2014). Speech perception measures included speech reception threshold (SRT) in noise, vowel recognition, consonant recognition, and BKB-SIN.
Results: Aim 1 preliminary results showed performance was poorer than baseline in the all-blurred condition, suggesting that an increase in channel interaction was achieved. Performance with the regional blurred programs fell between the all-blurred and baseline conditions, with apical blurring causing more detrimental effects on performance. Aim 2 preliminary results showed performance with the image-based electrode selection program was better than baseline for all measures except the SRT in noise, suggesting that a reduction in channel interaction was achieved. The impact of electrode placement factors on these results will be investigated once data collection is complete.
Conclusions: Spectral blurring is a useful tool for identifying areas most affected by channel interaction for individual CI recipients, which could be used to optimize image-based electrode selection protocols.

Comparison of Performance for Cochlear-Implant Listeners Using Audio Processing Strategies Based on Short-Term FFT or Feature Extraction

Peter Johannesen[1], Yue Zhang[2], Benham Molaee-Ardekani[2], Aswin Wijetillake[3], Alejandro Soler Valcárcel[4], Manuel Segovia-Martínez[2], Enrique Lopez-Poveda*[5]
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Background: We investigated if and to what extent a feature-extraction (FE) audio processing strategy for cochlear implants (CIs) improves hearing performance and comfort compared to a conventional short-term FFT (STFT) based approach. In the FE strategy, acoustic events (or spectral peaks) were extracted using a synthetic feature extractor and mapped into the 20 CI stimulation channels. FE was hypothesized to reduce frequency smearing and improve frequency resolution because spectral peaks are detected and narrower filter spacing can be achieved without the constrains of the STFT bin width.

Methods: For six users of Oticon Medical Digisonic CIs, we compared performance with the FE and STFT strategies on various aspects: word recognition in quiet, sentence reception threshold in noise (SRT), consonant discrimination in quiet, listening effort, melody contour identification (MCI), and subjective sound quality. Word recognition and SRTs tests were conducted on the first and last day of testing to assess potential learning and/or accustomization effects. Listening effort was assessed in quiet and in a low-noise condition (individual SRT+15 dB) by measuring pupil dilation. MCI involved identifying a pattern of five tones among five possible patterns that formed either a constant, rising, falling, rising-falling, or falling-rising melody. MCI was measured for tone distances of two and four semitones and for fundamental frequencies of 131 and 262 Hz. Subjective sound quality was assessed using the multiple stimulus with hidden reference and anchor (MUSHRA) paradigm for three groups of sounds: sentences, music, and ambient sounds.
Results: Word recognition was similar for the two strategies on the first day testing, while it became (unexpectedly) better with STFT than the FE strategy on the last day of testing. SRTs were worse with FE than STFT strategy on the first day of testing but became comparable for the two strategies on the last day of testing. Consonant discrimination scores were higher for the STFT than for the FE strategy. MCI scores were similar for the two strategies in all test conditions. Subjective sound quality scores tended to be lower for the FE than for the STFT strategy. Listening effort was not substantially different across strategies.
Conclusions: We conclude that CI-user performance is similar with feature extraction as with a more standard short-term FFT based approach. However, longer accustomization times may be required to reveal the full potential of feature extraction. [Work supported by Oticon Medical].

Neuroplasticity in Cochlear Implant Users: From Synapse to Speech Perception

Ariel Hight*[1], Michele Insanally[2], Silvana Valtcheva[3], Erin Glennon[1], Julia K. Scarpa[4], Jonathan Neukam[1], Nicole Capach[1], Yew-Song Cheng[5], Robert C. Froemke[1], Mario Svirsky[1]
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Background: Addressing scales of neuroplasticity is challenging. Neuroplasticity and resulting timescales of learning and memories can range from milliseconds to years, from minuscule synapses to neuron conglomerates, and from sound detection to speech perception. We address this challenge by performing comparative studies of neuroplasticity between adult-deafened human and rat cochlear implant (CI) users. CIs are neuroprosthetic devices that successfully restore hearing and speech perception, but significant adaptation periods are required before human subjects attain maximum benefit from their devices. To connect human and rodent studies of CI use, we focused on types of adaptations that may generalize to both speech perception (humans) and cortical encoding of CI stimulation (rodents).
Methods: (Human Studies) We longitudinally tracked speech perception and auditory psychophysical abilities following activation of cochlear implants in adult deafened humans (N=3). Subjects were sent home with a tablet loaded with validated tests of spectral acuity (quick-spectral modulation detection, QSMD) and temporal acuity (modulation-detection test, MDT; gap-detection). These tasks were completed every other day for the first ~30 days following initial activation of their CI. Speech perception was tested periodically in-lab using standard tests of word and sentence recognition.
(Rodent Studies) We trained rats on a 2-alternative forced choice (2AFC) task for sound frequency discrimination. Next, excitatory and inhibitory postsynaptic currents (E/IPSCs) evoked by individual CI electrodes were measured in A1 neurons. Lastly, we performed micro-electrocorticography (μECoG) recordings of A1 in normal hearing and deafened rats with CIs.
Results: (Human Studies) We found significant improvements in QSMD scores (frequency acuity) in 2 of 3 CI users. In all 3 tested CI users, we found improvements in speech perception.
(Rodent Studies) Rats completed 2AFC frequency discrimination task with high discrimination performance (d'>1) after ~3 weeks of acoustic training (N=18) and ~1 week of CI training (N=5). Measuring postsynaptic currents, we found excitatory-inhibitory correlation to be initially poor prior to and improved significantly after 2AFC training. Measuring far-field evoked potentials across the auditory cortex, we found cochleotopic encoding of both tone-evoked and CI-evoked stimuli, but a supervised PCA/LDA decoder suggests encoding of CI stimuli may be degraded compared to acoustic stimuli.

Conclusions: We found CI-use induces an improvement in both speech perception and spectral discrimination in humans and both spectral discrimination and cortical encoding (A1) in rats. Our next steps are to further elucidate potential relationships between different types of adaptations ranging from speech perception to synaptic encoding.

What is the Best Frequency Allocation Table for Experienced Cochlear Implant Users With Single-Sided Deafness?

Emily Spitzer*[1], Jonathan Neukam[1], Nicole Capach[1], David Landsberger[1], Elad Sagi[1], Susan Waltzman[1], Mario Svirsky[1]
[1]*New York University Grossman School of Medicine*

Background: Research with cochlear implant users with single sided deafness (SSD CI) has shown there is a mismatch between the place of stimulation in the cochlea by a given frequency when presented through the CI and the place stimulated in the normal-hearing cochlea by the same frequency (a "place-pitch mismatch"). This may explain why most CI users require several months to adapt before achieving asymptotic performance. Because of their normal contralateral hearing, this process may be even more difficult for SSD CI users who continuously receive mismatched input, possibly leading to device rejection, poor speech perception, and delayed asymptotic performance. In the present study, we investigate whether a frequency allocation table (FAT) designed to reduce place-pitch mismatch improves performance and sound quality measures for experienced SSD CI users. All subjects were implanted with an electrode designed to go approximately one turn into the cochlea.
Methods: 15 CI subjects with SSD were tested on measures of unilateral and bilateral speech perception, localization, and sound quality using frequency allocation tables (FATs) starting at 188 Hz, 563 Hz, 688 Hz, and a value selected by each subject. Subjects were then mapped with one or both alternative FATs: 438-7938 Hz and 688-7938 Hz. These FATs were chosen to reduce place-pitch mismatch. Subjects returned to complete the same measures after a one month adaptation period.
Results: Bilateral speech perception and localization remained unchanged for the maps with alternative FATs, suggesting that removing low frequency information from the CI is not detrimental to performance in an everyday listening condition. For the CI alone, speech perception and subjective sound quality ratings improved for FATs with 563 and 688 Hz low-frequency cutoffs, despite no adaptation period with the 563 Hz FAT. To answer the question contained in the title, the FAT generating the best speech perception was different across subjects. For some, the standard FAT (188-7938 Hz) was optimal, where others obtained clear speech perception and/or sound quality benefit from alternative FATs. All participants but one chose to go home with at least one alternative FAT.
Conclusions: Raising the low-frequency cutoff of the FAT may reduce place-pitch mismatch, leading to improved speech perception and sound quality ratings for users of CI electrodes inserted approximately one turn into the cochlea. These results may generalize to FATs that are similar but with which the user has no listening experience. CI users with SSD likely require a specialized programming method that differs from traditional CI users. These listeners may benefit from an alternative FAT.

Podium #30 - Non-Sensory Processes in Speech Perception

2:00 p.m. - 4:00 p.m.
Crystal Ballroom D-E

Moderators: Jonathan Peelle and Karen Banai

Selective Attention and Familiarity at the Musical Cocktail Party

Jane Brown*[1], Gavin Bidelman[2]
[1]*University of Memphis,* [2]*Indiana University*

Background: The cocktail party problem – how a listener perceives speech in a noisy environment – is typically studied using speech (multi-talker babble) or noise maskers. However, realistic "cocktail party" scenarios often include background music (e.g., coffee shops, concerts). Previous studies from our lab suggest speech listening is poorer during concurrent background music containing vocals than instrumentals alone, but is also affected by how familiar the music is to the listener. Yet, music familiarity effects have been somewhat ambiguous, with studies finding both enhancements and detriments in speech processing

amidst well-known (pop song) music backdrops. We posit such equivocal findings may stem from unmeasured attentional allocation to the background music.
Methods: Here, we aimed to disambiguate the role of familiarity on concurrent speech perception, as well as to further understand the role of attention and distraction in musical cocktail party listening. During EEG recordings, participants listened to an audiobook while (un)familiar popular songs played in the background (0 dB SNR) and attended to either the speech (audiobook) or music (song lyrics) via word-identification tasks.
Results: Preliminary behavioral results suggest listeners are more successful at monitoring song lyrics as compared to speech. Ongoing temporal response function analysis applied to continuous EEGs are being used to model the cortical tracking of target speech (or lyrics) and (i) determine the degree to which listeners follow the attended vs. unattended speech/music streams and (ii) show differential encoding of speech as a function of music familiarity.
Conclusions: Our results may provide insight into interference or facilitation of background music during concurrent linguistic tasks, as well as the role of selective attention.

Attention Increases the Odds of Expectation-Induced Misperception: Behavioural and Pupillometric Evidence

Alina Kuimova*[1], Chandan Narayan[2]
[1]University of Western Ontario, [2]York University

Background: Misperceptions are common in everyday conversation, particularly as listening conditions degrade. Previous work shows that such misperceptions derive from overreliance on prior expectations—especially when the predicted version is phonetically close to the degraded target. Hearing-impaired individuals are particularly prone to misperception, as they tend to rely heavily on prior knowledge to offset reduced audibility. In these listeners, frequent misperceptions may lead to a failure of speech comprehension, breakdown in communication, and social withdrawal—with severe consequences for cognitive health and well-being. Increased attention and cognitive control are critical for comprehension of degraded speech (Ritz et al., 2022; Vaden et al., 2013), suggesting that they may reduce the frequency of misperceptions driven by plausible yet misinformative predictions. Here, we investigated how (in)accurate prior expectations and attention jointly determine perceptual outcomes during listening.
Methods: We induced frequent misperceptions with degraded spoken words preceded by matching (KIT–/kit/), mismatching (BAN–/kit/), and partially mismatching (TIT–/kit/) text, and used monetary incentives to manipulate listeners' attention. Participants (N = 47) reported whether the two words were same or different in two blocks: a baseline block performed without any knowledge of incentives and a reward block consisting of intermixed incentive and non-incentive trials. On incentive trials, participants could earn or lose 10¢ CAD for correct and incorrect judgements respectively. On non-incentive trials, there was nothing to gain or lose. Besides behavioural report, we tracked listeners' pupil size and used generalized additive models to assess the effect of incentives and prior knowledge on perceptual processing leading up to each perceptual outcome (correct perception vs misperception).
Results: Listeners correctly reported total mismatch pairs as 'different' (99.3% accuracy) but misperceived many partial mismatch pairs as 'same' (37.3% misperception) and some total match pairs as 'different' (20% misperception). Contrary to our predictions, incentives increased misperception on partial mismatch trials (to 39.9%) but improved perceptual accuracy on match trials (to 84.7%)—suggesting an increased bias towards responding 'same'. When listeners were intrinsically (baseline trials) and extrinsically (incentive trials) motivated, their pupil was more dilated relative to periods of disengagement (non-incentive trials). Incentive trials additionally triggered a dramatic increase in phasic dilation compared to non-incentive trials. These transient effects emerged retroactively, in response to degraded words.
Conclusions: We conclude that higher attentional engagement driven by extrinsic incentives increases reliance on prior knowledge when sensory detail is insufficient. Pupillometry data show that incentives load both proactive (preparatory) and reactive ("late correction") control processes, suggesting increased involvement of top-down predictive mechanisms. While this strategy only exacerbated prediction-induced mishearing in word discrimination tasks, adjusting the intensity of cognitive control via incentives could, nonetheless, improve speech perception in real-life listening situations where listeners can directly benefit from increased use of contextual, semantic, and other linguistic cues.

Lexical Bias in Phonemic Categorization: Effects of Spectral Degradation, Cognitive Load, and Aging

Zilong Xie*[1]
[1]*Florida State University, School of Communication Science and Disorders*

Background: Speech perception involves the integration of bottom-up acoustic cues and top-down linguistic knowledge. A classic illustration is the Ganong phenomenon. For example, if an acoustic cue continuum (e.g., a /g/-/k/ continuum with varying voice onset time) is embedded in a word-nonword context (e.g., GIFT-kift), listeners' responses are biased toward the word than the nonword (e.g., making more 'gift' responses). This lexical bias effect may increase under conditions with degraded input signals (e.g., listening through a cochlear implant) or under cognitive load conditions that do not directly impact signal quality (e.g., performing concurrent nonauditory tasks). Aging may also exaggerate the lexical bias effect. To date, how these factors (signal quality, cognitive load, and aging) interact in modulating the lexical bias effect is less understood.

Methods: Stimuli were speech tokens from an eight-step stop-consonant (/g/-/k/) continuum of varying voice onset times (18 to 70 ms). The continuum was embedded in both word-to-nonword (GIFT-kift) and nonword-to-word (giss-KISS) contexts. Younger to older adults with normal hearing performed a phonemic categorization task on unprocessed and 8-channel vocoded versions of the two continua. Specifically, participants were instructed to categorize the word-initial phonemes as being /g/ or /k/ and ignore the words' meaning. Participants performed the categorization task with a concurrent visual working memory task of low (remembering three same images) or high load (remembering three different images). The dual-task conditions were compared with an auditory-only condition wherein participants only performed the categorization task.

Results: Consistent with prior work, participants' responses were biased toward the words. They reported more /g/ responses for the word-to-nonword (GIFT-kift) context and more /k/ responses for the nonword-to-word (giss-KISS) context. The lexical bias effect appears to be exaggerated in the dual-task conditions than in the auditory-only condition, particularly for older adults and vocoded stimuli. Data collection will continue to determine how spectral degradation, cognitive load, and aging interact in modulating the lexical bias effect.

Conclusions: Our findings have implications for understanding how older listeners with cochlear implants utilize top-down information to compensate for reduced bottom-up sensory inputs during speech perception in multisensory environments.

Effect of Feedback Reliability for Neurofeedback Training of Auditory Selective Attention

Jusung Ham*[1], Leah Gibbs[1], Karsyn Rush[1], Hwan Shim[2], Inyong Choi[1], Inyong Choi[3]
[1]*Dept. Communication Sciences and Disorders, University of Iowa,* [2]*Dept. of Electrical and Computer Engineering Technology, Rochester Institute of Technology, Rochester, NY,* [3]*Dept. Otolaryngology – Head and Neck Surgery, University of Iowa Hospitals and Clinics*

Background: Understanding speech in a noisy environment is essential for everyday communication. However, even young adults with normal hearing thresholds struggle with hearing in noise. It has been shown that the neural process of selective attention that enhances the target voice and suppresses noise is critical for understanding speech in noise. Yet, no rehabilitation method that improves the neural process of attention has been developed. Our former research revealed that perceptual training with feedback from neural activity has a significant positive effect on speech-in-noise performance. However, it was not clear whether the effect was due to the neurofeedback or the behavioral training itself.

Methods: To tackle this issue, we conducted an additional study with a "fake" neurofeedback training paradigm and compared the improvement of speech-in-noise performance with the former study. For each trial, participants had to selectively attend to one voice stream between two competing voices; a female voice saying "up" 5 times, and a male voice saying "down" 4 times. Visual feedback was given with a rocket figure on a computer screen, which moves up or down according to the participant's attention to the target voice stream. Unlike the treatment group in our previous study who received feedback based on their attention, this placebo group received randomized feedback. The speech-in-noise performance was measured by the ISNT (Iowa Speech-in-Noise Test). For this test, participants had to choose a target word out of four, which is provided with background noise.

Results: As a result, the speech-in-noise performance has significantly improved for the treatment group but not for the placebo group. Additionally, the decoding accuracy of attention, which reflects the cortical activity of the attentional network modulation, has not been improved for the current study.
Conclusions: These results suggest that valid neurofeedback is a critical component for the success of attention training. This supports the necessity of neurofeedback training for improving the attentional resource allocation for hearing speech in a noisy environment.

Listening Effort: Separating the Subjective From the Objective

Joseph Rovetti*[1], Jaimy Hannah[1], Stephen Van Hedger[2], Hasan Saleh[1], Susan Scollie[1], Ingrid Johnsrude[1]
[1]Western University, [2]Huron University College

Background: Background noise, or other challenges to speech perception, may increase "listening effort." Listening effort has been defined in two ways: the recruitment of cognitive resources during listening ("objective" effort), and the self-reported feeling of effort during listening ("subjective" effort). These constructs are often assumed to be interchangeable, despite rarely correlating with one another when used together. Few attempts have been made to compare these two constructs. As a result, it is unclear if they respond in the same way or differently as the signal-to-noise ratio (SNR) becomes less favorable.
Methods: In the current study, we compared these two dimensions of listening effort directly. In an online experiment with recruitment via CloudResearch, 224 adult participants with normal hearing listened to highly intelligible speech passages across several SNRs, ranging from +11 dB to -1 dB in steps of 1 dB (as well as a clear condition). In one block, reaction time on a secondary case-judgment task served as a measure of objective effort; and in another, separate block, average effort rating on a 1-7 scale served as a measure of subjective effort. Participants' understanding of the gist of each passage, assessing speech intelligibility, was also measured in both blocks.
Results: Across the vast majority of SNRs, gist understanding exceeded 95%. As the SNR became less favorable, effort ratings increased linearly, with each 1-dB decrease in the SNR always leading to a significant increase in effort ratings. In contrast, as the SNR became less favorable, case-judgment reaction time only began to increase after an SNR of approximately +3 dB was reached. Over the range of SNRs used, this increase in reaction time was exponential. Finally, gist understanding only began to decrease after an SNR of approximately +2 dB, again doing so exponentially.
Conclusions: Objective and subjective effort both increased as the SNR became less favorable, and more cognitive resources may have been recruited to maintain high levels of speech intelligibility. Although subjective effort appeared to be more responsive to decreased SNR than objective effort, it is unclear what this increase in subjective effort would be in response to if not objective effort. Thus, it is possible that participants engaged in attribute substitution, replacing the difficult question of "How much effort do I feel?" with the easier question of "How hard does the task seem?"

Listening Effort Reduces Eye-Movement Behaviors in Younger Adults

Mo Cui*[1], Björn Herrmann[2]
[1]U of Toronto and Rotman Research Institute, [2]Rotman Research Institute

Background: Comprehension of speech in the presence of background noise requires a listener to invest substantial cognitive resources, making listening effortful. Listening effort is increasingly considered as an important marker of hearing impairment in older adults beyond standardized hearing diagnostics. However, the current measures of listening effort – such as pupillometry – have practical limitations (e.g., affected by environmental conditions; less useable with naturalistic speech). The current study proposes that gaze behaviors, specifically the reduction is eye movements with increasing listening challenges, provide a reliable and valid way to assess listening effort.
Methods: Three eye-tracking experiments investigated whether eye movements decrease with the increasing listening effort induced by background noise masking. In Experiment 1 (N=22), participants listened to sentences masked by a 12-talker babble at three signal-to-noise ratios (SNRs; -2, 3, and 8 dB) while either fixating on a screen or exploring a blank screen (free-viewing). They performed a semantic-relatedness judgment task on a probe word presented after each sentence. In Experiment 2 (N=22), participants listened to sentences in babble at two SNRs (-3 and 10 dB), while being presented concurrently with a task-irrelevant moving object; they performed the semantic-relatedness task. In Experiment 3 (N=24), participants listened to masked spoken stories, with SNRs changed in every 30 s across five levels (-4, 1, 6,

11, and 16 dB). The stories were either intact or scrambled (shuffled phrases/sentences). Eye-movement analyses focused on the time participants fixate in one location and on the spatial dispersion of their gaze.
Results: In Experiment 1, the sensitivity of behavioral measures of speech comprehension was reduced in the free-viewing condition, whereas fixation improved comprehension for the most challenging SNR. Notably, eye fixation times increased and gaze dispersion decreased with increasing speech masking. Experiment 2 showed similar reductions in eye movements as well as reduced incidental object tracking during effortful listening. Experiment 3 also shows that fixation time increased and gaze dispersion decreased with increasing speech masking during story listening. This was not the case for scrambled stories. Although pupil dilation was sensitive to speech masking of individual sentences, it was less sensitive to masking during story listening.
Conclusions: The current study demonstrates that gaze behaviors could be a valid and sensitive measure of listening effort. Individuals' eye movements decrease as a result of increasing background masking during speech listening, and this eye-movement reduction was consistently observed under different listening conditions (free viewing, incidental object tracking, story listening). Our findings highlight the reliability of the novel measure across various visual and auditory contexts. Our study emphasizes the relationship between cognitive demands and gaze behaviors, and that changes in gaze behaviors may be a particularly useful measure of listening effort where pupillometry is less feasible (e.g., light conditions; naturalistic speech).

Deficits in Sensory Decision-Making Underlie Self-Perceived Hearing Difficulties

Jacie R. McHaney*[1], Leslie Zhen[1], Sarah Anthony[1], Zilong Xie[2], Aravindakshan Parthasarathy[1], Bharath Chandrasekaran[1]
[1]University of Pittsburgh, [2]Florida State University
Background: One in ten adults seeking help at audiology clinics presents with a primary complaint of listening difficulties yet have normal audiograms. These adults (~20 to 50 years) are an often-overlooked population, despite being an age when these self-perceived hearing difficulties (SPHDs) arise, yet hearing acuity remains relatively intact. Currently, objective clinical assessments that elucidate neurophysiological insights, and that complement traditional audiometry, are not available. Prior work suggests that individual differences in subcortical temporal encoding and increased cortical reliance on envelope cues to compensate for declines in encoding explain some of the SPHDs in individuals with normal audiograms, but only a small variance. Here, we examined SPHDs in adults without overt hearing loss using a neurobiologically-driven computational model, Going above and beyond prior speech perception work that primarily focuses on behavioral accuracies alone, we examined SPHDs using a novel drift-diffusion model (DDM). DDMs break down accuracies and reaction times from behavioral tasks into neurobiologically-salient parameters related to the efficiency of sensory evidence accumulation and response-caution (i.e., favoring accuracy over speed of decision-making). These parameters are related to neural network activity, can inform how well a listener extracts information from a stimulus, and how much information they require to make a decision. Our goal is to develop scalable metrics for the clinic that explain the source(s) of SPHDs.
Methods: Participants underwent a comprehensive battery of behavioral and electroencephalography (EEG) assessments. A DDM was applied to accuracies and reaction time data from a speech categorization task, where participants were required to categorize phonemes in quiet and in speech-shaped noise. The EEG battery included frequency-following responses (FFRs), which are phase-locked responses to periodic stimuli, as a marker of speech temporal processing fidelity, and responses to continuous speech as a marker of cortical envelope tracking. SPHDs were quantified via a questionnaire.
Results: Our results show that individuals with more SPHDs have less efficient sensory evidence accumulation and are more cautious responders when categorizing speech in noisy listening conditions, compared to those with fewer SPHDs. Further, individuals with more SPHDs demonstrated poorer speech discriminability based on the FFR and poorer cortical tracking of the speech envelope than individuals with fewer SPHDs. Cautious responding in individuals with more SPHDs also related to objective EEG measures (encoding fidelity). Crucially, accuracies on the speech in noise categorization task do not relate to SPHDs.
Conclusions: Our results indicate that DDMs to speech in noise categorization reveal novel insights about SPHDs that accuracies and self-report do not. Individuals with more SPHDs tend to be more cautious in their sensory decisional processes than those with fewer SPHDs, which may partially reflect downstream effects on poorer sensory encoding fidelity. These results are promising for developing clinically-viable markers of SPHDs in listeners without overt hearing loss.

Short Course- Part 2: Single Cell "Omics"; A Practical Introduction to Molecular Analysis at the Single Cell Level

4:00 p.m. - 5:00 p.m.
Biscayne 1

This course will provide a practical introduction to molecular analyses at the single cell level. Part 2 will include presentations on bioinformatic packages that can be used to analyze and synthesize the large data sets that are generated from single cell studies. Part 2 will also include additional information on useful websites and online tutorials

Matthew Kelley*, NIH/NIDCD*

Seurat
Michael Hoa*, NIDCD*

Trajectory Analysis
Tessa Sanders*, NIDCD, National Institutes of Health*

Transcriptional Analysis
Mai Eshel*, Department of Human Molecular Genetics and Biochemistry, Faculty of Medicine, Tel Aviv University, Israel*

Cell-Cell Communication (Cell-Chat)
Kevin Rose*, University of Maryland, Baltimore*

gEAR
Ronna Hertzano*, University of Maryland School of Medicine*

gEAR Workshop - Analysis - Advanced: Transfer Learning and Data Upload

4:15 p.m. - 5:45 p.m.
Biscayne 2

Transfer learning is a technique used to apply patterns or features learned from one dataset to another. Discover intersecting molecular dynamics across the various studies, samples, and cells - without requiring programming skills. This workshop includes a hands-on component, bring your laptop along.
Data upload (45 min)
Users can upload their own datasets to the gEAR and view/analyze them privately, share with others specifically, or make them public entirely. Here we cover the required input formats and steps for uploading. This workshop includes a hands-on component, bring your laptop along.

Ronna Hertzano*, University of Maryland School of Medicine*
Joshua Orvis*, Institute for Genome Sciences*

Symposium Honoring the Contributions of Steve Colburn

4:15 p.m. - 6:15 p.m.
Oceans Ballroom 1-4

Ruth Litovsky*, University of Wisconsin*
For over 50 years, Steve Colburn's thoughtful, critical thinking has helped shape the field of Binaural Hearing. Throughout his career, Steve inspired his students and colleagues to think about spatial percepts arise, from how the auditory system encodes the signals arriving from the two ears to how this improves the ability to detect signals in noise. He also dedicated significant effort to understanding why people with hearing loss struggle in situations where normal-hearing listeners would benefit from spatial hearing, shining a bright light on some of the most perplexing and important questions in human communication. Through his teaching, mentoring, and advocacy for great science, Steve's approach — integrating psychophysical,

physiological, theoretical, and computational approaches — has fundamentally changed and enriched our field. In addition to his scientific contributions, Steve stands out as one of the most approachable, supportive, and kind humans on this planet. This symposium honors Steve's decades of contributions to the field of Binaural Hearing, reflected through the lens of Steve's progeny's progeny.
Barbara Shinn-Cunningham*, Carnegie Mellon University*

Binaural Phenomena in Asymmetric Hearing
Inyong Choi*, University of Iowa*
Dr. H. Steven Colburn has made a huge contribution to our understanding of binaural hearing: The benefit of having two ears and its underlying physiological mechanisms. As his academic progeny, we observe binaural phenomena in an extreme case of asymmetric hearing: Listeners with single-sided deafness (SSD) who received a cochlear implant (CI) in the deafened ear. The SSD-CI can potentially provide three binaural benefits: 1) Binaural release from masking, 2) The head shadow effect – relying on the ear with the better signal-to-noise ratio, and 3) Binaural summation of redundant auditory inputs. The former two mechanisms (binaural release from masking and head shadow) are questions of where – to what degree can SSD-CI users harness binaural localization. Equally important is the third benefit: Integrating the content of the two ears (what), which can be especially useful for the understanding of quiet speech. Using a word-in-noise and a soft-speech task, we studied binaural integration for both localization and content. Our results from a word-in-noise task demonstrated that SSD-CI provides the head shadow effect at the group level. However, no group-level effect of binaural release from masking was observed in SSD-CI, indicating that the interaural disparity in SSD-CI does not reach the fidelity that the binaural localization mechanism requires. In contrast, our results from a soft-speech task exhibited a statistically significant CI benefit in recognizing low-intensity speech contents, indicating that SSD-CI can provide binaural summation. Our study offers a multi-level and fresh perspective on binaural benefit recovery in SSD-CI and will provide insight into mechanisms underlying either successful or poor outcomes.

Steve Colburn's Advances in the Understanding of Binaural Unmasking
Bobby Gibbs*, Univesity of Maryland College Park*
Steve Colburn has made important advances in the understanding of how the auditory system uses interaural differences to improve signal-in-noise detection, a process called binaural unmasking. Through decades of work on this topic, his Binaural Hearing Laboratory has identified shortcomings in binaural unmasking models, incorporated important refinements, and suggested fertile areas for future research. This talk will first overview Steve Colburn's contributions to identifying potential mechanisms of binaural unmasking in the typically functioning auditory system. The impact of this work for understanding binaural unmasking with degraded inputs (such as with cochlear implants) will then be reviewed.

Subcortical Modeling and Neural Responses to Naturalistic Speech
Melissa Polonenko*, University of Minnesota*
Binaural hearing is especially important for complex communication situations involving speech. But traditionally it has been difficult to evaluate the subcortical underpinnings of speech communication. This talk will highlight the modeling and brainstem work undertaken towards creating subcortical measures of speech processing and spatial hearing, which have been possible through Steve Colburn's legacy as a mentor and researcher.

The Role of Hearing in Development: Lessons from Science and Life
Tina Grieco-Calub*, Rush University Medical Center*
The ability to hear enables children to develop skills necessary for navigating the world of sound, including the ability to localize sound sources in the environment, to acquire spoken language, and to manage interpersonal communication. When children's access to sound, and to speech, is disrupted due to hearing loss or due to an acoustically complex environment, there are downstream consequences on development. This presentation will highlight interdisciplinary work that illustrates this relation.

Steve Colburn's contributions to physiology-based modeling of auditory responses
Jayaganesh Swaminathan*, Eargo Inc*
Steve Colburn has made seminal contributions to the development of powerful and elegant auditory models. These models have been critical in advancing our understanding of the mechanisms of monaural and

binaural neural coding and perception of sound. In this talk, I will review some of the applications of these models in basic and applied research.

spARO Town Hall

6:15 p.m. - 6:45 p.m.
Canaveral 3

Join the spARO Chair, Kevin Booth, at the Town Hall to learn about spARO, the year-round initiatives, and how to get involved!
Kevin Booth, *Indiana School of Medicine, Dept. Medical and Molecular Genetics*

Wednesday, February 15, 2023

Podium #31 - Cochlear Modeling and Mechanics

8:00 a.m. - 10:00 a.m.
Ocean's Ballroom 5-12

Moderators: Rob Raphael and Lisa Olson

The Nonlinear Mechanics and Dynamics of a Three-Row Hair Bundle Model

Varun Goyal*[1], Karl Grosh[1]
[1]*University of Michigan*

Background: The mammalian cochlear outer hair cells (OHCs) are each equipped with a hair bundle (HB) consisting of roughly three rows of stereocilia. The role of each row of stereocilia in governing the bundle stiffness, sensitivity, transduction current, and related biophysical quantities is unclear. Therefore, we are developing a three-row model of an isolated HB to quantify each row's contribution to the passive and active mechanics of the HB.
Methods: We derived the three-row model equations by including the nonlinear kinematics, viscoelastic HB mechanics, and the nonlinear response of the mechano-electric transducer (MET) channels coupled to an adaptation mechanism. We also constrain the system with sliding contacts between adjacent rows. A two-state Boltzmann function is used to model the gating mechanics for each MET channel (representing the tip links between rows 1 and 2 and between rows 2 and 3, where stereocilia rows 1, 2, and 3 are numbered in the order of their decreasing heights). The system is stimulated by applying a horizontal external force to the tallest stereocilia. The nonlinear equations are solved using the Runge-Kutta algorithm. Finally, we perform a linearized perturbation analysis about a nonlinear equilibrium point to obtain system stability and the rate constants.
Results: A biasing, static force of 200 pN was used to bring the first MET channel (between rows 1 and 2) to a resting open probability of 0.51 and the second channel to an open probability of 0.48. We then varied the amplitude of additional external force and plotted the force-displacement relations. We repeated the same procedure, first severing the second gating spring (between rows 2 and 3) and then severing both gating springs. We found that the bundle stiffness reduced by about 6% and 13%, respectively, when only the second and both gating springs were cleaved. We also observed that the MET current is higher through the first channel than the second channel. Further, linearization of the model revealed a stable system with three time constants as 5.96 μs, 0.11 ms, and 0.13 ms.
Conclusions: We predict a larger current influx through the first MET channel than the second. We estimate that the tip link between rows 1 and 2 increases the bundle stiffness more than the second tip link does alone. Lastly, we predicted one fast component and two slow components of adaptation, as seen in experiments. We will next investigate their effects on HB displacement and MET currents. Further, this model can be used to explore the consequences of different hypotheses for adaptation mechanisms in the stereocilia and to provide mechanistic explanations for pharmacological or genetic interventions.

The Effects of BAHA Force Directions on BC Hearing

Jongwoo Lim*[1], Namkeun Kim[2]
[1]*Korea Advanced Institute of Science and Technology,* [2]*Sogang University*

Background: Bone anchored hearing aid (BAHA) is a device to help patients who suffer conductive hearing loss. There have been many studies to achieve better hearing from BAHA. For example, Eeg-Olofson et al. (2008) and Dobrev et al. (2020) have investigated the effect of stimulation position on cochlear responses in bone-conducted (BC) hearing. In addition, the effects of the bilateral stimulation from BAHA on BC hearing have been also studied and compared with results from unilateral stimulation. For instance, Priwin et al. (2004) investigated the benefits and drawbacks of bilateral BAHA stimulations. However, most studies did not consider the effect of the stimulated force direction on BC hearing. The main objective of this study was to clarify the force direction causing the maximum cochlear responses in both unilateral and bilateral BAHA stimulation.
Methods: A three-dimensional-finite-element model of a human head including auditory periphery was used to investigate the stimulation direction deriving the maximum basilar membrane (BM) velocity. In the unilaterally stimulated simulations, the BC force was applied in the anatomically medial, anterior, and superior directions, and the results were compared with those in the typical direction obtained from a commercial BAHA. In the bilateral BC stimulation, three different types of input were used, which are forces 1) with the same phase toward the center of the head, 2) with 90 degrees phase differences, and 3) in the medial and anterior directions at the ipsilateral and contralateral sides, respectively.
Results: The BM velocities induced in three orthogonal directions in the unilateral simulation showed generally similar levels within 10 dB differences to those obtained from the typical BAHA. However, while the medial and superior directional inputs induced about 20 dB higher BM velocities at 3.5 kHz, the anterior direction caused 20 dB lower BM velocities at 4.5 kHz. In the bilateral case, all the simulated inputs caused similar BM velocities with those in the unilateral stimulation using the typical BAHA, but exceptionally only 90 degrees phase difference case showed 17 dB less BM velocity than those in the unilateral stimulation.
Conclusions: In general, the BM velocities were not significantly affected by BC force directions in both unilateral and bilateral stimulation. However, since three orthogonal directions in the unilateral stimulation and three different phase differences in the bilateral stimulation were simulated, a specific direction causing the maximum BM velocity may be able to exist.

The Effect of Nonlinearity in a Tapered, Viscous Cochlear Model

Vipin Agarwal*[1], Karl Grosh[1]
[1]*University of Michigan*

Background: The mammalian cochlea is responsible for transforming incoming acoustic energy into neural signals. With a goal of predicting response of a healthy cochlea to variety of sounds, researchers have continuously enriched mathematical models to include the anatomy, comprehensive representations of the electromechanical processes, and mechanics within the cochlea.

Efficient modeling of the cochlear response is extremely challenging for a variety of reasons. For instance, both the length scales, which vary from the sub-micron to centimeters, and the time scales, which vary from microseconds to seconds, that must be resolved increase compute times and constrain the choice of algorithms. Furthermore, the fundamental physics of the cochlea involves propagation speeds ranging from 1500 m/s (the speed of sound in water) to 2 m/s (the speed of the traveling wave near its peak response location) as well as viscous boundary layers associated with the fluid dynamics likewise challenge even the most efficient and stable computational methods. Furthermore, the cochlea is a nonlinear system, and nonlinear models require time-consuming simulation. In the current work, we study the prediction of stationary nonlinear response of a base-to-apex cochlear model to a harmonic input, considering taper of the cochlear scalae. We seek to understand the influence of bulk fluid viscosity and the geometric fluid-duct tapering on the nonlinear response of the system.
Methods: Coupled equations are derived from a kinematically constrained Langrangian dynamics formulation. To solve these equations, we have used an iterative algorithm, the alternating frequency-time method, where nonlinear forcing from transduction current is calculated in the time domain followed by solution computed in the frequency domain. The algorithm swaps between frequency and time domains using the Fourier and inverse Fourier transforms, and is based on a fixed-point iteration.
Results: We analyzed the solutions for nonlinear models where the frequencies and stimulus levels are varied from 6kHz to 20kHz, and 10 dB SPL to 90 dB SPL, respectively. The input-output growth curves for the basilar membrane (BM) and reticular lamina (RL) at the fundamental frequency are derived and

nonlinear compression at different stimulus levels is seen. The I/O growth curves for the RL show hyper-compression instead of the usual compressive behavior. Further, for different stimulus levels, the BM amplification at low levels is localized to the characteristic frequency (CF) of the measurement location. In addition, the scala tympani voltage (the local cochlear microphone or LCM) shows a linear growth below the CF, saturating at high levels. The LCM is highly nonlinear near the CF, similar to experimental results.
Conclusions: We performed alternating frequency-time analysis, considering viscous boundary layer for a tapered cochlear model. The responses are compared with non-tapered model and significant differences are observed, especially in the lower frequency region. This work was supported by NIH-NIDCD R01 04084.

Determining the Mechanical Properties of Hair-Bundle Components From Physiological Measurements in Outer Hair Cells

Rayan Chatterjee*[1], Daibhid O Maoileidigh[2]
[1]Stanford University School of Medicine, [2]Stanford University

Background: Hair bundles in the outer hair cells (OHCs) are required for hearing, and dysfunction in their mechanics causes hearing loss. However, there are uncertainties in the values of OHC hair bundles' mechanical properties because estimates make assumptions about bundle morphology.
Hair bundles comprise a staircase pattern of rod-like projections, the stereocilia, which pivot on the hair- cell surface and are connected by filamentous gating-springs that regulate the opening of ion channels at the stereociliary tips. Sound-induced forces deflect the stereocilia, which extends the gating-springs, generating ionic currents through the channels. Differences in stereociliary heights within a bundle should cause differences in their stiffness, causing unequal deflections, gating-spring extensions, and channel currents, which sum to equal the receptor current.
We hypothesize that uncertainty and assumptions about OHC hair bundle morphology cause uncertainty in the estimated values of bundle mechanical properties.
Methods: We test this hypothesis by building a computational model that accounts for the morphology (stereociliary heights, widths, and pivot positions) of the OHC hair bundle, which are assigned from published experimental data. We calculate the pivot stiffness of each stereocilium by fitting our model to experimental data for deflection of the hair bundle without gating-springs versus applied force. We use the value of pivot stiffness to determine the gating-spring stiffness by fitting our model to force- deflection data of the hair bundle with gating-springs.
Results: We show that stereocilia and gating-springs do not contribute equally to hair bundle stiffness. The row 3 (shortest) stereocilium is the stiffest (i.e. displaces least), followed by 2 and 1. Consequently, the row 2 channel currents differ from the row 3 channel currents.

Uncertainties in the heights and spacing of stereocilia create uncertainties in the estimated values of the pivot and gating-spring stiffness. To determine the sizes of these uncertainties, we change the morphological dimensions of our model within one standard deviation of the experimental mean and calculate how the pivot and gating-spring stiffness vary. Uncertainties in pivot and gating-spring stiffness, in turn, cause uncertainties in the bundle's output, the receptor current. The variations show that estimates of mechanical properties are highly sensitive to uncertainty in and assumptions about the bundle morphology.
Conclusions: We show that it is incorrect to assume equal contributions from stereocilia and gating-springs to hair bundle stiffness, as assumed in previous work. It is important to constrain hair bundle morphology accurately using experimental measurements in order to accurately estimate the values of pivot and gating-spring stiffness. Understanding hair bundle mechanics better will help us determine how changes in bundle mechanics cause changes in receptor current that lead to hearing loss.

Otoacoustic Constraints on Anisotropic Wave Amplification in the Cochlea

Christopher Shera*[1], Alessandro Altoe[2]
[1]University of Southern California, [2]Caruso Department of Otolaryngology—Head and Neck Surgery, University of Southern California

Background: The oblique, micromechanical geometry of the organ of Corti seems likely to play an important role in mammalian cochlear amplification. Some models incorporating this geometry suggest that the cochlear amplifier operates, at least in part, through mechanisms involving the spatial feed-forward and/or feed-backward (FF/FB) of OHC somatic forces. In these models, OHCs sense stereociliary displacement at one location but produce forces and motion at another, their somatic forces being

transmitted along the organ of Corti via the Y-shaped cytoarchitecture of the OHCs and phalangeal processes of the Deiter's cells. Because of traveling-wave propagation, the longitudinal separation between the locations of OHC sensing and forcing introduces phase shifts that can yield a form of negative damping, amplifying traveling waves as they propagate.
Methods: Heuristic arguments suggest that push-pull amplification may suffer from the very virtue that makes it so attractive and stable from an engineering perspective: the amplification it provides depends on the direction of wave propagation. These arguments suggest that the oblique geometry introduces a directionality to the amplifier---whereas forward-traveling waves are boosted, reverse-traveling waves are squelched. Unlike other forms of negative damping, spatial push-pull amplification may therefore be effectively "one-way." This anisotropy would not be a problem---indeed, it might be an advantage for cochlear signal processing near threshold, where it would alleviate complications due to standing waves---were it not for the existence of otoacoustic emissions (OAEs), which indicate that amplified energy escapes from the cochlea via mechanisms involving reverse traveling waves.
Results: In this theoretical study we develop and apply a family of "hybrid" push-pull/classical models to identify the major constraints that the existence and properties of OAEs place on the role of anisotropic wave amplification in the cochlea.
Conclusions: Please come to the talk to find out!

A Graph Signal Processing Model of the Cochlea

Melia Bonomo*[1], Santiago Segarra[1], Robert Raphael[1]
[1]Rice University
Background: Sound encoding in the cochlea has traditionally been understood to be the product of individual cells being excited at characteristic frequencies. However, this representation does not appreciate the relationships in the functional activity among all of these cells, which may be especially important while they are encoding music with many simultaneous pitches and timbre mixtures. Here, we take a novel graph approach to study sound encoding in the cochlea. Graph signal processing is used to look at how the response of individual cells is coordinated at the level of the whole-cochlea to pass complex information to the auditory nerve fibers.
Methods: We use a simulation (UR EAR 2020b) developed by the Carney Lab at the University of Rochester that incorporates the nonlinear response properties of the inner ear to compute hair cell voltages. Audiograms from patients with normal hearing, conductive hearing loss, sensorineural hearing loss, and both conductive and sensorineural hearing loss are extracted from the AudGenDB database (Children's Hospital of Philadelphia) and input as hearing health parameters in the simulation. We use the simulation to generate inner hair cell responses for various musical stimuli. The GSPbox Graph Signal Processing package (Version v0.7.5, 2018) is used to learn the graph links between hair cells from smoothness in their voltage signals and determine a cochlea graph for each patient. Graph theoretic metrics are then calculated to quantify differences among patients.
Results: There are significant differences in graph density, node weighted degree, and modularity between patients with normal hearing and those with hearing loss. Patients with normal hearing have lower graph density and higher weighted degree, meaning that these graphs generally have less links between hair cells, but these links are strong. These normal hearing graphs also have more modules, where a module is a group of tightly interacting hair cells. The graphs of patients with both conductive and sensorineural hearing loss were generally the noisiest (i.e., many weak links) and had fewer, less-specialized modules.
Conclusions: This project is the first to explore the representation of cochlear sound encoding as a graph using graph signal processing. Using this approach with patient data, we observe various graph-theoretic features that distinguish hearing loss diagnoses. Our method has the potential to improve cochlear implant signal processing of music. In particular, patient cochlea graphs can be used to develop a graph deep learning model for learning individualized signal processing settings, to ensure that melody information is not lost between channels.

Two-Tone Suppression in the Reticular Lamina in the Basal Turn of the Gerbil Cochlea Challenges Conventional Views of Otoacoustic Emissions Evoked by Sngle Tones

Jonathan Siegel*[1], Wenxuan He[2], Tianying Ren[2]
[1]Northwestern University, [2]Oregon Health and Science University

Background: There has previously been no compelling explanation for why the ear canal acoustic response for low level (probe) tones is subject to nonlinear interaction with more intense suppressor tones much higher in frequency, as this phenomenon has not been detected in basilar membrane vibrations (Charaziak and Siegel JARO 2015). The probe tone evokes a signal commonly referred to as stimulus frequency otoacoustic emission (SFOAE). The advent of optical techniques allowing vibration measurements of structures within the organ of Corti as well as the basilar membrane, the response measured in the reticular lamina (RL) motion to probe tones an octave or more below the local characteristic frequency (CF) in the apex of mice is suppressed by more intense tones near CF, with no corresponding suppression in the basilar membrane (BM) (Dewey et al, J Neurosci 2019). Similar suppression of RL vibrations in mice has been reported to correspond to changes in ear canal pressure, again with no detectable nonlinear suppression in the BM vibrations (Charaziak, Mechanics of Hearing Workshop, 2022). We report here two-tone suppression similar to the Dewey et al study, but in the basal turn of the Gerbil cochlea.
Methods: Young Mongolian gerbils of either sex with normal hearing were used in this preliminary study. Reticular lamina and basilar membrane responses to two tones were measured from the base of the gerbil cochlea through the round window using a heterodyne low coherence interferometer. The CF of the location where measurements were made was in the range of 18-22 kHz.
Results: The response measured in the RL motion to a probe tone of 5 kHz and 40-50 dB SPL was suppressed by a second tone varied widely in frequency at levels of 40-70 dB SPL. Maximal suppression corresponded to the frequency producing maximal displacement of the response to the suppressor tone and suppression was detected for suppressor levels as low as 40 dB SPL. In stark contrast, no suppression of the probe was detected in the BM response for any suppressor condition.
Conclusions: The suppression of RL vibrations to probe tones well below CF appears to generalize from mice to other species and more basal locations. We expect that nonlinear interactions measured in the ear canal sound pressure similar to those reported by Charaziak (2022) will also generalize. There is now a well-supported basis to explain the acoustic suppression reported previously (Charaziak and Siegel, 2015). The fact that no suppression of BM vibrations is seen appears to negate the role of conduction of SFOAE signals through reverse slow BM waves. SFOAEs appear to originate over a broad region of the cochlea. A similar conclusion regarding reverse propagation of intermodulation distortion signals was reported previously (e.g. He and Ren, Sci Reports 2013).

Triobp Promotes Unbalanced Bidirectional Radial Stiffness Gradients Within the Mammalian Organ of Corti

Hesam Babahosseini[1], Inna A. Belyantseva[2], Rizwan Yousef[2], Risa Tona[2], Shadan Hadi[3], Sayaka Inagaki[2], Elizabeth Wilson[2], Shin-ichiro Kitajiri[4], Gregory I. Frolenkov[3], Thomas B. Friedman[2], Alexander X. Cartagena-Rivera*[1]
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Background: Hearing depends on an elaborate architecture and mechanical properties of diverse inner ear cell types. The individual contributions of various inner ear cell types to mechanical properties of the organ of Corti and the mechanisms of their integration are largely unknown. Pathogenic variants of TRIOBP/Triobp are associated with deafness in human and mouse.
Methods: Using sub-100-nanometer spatial resolution Atomic Force Microscopy (AFM), we mapped the Young's modulus (stiffness) of the apical surface of the different cells of freshly-dissected P5-P6 cochlear epithelium from wild-type and mice lacking either Trio and F-actin binding protein (TRIOBP) isoforms 4 and 5 or isoform 5 only.
Results: Remarkably, nanoscale AFM-mapping revealed unrecognized bidirectional radial stiffness gradients of different magnitudes and opposite orientations between rows of wild-type supporting cells and sensory hair cells. Moreover, the observed bidirectional radial stiffness gradients are unbalanced with sensory cells being stiffer overall compared to neighboring supporting cells. Deafness-associated TRIOBP deficiencies significantly disrupted the magnitude and orientation of these bidirectional radial stiffness gradients. In addition, FIB-SEM tomography shows that a TRIOBP deficiency results in ultrastructural changes of supporting cell apical phalangeal microfilaments and bundled cortical F-actin of hair cell cuticular plates, correlating with mRNA and protein expression levels. Concurrently, AFM stiffness measurements showed a softening of the apical surface of the sensory epithelium in mutant mice.

Conclusions: Altogether, this additional complexity in the mechanical properties of the sensory epithelium is hypothesized to be an essential contributor to frequency selectivity and sensitivity of mammalian hearing. [1, 2]

References:
[1] Babahosseini, H., Belyantseva, I. et al. Unbalanced bidirectional radial stiffness gradient within the organ of Corti promoted by TRIOBP. PNAS 119, (2022)
[2] Katsuno, T. et al. TRIOBP-5 sculpts stereocilia rootlets and stiffens supporting cells enabling hearing. JCI Insight 4, (2019)

Podium #32 - Aging: Sensory Disorders and Cognitive Impairments

8:00 a.m. - 10:00 a.m.
Oceans Ballroom 1-4

Moderators: Sally Dawson and Hong-Bo Zhao

Auditory Biomarkers for Cognitive Decline

Yasmeen Hamza*[1], Ye Yang[2], Janie Vu[2], Antoinette Abdelmalek[2], Carol Barnes[3], Fan-Gang Zeng[2]
[1]Florida State University, [2]University of California, [3]University of Arizona

Background: Sensory impairments, including hearing loss, are a hallmark of aging and are associated with cognitive decline. However, there is a knowledge gap about the mechanisms underlying the sensory-cognitive relationships. Gray et al. (2019) investigated how auditory and visual evoked potentials are associated with cognition in adult and aged macaques. Cognitive performance was associated with a temporal measure of the Auditory Brainstem Response (ABR), defined as the latency difference between two stimulus presentation rates. The present study assessed 1) whether the association between the ABR temporal measure and cognition holds in humans, 2) whether a psychophysical gap detection measure reflects the same association, and 3) whether the ABR and gap detection measures reflect cognitive decline even after the relationship between age and hearing loss is accounted for.

Methods: The study included one hundred eighteen adults aged 18-92 years. Linear regression models assessed the association between auditory measures (predictor) and cognition (outcome). Cognition was a composite z-score of ten measures from eight cognitive tests that assessed episodic memory, working memory, processing speed, attention, executive function, spatial short-term memory, and visual discrimination. The absolute and relative (difference between two stimulation rates) ABR wave V latency at 60 dB SL served as an objective predictor. A gap detection threshold at 40 dB SL served as a subjective predictor. The pure-tone-average threshold at 0.5, 1, 2, and 4 kHz in the better ear was the hearing-loss predictor.

Results: Only the absolute ABR latency measures, not the relative, were associated with cognition (51Hz: B, -0.212; 95% CI, -0.322 to -0.101; $p<0.0001$). Gap detection threshold was also associated with cognition (B, -0.334 95% CI, -0.433 to -0.235; $p<0.0001$). A Composite temporal z-score measure (average z-score of the ABR latency at 51Hz and the gap detection threshold) yielded a stronger association with cognition (B, -0.500; 95% CI, -0.629 to -0.371; $p<0.0001$). Pure-tone average z-scores in the better ear were associated with cognition (B, -0.324; 95% CI, -0.425 to -0.223; $p<0.0001$). After adjusting for age, the composite temporal auditory measure was associated with cognition (B, -0.260; 95% CI, -0.384 to -0.136; $p<0.0001$), but not the pure-tone-average z-score (B, -0.050 95% CI, -0.162 to -0.063; $p=0.385$).

Conclusions: The present study found an age-independent auditory temporal biomarker for cognitive decline. The age-independent association between auditory temporal processing and cognitive function suggests common underlying central neural mechanisms that are not related to peripheral age-related hearing loss. The validity of the biomarker in the detection and monitoring of cognitive impairments needs to be addressed in future studies.

Baseline Hearing Characteristics of Participants Enrolled in the Aging and Cognitive Health Evaluation in Elders (ACHIEVE) Randomized Controlled Trial: Evaluation of Site and Recruitment Route Differences

Victoria Sanchez*[1], Michelle Arnold[1], Haley Neil[1], Sarah Faucette[2], Nick Reed[3], Frank Lin[3], Theresa Hnath Chisolm[1], For the ACHIEVE Study Investigators .[4]

[1]University of South Florida, [2]University of Mississippi Medical Center, [3]Johns Hopkins, [4]Multiple Institutions

Background: Hearing loss is associated with accelerated cognitive decline even after adjusting for potential confounding factors (e.g., age, sex, education, co-morbidities). Although a causal association remains to be determined, hearing loss is hypothesized to be the largest potentially modifiable risk factor for dementia. Approaches for reducing cognitive decline in older adults are needed given the aging of the population and the personal, socioeconomic, and public health implications of cognitive impairment. Here we will describe an ongoing multi-site clinical trial, the "Aging and Cognitive Health Evaluation in Elders randomized trial (ACHIEVE RCT; ClinicalTrials.gov Identifier: NCT03243422). The ACHIEVE RCT is the first large-scale trial to evaluate how best-practices hearing intervention, versus a successful aging education control intervention, reduces cognitive and other functional declines in older adults. We will describe the baseline hearing characteristics of the enrolled ACHIEVE participants as such detail is often lacking in studies examining the relationship between hearing loss and cognition.
Methods: We recruited 977 adults with untreated mild-to-moderate hearing loss between 2018-2019 from four study sites located in Washington County, MD; Jackson, MS; Forsyth County, NC; and Minneapolis, MN. Participants were recruited through either the ongoing Atherosclerosis Risk in Communities (ARIC) longitudinal observational study, which has been ongoing since 1987, or de novo from the community. Participants were randomized to receive either a control intervention consisting of the standardized 10 KeysTM to Healthy Aging program, or a best-practice hearing intervention. We will describe the baseline hearing characteristics of the enrolled participants (i.e., pure-tone audiometric thresholds, word recognition in quiet, speech perception in noise) and self-reported handicap.
Results: Participants better ear median 4-frequency (i.e., 0.5, 1, 2, 4 kHz) pure-tone average was 38.8 dB HL [interquartilesQ1, Q3: 33.8, 43.8], while the poorer ear pure-tone average was 42.5 dB HL [Q1, Q3: 37.5, 47.5]. In the better ear, word recognition scores in quiet ranged from 60% to 100%. Mean speech in noise score measured with the Quick Speech in Noise Test (QuickSIN) in soundfield was 7.1 dB SNR Loss (SD= 5.2), and the Hearing Handicap Inventory for the Elderly – screening version (HHIE-S) showed the majority of participants had at least a "Mild-to-Moderate" handicap (n=487). There were strong and statically significant correlations between these hearing function measures and self-reported hearing ability and handicap. Differences between participant geographical location and study recruitment route (ARIC vs. de novo) were observed.
Conclusions: Enrolled participants are currently being followed for three years with final study visits scheduled to finish by the end of 2022. These baseline audiometric and hearing-related self-report characteristics of ACHIEVE participants will help inform study outcome analyses that will be conducted in 2023.

Oxidative Stress is Exacerbated in the Aging Cochlea of Alzheimer´s Disease Mouse Models

Jose Juiz*[1], Veronica Fuentes-Santamaría[2], Juan C Alvarado[2], Thomas Lenarz[3], Zaskya Benitez[2], Maria C. Gabladón-Ull[2]
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Background: Age-related hearing loss (ARHL) is the major risk factor of Alzheimer disease (AD) in midlife. Neuropathogenic interactions between ARHL and AD are unknown. We test the hypothesis that the beta-amyloid proteinopathy linked to AD in the central nervous system has effects on the auditory receptor, which may exacerbate preexisting hearing loss, thus generating an unsuspected vicious circle between ARHL and AD.
Methods: We have used APPNL-F "knock-in" mice, in which a mutated "humanized" amyloid precursor protein (APP) gene inserted in its original locus induces abnormal APP processing with beta-amyloid deposits akin to those of human AD but no massive overexpression. Controls were age-matched wild type (WT) mice. Auditory brainstem response recordings were carried out, followed by immunoperoxidase and immunofluorescence immunocytochemistry for the detection of the "antioxidant" enzymes superoxide dismutase (SOD) and catalase (CAT) and the oxidative stress marker 3 nitrotyrosine were carried out.
Results: Hearing thresholds were increased in APPNL-F mice aged 12-14 months. SOD and CAT immunoreactivities were diminished in the cochlea, relative to that observed in WT. This was particularly

evident in the stria vascularis and spiral ligament, as well as in regions of the spiral limbus, along with spiral ganglion cell bodies. This, along with immunolabeling for the oxidative stress marker 3-NT indicates increased oxidative stress.
Conclusions: Lower immunoreactivity levels of SOD and CAT in the auditory receptor and spiral ganglion of aged APPNL-F mice, along with levels of 3-NT, compared with age-matched WT mice of the C57BL/6J strain, supports diminished antioxidation capacity and exacerbation of oxidative stress in the cochlea of mice carrying traits of AD beta-amyloid proteinopathy. This correlates with previously reported increase in inflammatory markers in the cochlea of APPNL-F mice (Juiz et al., ARO 2022), suggesting that altered proteostasis in AD affects the peripheral auditory receptor through yet unknown mechanisms. If preexisting ARHL is thus exacerbated, this may in turn negatively affect the course of AD, leading to a vicious circle of important consequences.
Supported by PID2020-117266RB-C22 from AEI, Ministerio de Ciencia e Innovación, Spain and by Cluster of Excellence "Hearing4all", EXC 2177/1, ID: 390895286, part of the framework of the Germany's Excellence Strategy of the German Research Foundation, DFG

Investigating the Link Between Hearing Loss and Dementia Utilising Brain Imaging and Genetic Analyses in ADNI and UK Biobank Cohorts

Fatin Zainul Abidin*[1], Helena Wells[1], Andre Altmann*[1], Sally Dawson*[1]
[1]*University College London*

Background: Age-related hearing loss (ARHL) was recently established as the largest modifiable risk factor for dementia although the reasons underlying the link remain unclear. It has been hypothesised that ARHL may increase dementia risk through effects on cognitive load, structural changes in brain, and/or social isolation mechanisms or there could be common neuropathological process to both traits. Here, we took an exploratory approach to finding the link between hearing loss and dementia through genetic analyses as well as exploring the hearing loss neural correlates using brain images from large datasets such as the Alzheimer's Disease Neuroimaging Initiative (ADNI) and the UK Biobank Cohorts.
Methods: Data from previously published genome-wide association studies on self-reported hearing difficulty from the UK Biobank cohort (87,056 cases and 163,333 controls) and Alzheimer's disease from the International Genomics of Alzheimer's Project (IGAP) (21,982 cases and 41,944 controls) were used in our study. We used genetic correlation, simple Mendelian randomisation (MR) and shared pathway analysis on both GWAS risk variants. In addition, we also performed a systematic MR analysis to screen for shared causal traits between ARHL and AD. For imaging analysis, we used FDG-PET images from ADNI (162 cases and 742 controls) and voxel-wise statistics of FDG uptake differences between cases and controls were computed using the generalised linear model. We also investigated the volumetric changes in the brains of participants with self-reported hearing difficulty by analysing MRI images from the UK Biobank (9,573 age-matched cases and controls). Regional analyses were conducted to evaluate how regional areas, mean thicknesses and volumes of the brain grey matter vary with hearing status.
Results: Genetic correlation and MR analysis do not support a genetic correlation between the disorders but suggest a direct causal link from AD genetic risk to ARHL, driven by APOE. Systematic MR analyses on the effect of other traits revealed shared effects of glutamine, gamma-glutamylglutamine, and citrate levels on reduced risk of both hearing difficulty and AD. Pathway analysis on GWAS risk variants suggests shared function in neuronal signalling pathways as well as aetiology of diabetes and cardiovascular disease. Neuroimaging analysis show the hearing loss group exhibited glucose hypometabolism in the bilateral Heschl's gyrus, the inferior colliculus and cochlear nucleus after age correction. Cortical mean thicknesses and volumes were increased in the occipital lobe and decreased in the Heschl's gyrus regions.
Conclusions: Our genetic-driven analysis suggests hearing difficulty and AD are linked by a shared vulnerability in molecular pathways rather than by a shared genetic architecture. Both imaging analyses confirm the neural correlates of hearing loss along the auditory processing pathway in the brain and from MRI imaging analysis, that visual processing regions may be enlarged to compensate for reduced auditory input in older adults with ARHL.
*Joint senior authors

Synchronization of the Brain Wave of Young and Elderly People When Listening to 40-Hz Amplitude-Modulated Sound

Rara Shimauchi*[1], Mami Suezaki[1], Masajiro Chikamori[1], Eriko Aiba[1], Akinori Yamashita[2], Kazuma Maeda[3], Kazuki Takazawa[4], Yoshiki Nagatani[4]
[1]University of Electro-Communication, [2]Nara Medical University, [3]Shionogi Pharmaceutical, [4]Pixie Dust Technologies
Background: Improvement of cognitive impairment by presenting sound stimuli with a repetition period of 40 Hz has been reported along with synchronization of electroencephalography (EEG) while hearing various amplitude-modulated sounds, especially in younger participants. However, a comparison of EEG synchronization between young and elderly participants has not been reported.
Methods: Based on previous studies, we used a 1 kHz sinusoidal wave and a 40 Hz pulse train (each pulse contains one cycle of 1 kHz sinusoidal wave) as the stimuli. In addition, we also used recordings of news programs and music programs broadcasted on Japanese TV. From these stimuli, we created one with no modulation, one modulated 100 % with a 40 Hz sinusoidal wave, and one modulated 100 % with a 40 Hz inverse-sawtooth wave. The length of each stimulus was 15 seconds, and each pattern was presented randomly four times per pattern. Prior to the experiment, hearing tests were conducted in a soundproof room, and the presentation levels were adjusted using the obtained trichotomous hearing levels. The presenting level was the equivalent noise level of 60 dB plus the hearing level (dB). Participants with hearing levels greater than 30 dB were excluded. The audiograms of participants suggested that their hearing might not be affected by any diseases other than presbycusis. Stimuli were presented through earphones. While the stimuli were presented, EEG was measured with active electrodes placed at Cz. The phase locking index (PLI) was calculated for each stimulus pattern on the EEG waveforms obtained. Data from four young and four elderly participants was employed. Experimental participants were instructed to watch a silent animation. The experiment was approved by the Ethics Review Board and was conducted with informed consent.
Results: The results showed that all modulated sounds improved the PLI relative to unmodulated sounds for both young and elderly participants (PLI of all unmodulated sounds was less than about 0.05). For example, the PLI for pulsed sounds was about 0.40, compared to about 0.20 for sinusoidal wave modulation and about 0.30 for inverse-sawtooth modulation, showing clear synchronization. The modulated music and news program stimuli had around 0.15~0.20 as same as sinusoidal and inverse-sawtooth modulation. Although weaker than the pulse tones, clear synchronization was observed. These values showed no clear difference between the younger participants and elderly participants. Between young and elderly participants, we found no apparent differences in these values.
Conclusions: The EEG was synchronized to the modulation cycle in young and elderly participants by presenting sound stimuli with 40 Hz amplitude modulation of sinusoidal waves, pulsed sounds, music, and news programs. Our results indicate the possibility of improving cognitive impairment by presenting amplitude-modulated sound stimuli regardless of age, and holds promise for clinical application.

The Effect of ATP Purinergic P2x Receptor Deficiency on Alzheimer's Disease Development and Progression

Hong-Bo Zhao*[1], Liman Liu[2], Ling Mei[2]
[1]Yale University Medical School, [2]University of Kentucky Medical Center
Background: Alzheimer's disease (AD) is a neurodegenerative disease with a progressive loss of memory and cognitive decline. Hearing is an important neural-sensory input for cognition. Previous studies demonstrated that hearing loss could accelerate AD and dementia generation. Our previous study demonstrated that ATP-purinergic P2x2 receptor mutations can exacerbate age-related hearing loss (Yan et al., 2013). In this study, we tested whether deficiency of P2X receptors can exacerbate/accelerate AD development and progression.
Methods: An AD mouse model (APP/PS1; Jackson Lab: Stock No: 004462) and P2X2 KO mice (Stock No: 004603, Jackson Laboratory) were used. APP/PS1 mice were crossed with P2x2 KO mice to generate APP/P2x2 KO double transgenic mice. Hearing function was assessed by ABR, DPOAE, and other hearing tests. Acoustic startle response (ASR) and gap-detection tests were also recorded to assess behavior changes.
Results: Preliminary data showed that P2x2 deficiency could exacerbate hearing loss in AD mice, although P2x2 KO mice themselves had no apparent hearing loss. APP/PS1 and P2x2 KO double transgenic mice had more significant increase in ABR threshold in comparison with that in "pure" APP/PS1 AD mice.

Conclusions: Our preliminary data suggest that deficiency of ATP-purinergic signaling function could accelerate AD development and progression. This study may also provide a clue to prevent or delay AD development and progression.
Supported by NIH R01 DC 019687 and DC019687 AD supplement to HBZ

The Prevalence and Recognition of Cognitive Impairment in Older Individuals Visiting a Hearing Instrument Specialist in the Netherlands

Sander Ubbink*[1], Iris Haaijer[2], Rianne Louwes[2], Marissa Niedorff[2], Arne van den Bosch[2], Barbara van Munster[3]
[1]University of Groningen, University Medical Center Groningen, Dept. of Otorhinolaryngology / Head and Neck Surgery, The Netherlands, [2]University of Groningen, [3]University of Groningen, University Medical Center Groningen, Dept. of Internal Medicine
Background: Because of increasing life-expectancy the prevalence of individuals with dementia and hearing loss are both increasing. Hearing loss is the largest modifiable risk factor for dementia and there are indications that hearing rehabilitation slows down the cognitive decline. At the same time, the presence of cognitive impairment can have a negative effect on hearing rehabilitation. To improve hearing rehabilitation for people with cognitive impairment, hearing specialists need to adapt their procedures to the needs of people with cognitive impairment. We investigated the prevalence of cognitive impairment in older individuals visiting hearing instrument specialists and investigated to what degree these specialists could recognize cognitive impairment within their clinical routine.
Methods: This observational study was conducted in collaboration with Otorhinolaryngology and Geriatrics departments of UMCG and four hearing aid retail stores in the Netherlands. We determined the prevalence of cognitive impairment in older individuals (60 years or older) visiting hearing instrument specialists. Cognitive impairment was assessed by use of the Mini-Cog. The Mini-Cog is a cognitive screening test that can be administered in three minutes. It combines a three-item recall task (0-3 points) with an elective clock drawing task (0-2 points). In this study a score of ≤2 points was defined as abnormal, indicating that a cognitive problem may be present. Additionally, hearing instrument specialists were asked for their professional assessment if they suspected cognitive impairment.
Results: We screened 239 older individuals. The mean age was 79 years (range 60-96, std 8.6 years) and 133 (56%) of the individuals were male. In 51 out of 239 (21.3%) screened older patients an abnormal outcome on the Mini-Cog was found. The recognition of cognitive impairment by the hearing instruments specialists compared to the outcome of the Mini-Cog resulted in a sensitivity of 66% and a positive predictive value of 50%.
Conclusions: Results from our cohort indicate that the prevalence of cognitive impairment in hearing impaired older individuals visiting a hearing instrument specialist could be as high as 1 in 5. Hearing instrument specialists experience difficulty recognizing cognitive impairment and miss 1 in 3 older individuals with cognitive impairment. The lack of good recognition may lead to suboptimal hearing rehabilitation for older people with cognitive impairment.

Sensorimotor Deficits as Contributors to Early Cognitive Impairment

Zahra Sayyid*[1], Yuri Agrawal[1]
[1]Johns Hopkins University School of Medicine
Background: It is increasingly recognized that age-related sensory and motor dysfunction are associated with an elevated risk of mild cognitive impairment and Alzheimer's disease and related dementias. However, the relationship between early cognitive impairment with multi-sensory impairment as well as specific domains of motor function has yet to be systematically studied.
Methods: Participants in the Baltimore Longitudinal Study of Aging (N=693, aged 50-99) completed motor and physical function tests including pegboard, grip strength, standing balance, chair stands, 400-meter walk lap time, and usual gait speed as well as multi-sensory function tests including hearing, vision, vestibular function, proprioception and olfaction. The association between sensorimotor factors and early cognitive impairment was examined using structural equation modeling methods. Three latent factors corresponding to fine motor function, gross motor function, and multi-sensory system function were identified, and measurement models were estimated for these factors using confirmatory factor analysis. The three latent factors were then entered as predictor variables with early cognitive impairment as an outcome in a full model.

Results: In the confirmatory factor analysis model, the fine motor factor was correlated with the gross motor and multisensory system factor (r = 0.76 and r = 0.78), respectively. The gross motor factor and multisensory system factor was correlated (r = 0.82). In the full structural equation modeling model, after adjusting for age, sex, race, education, and body mass index, the odds of early cognitive impairment decreased 34% (95% CI: 17-47%), 32% (95% CI: 17-43%) and 15% (95% CI: 2-26%) given one unit increase in the multisensory system factor score, fine motor factor score and gross motor factor score respectively.
Conclusions: Our study shows that multiple sensory function, fine motor, and gross motor function are associated with early cognitive impairment. Future longitudinal studies on the effect of changes in these factors are warranted. These findings have the potential to guide future intervention studies aimed at preventing and/or treating cognitive decline at an early stage through modifiable mechanisms.

Symposium #33 - Hyperacusis: Diversity in Cause, Expression, and Advocacy

8:00 a.m. - 10:00 a.m.
Crystal Ballroom D-E

Hyperacusis: Diversity in Cause, Expression, and Advocacy

Chair: Benjamin Auerbach, *University of Illinois at Urbana-Champaign*
Co-Chair; Fatima Husain, *University of Illinois at Urbana-Champaign*

Hyperacusis is a complex hearing disorder that encompasses a wide-range of reactions to sound, including excessive loudness, increased aversion/fear of sound, or even pain. While often associated with hearing loss and tinnitus, sound tolerance disturbances are observed across a broad spectrum of neurological disorders, including autism, chronic pain and post-traumatic stress disorder. Thus, hyperacusis is diverse in both its etiology and phenotypic expression, and it is imperative to consider this diversity when attempting to elucidate its physiological mechanisms and advance diagnostic and treatments. Bryan Pollard, founder and president of the Hyperacusis Research Ltd., was a champion for this cause. Bryan was a tireless advocate for hyperacusis research, working to connect researchers from diverse backgrounds, often through formal and informal workshops and dinners at the ARO midwinter meeting. He was the first non-researcher to present at ARO and was instrumental in creating a new diagnosis of pain hyperacusis. Bryan unfortunately passed away earlier this year.

This goal of this symposium is two-fold. First, we hope to update the hearing community on the status of the hyperacusis field and the progress that has been made towards diagnosing, treating, and modeling this disorder. Hyperacusis is a rapidly growing research field that intersects with many other aspects of hearing research, including central auditory processing and plasticity, tinnitus, and autism. As such, this symposium would have a broad target audience, from basic auditory neuroscientists interested in the neural mechanisms of perception and experience-dependent plasticity to audiologists seeking to understand and perhaps better treat a host of auditory processing disorders. In addition, we hope to use this symposium to commemorate the impact that Bryan Pollard had on the field, starting with a short introduction and in memoriam to highlight the ways he and the Hyperacusis Research foundation have helped move hyperacusis research forward.

Connecting Researchers to Clinicians and Patients: Bryan Pollard's Roadmap to a Cure for Hyperacusis With Pain
Michael Maholchic, *Hyperacusis Research, Ltd*

In memoriam to Bryan Pollard, founder of Hyperacusis Research Ltd., and his impact on the field.

A Need to Better Define and Characterize Loudness and Hyperacusis
Sylvie Hebert, *Universite de Montreal*

Hyperacusis is a hearing disorder wherein everyday sounds are considered too loud, fearful, annoying, or painful. In its most accepted definition, hyperacusis designates sounds of moderate intensities that are judged louder than normal perception. A deeper understanding of what loudness is would contribute to a

better definition of what hyperacusis is, and how it can be defined and diagnosed. Indeed, although loudness involves the perception of the intensity of sounds, i.e. its sensory dimension, loudness may also be modulated through its affective dimension. In this talk I will present recent data aiming at a better understanding of loudness, namely its sensory and its affective dimensions, and how these two dimensions can be dissociated within the same stimuli. I will also report the findings of a recent scoping review on the electrophysiological correlates of hyperacusis, in which we found a diversity in terms and definitions used to describe hyperacusis and several identifiable aetiologies, among which developmental disorders, neurological disorders, induced hearing damage, and idiopathic aetiology. Broader consensus around definitions and diagnostic criteria considering aetiologies may guide researchers to ask better questions and clinicians to more efficiently manage the patient who complain about hyperacusis.

Stressful Hearing: Hyperacusis Can Be Induced by Chronic Stressful Noise Exposure or Chronic Pharmacological Stress That Disrupts the HPA Axis

Richard Salvi, *Center for Hearing and Deafness, SUNY at Buffalo*

We live in a noisy, stressful environment largely oblivious to its negative hearing-health consequences. One debilitating hearing impairment associated with noise-induced hearing loss is hyperacusis, a loudness intolerance disorder in which everyday sounds are perceived as excessively loud, aversive and stressful. To investigate the biological bases of hyperacusis, we developed a reaction time-intensity (RT-I) paradigm to test for loudness hyperacusis. To assess the aversive quality of hyperacusis, we developed an active sound avoidance paradigm (ASAP) to measure sound avoidance behaviors. To trigger the induction of hyperacusis, we exposed rats to intense high-frequency noise for several months; these conditions were expected to induce chronic noise-stress in addition to high-frequency loss. After this prolonged, stressful noise exposure, rats developed clear signs of sound avoidance hyperacusis as well as loudness hyperacusis with a low-frequency spectral profile. This prolonged, stressful noise exposure disrupted the hypothalamic-pituitary-adrenal (HPA) axis; it did not alter basal corticosterone (CORT) levels, but instead, greatly reduced the rise in corticosterone (CORT) triggered by restraint stress (i.e., it blunted the stress response). This noise exposure also chronically increased the expression of glucocorticoid receptors (GR) in the auditory cortex, part of the negative feedback network that suppressed the continued release of CORT. To determine the role of chronic stress independent of hearing loss, rats were chronically stressed by pharmacologically treating the rats with CORT-stress hormone. After chronic pharmacologic stress, rats developed behavioral evidence of loudness hyperacusis and sound avoidance hyperacusis. Consistent with the previous noise studies, basal CORT levels remained normal; however, rats exhibited a blunted CORT response to restraint stress and GR expression in the auditory cortex increased. Chronic pharmacologic CORT stress did not alter DPOAEs or the cochlear compound action potential; however, sound-evoked responses from chronically implanted electrodes on the auditory cortex were greatly enhanced. These results show for the first time that chronic pharmacologic stress, in the absence of hearing loss, is sufficient to induce behavioral evidence of hyperacusis, which is associated with auditory cortex neural hyperactivity and enhanced central gain.

Behavioral and Physiological Measures of Sound Intolerance

Sarah Theodoroff, *Department of Veterans Affairs, NCRAR*

The experience of individuals who have decreased tolerance to everyday sounds is heterogeneous. No consensus exists regarding what constitutes hyperacusis or noise sensitivity. A direct consequence of this is the lack of a “gold standard” regarding how to diagnose, assess, and treat these conditions. The purpose of this presentation is to address this gap in clinical knowledge.

Noise sensitivity refers to an increased reactivity to everyday sounds and encompasses a range of psychological attributes, often including annoyance or feeling overwhelmed by the sounds in the environment. An estimated 59% of patients with mild traumatic brain injury have noise sensitivity (Shepherd et al, 2019). Research is lacking both on the pathophysiology of noise sensitivity, its clinical treatment, and to what degree it may or may not be a similar phenomenon to hyperacusis. Hyperacusis describes a decreased sound tolerance driven by the perceived loudness of ordinary sounds. It often including physical discomfort or pain when listening to sounds that are at moderate or low intensity levels, which most people would find tolerable. Estimates are as high as 60-79% of tinnitus patients have comorbid hyperacusis (Andersson et al, 2001; Dauman and Bouscan-Faure, 2005).

When Veterans seek medical attention because ordinary sounds are painfully loud or there's "too much noise to function" many clinicians are uncertain how best to meet their patients' needs. This results in the focus being shifted to comorbid conditions hoping that the "hyperacusis" is a symptom of another condition. Research is needed to develop evidence-based tools that are capable of differentiating noise sensitivity from hyperacusis and from other health conditions that present with similar symptoms. This need motivated the ideas behind an on-going research project that is examining the relationship between auditory and psychological biomarkers to determine how well auditory and psychophysiological data predict self-reported decreased tolerance to everyday sounds. Behavioral and physiological measures are collected in order to detect where deficits exist in sensory and/or neurological structures associated with complaints of sound intolerance.

Results from this work will elucidate aspects of the underlying pathophysiology of "hyperacusis" and "noise sensitivity." Ultimately, outcomes from this avenue of research will guide the development of targeted rehabilitative treatments based on the etiology of these conditions and inform which disciplines should be working together to best meet the needs of this patient population (e.g., audiology, psychology, neurology).

A Rodent Model of Acoustic Trauma to Study Neural and Inflammatory Mechanisms of Hyperacusis
Megan Wood, *Johns Hopkins University School of Medicine*

The mechanism underlying painful hyperacusis is currently unknown. A subset of hyperacusis patients surveyed recently reported experiencing acoustic trauma before the onset of their symptoms. Therefore, we use acoustic trauma in rodents to induce neurological and inflammatory changes in the cochlea as a model for noise-induced hyperacusis. Acoustic trauma affects many cell types of the inner ear. We focus on the responses of type II auditory nerve fibers and immune cells as these cell types are the putative pain sensing neurons and chief responders to inflammation in the cochlea, respectively. Type II auditory nerve fibers exhibit changes in their calcium dynamics after acoustic trauma. New analysis of an existing dataset will be discussed to describe a sensitization of these fibers as a possible mechanism for hyperacusis. Another possible mechanism for hyperacusis is an enhanced inflammatory response following acoustic trauma. Neuroimmune crosstalk through neuropeptides found in the cochlea, such as CGRP, may play an important role in this possible mechanism. Recent studies following immune cell migration after blocking CGRP receptors during acoustic trauma will be discussed. Finally, the presentation will end with a discussion of the parameters needed to properly model painful hyperacusis in rodents including behavioral assays of pain perception to sound stimulation.

Imaging Hyperacusis: Past and Present
Fatima Husain, *University of Illinois at Urbana-Champaign*

Brain imaging of humans with hyperacusis remains one of the primary methods to identify neural networks sub serving this condition and dissociate them from those related to comorbid tinnitus or hearing loss. Despite the challenges associated with cost and noise of some tools, results of such studies have informed the existing experimental and theoretical framework of hyperacusis. In a pioneering fMRI study, Melcher and colleagues (2010) parsed out the contribution of co-occurring hyperacusis to brain imaging findings of tinnitus. In particular, they noted that while both subcortical and cortical auditory areas were responsive in those with hyperacusis (relative to controls), such an elevated response was only noted in the auditory cortex for those with tinnitus. Two recent studies (Koops and van Dijk, 2021; Hofmeier et al., 2021) further explored this dissociation. Koops and van Dijk confirmed the higher response in cortical and subcortical centers of auditory processing in response to external sounds but in addition found that there was reduced response to the frequencies of the internally-generated tinnitus sound. The Hofmeier study provided corroboration of the increased responsiveness of the tinnitus and hyperacusis group to external sounds in the cortical and subcortical auditory areas. Additionally they noted increased wave III amplitude in brain-stem response of the tinnitus and hyperacusis group relative to the control group; the tinnitus only group exhibited a prolonged and reduced wave V amplitude compared to the controls. These studies support the idea of central gain in the condition of hyperacusis but not necessarily if tinnitus occurs alone. They further point to the role played by attention in tinnitus, as noted in task-based fMRI studies in my own lab (Husain et al., 2011; 2015). What is not easily known is the contribution and interaction of clinically-significant hearing loss with hyperacusis. Koops and van Dijk noted the elevation of response at frequencies in the hearing loss

as well as normal hearing range in those with hyperacusis. The Melcher studies were primarily in those with normal hearing. In an ongoing study, we are collecting both auditory brainstem response and fMRI data on young adults with hyperacusis and normal hearing and their controls, results of which will be reported at the meeting. In summary, current and future non-invasive brain imaging studies continue to expand our understanding of pathophysiology of hyperacusis and eventually test therapies that help patients.

Investigating Hyperacusis in Rodent Models of Autism
Benjamin Auerbach, *University of Illinois at Urbana-Champaign*

Animal models are indispensable tools for identifying disease pathophysiology and developing objective biomarkers for hearing disorders like hyperacusis. However, care must be taken when attempting to recapitulate complex hearing disorders in model systems. Hyperacusis encompasses a wide range of reactions to sound and is observed across a broad spectrum of neurological disorders, and this diversity must be accounted for when attempting to measure and induce hyperacusis in animals. Recent progress has been made in developing rodent behavioral models that capture distinct aspects of sound perception disrupted in hyperacusis, with attempts to disentangle psychoacoustic (e.g. excessive loudness) from affective (e.g. decreased sound tolerance) aspects of the disorder. However, drug- and noise-induced hearing loss are still the primary methods used for inducing hyperacusis-like states in animals. Here we will discuss recent attempts to characterize sound tolerance disturbances in genetic models of neurodevelopmental disorders that present with high rates of hyperacusis, such as Fragile X syndrome and autism spectrum disorders (ASD). Comparing and contrasting hyperacusis associated with hearing loss and neurodevelopmental disruption has the potential to uncover convergent (or divergent) pathophysiological mechanisms across distinct forms of hyperacusis , which will help advance our ability to diagnose and treat this often devastating disorder.

Podium #34 - Auditory Processing, Challenges and Progress

10:30 a.m. - 12:30 p.m.
Ocean's Ballroom 5-12

Moderators: Brian Frost and Tobias Reichenback

Music Emotion Categorization in Typically Hearing and Cochlear Implant Users

Eleanor Harding*[1], Etienne Gaudrain[2], Imke Hrycyk[1], Robert Harris[3], Barbara Tillmann[2], Bert Maat[1], Rolien Free[1], Deniz Başkent[1]
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Background: Cochlear implant (CI) users who experience difficulties perceiving acoustically complex signals like music report a desire to improve their enjoyment of music. One important aspect of music enjoyment is perceiving music emotion. Previous studies investigating music emotion in CI users reported that whether a piece is relaxing or stimulating (i.e., related to emotional arousal) may be communicated via temporal cues related to tempo, which tend to be well preserved through electric hearing. Whether the music emotion is positive or negative — (i.e., related to emotional valence) — may often be communicated via spectral cues related to pitch and harmony, which are typically degraded in electric hearing. In the current study we compared emotion categorization in CI listeners to that in normal-hearing participants listening through vocoders that preserved more or less of the spectral and temporal information.
Methods: Normal-hearing and CI-user adults performed an emotion-categorization task (with choices joy, sadness, fear, serenity). The stimuli were excerpts from classical music pieces whose presented emotion was established in previous experiments. For the normal-hearing participants, the stimuli were either non-vocoded, or vocoded using 4 different vocoders: sinewave or noise carriers, low or wide simulated spread of excitation. Accuracy, sensitivity (d') and confidence of categorization, as well as the transmission of arousal and valence features, were evaluated.
Results: In normal-hearing participants, emotion categorization was good in the non-vocoded condition, and remained above-chance in vocoded excerpts but poorer than in non-vocoded control conditions. The data from the CI users resembles that of the vocoded conditions in normal-hearing participants. In particular, in both groups, the arousal was well transmitted while the valence information was essentially lost.

Conclusions: The findings are in line with the existing body of literature: temporal features of the envelope informed emotion categorization above and beyond spectral features, even in the optimal condition of spectral resolution, successfully conveying arousal but not valence categories. This salience of temporal features suggests that valence might be better conveyed if manifested in temporal as opposed to spectral structure of the signal, for example creating roughness in the envelope to convey dissonance.

Longitudinal Follow-Up of Children With Moderate Hearing Loss

Anna Persson*[1]
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Background: The universal newborn hearing screening followed by early intervention has led to an increased number of children with hearing loss reaching speech and language outcomes on par with typically hearing peers. Children with moderate hearing loss is a group that is presenting outcomes on both ends. Why is that? As poor vocabulary affects various other domains of learning, this specific outcome was of interest in this longitudinal follow-up of a group of children with moderate hearing loss from timepoint of hearing aid fitting (<6 months) to 6 years of age.
Methods: Size, development and complexity of expressive vocabulary from 18-36 months and then again at six years of age were analyzed and compared to a group of children with typical hearing. The vocabulary data was analyzed in relation to auditory variables and predictions on future development were made.
Results: All children with moderate hearing loss (HL) were fitted with hearing aids before six months of age and their use of amplification varied substantially over time. The hearing of some children progressed over time, resulting in some of them having cochlear implants at six years of age.
The children with hearing loss produced a similar number of words as the typical hearing (TH) at 18 months, but fewer at 24 and 30 months. The number of different true consonants at 18 months for the whole group showed a significant relationship to number of words produced at 24 months. No significant differences were found between children with HL and children with TH regarding phonological complexity of reported words. Hours of HA use was the main factor showing significant correlations to all speech and language measures. Hearing, speech and language data from the same cohort at six years of age have just been collected and results will be presented in relation to outcomes from earlier ages.
Conclusions: Meanwhile consonant proficiency were similar between the children with hearing loss and typical hearing, the difference in vocabulary outcomes were significant. The main factor affecting the vocabulary outcomes in the children with hearing loss the first three years was hours of hearing aid use. As all children had reached full-time hearing aids use at six years of age but still showed low outcomes on vocabulary suggests that monitoring and promoting hearing aid use during the first three years should be a main priority in the early intervention. This work demands cross-professional collaboration.

Environmental Analysis in Early Intervention Groups for Young Children With Moderate Hearing Loss

Aline Hoeve*[1], Annerenée Meijer[1], Ruben Benard[2], Evelien Dirks[3], Aart Woonink[4], Deniz Başkent[1]
[1]*University Medical Center Groningen,* [2]*Pento audiologisch centrum,* [3]*Nederlandse Stichting voor het Dove en Slechthorende Kind,* [4]*Cauberg-Huygen*

Background: The first years of a child's life are crucial for spoken language development. For children with moderate hearing loss (MHL), who have restricted access to sounds and speech, the development of spoken language is more at risk. Most children with MHL are hearing aid (HA) users. Even though these well-fitted HAs provide great improvement in all environments, a HL may not be entirely revalidated up to the level of normal hearing due to several factors. For example, the reduced dynamic range of a damaged inner ear needs to be compensated for by HAs, potentially affecting the fine structures of speech sounds. Non-optimal acoustic environments can be extra challenging for children with HL, since HL can alter the acoustic cues needed for recognizing speech in noise. Up to now, however, we do not have a complete picture of the acoustic environment of children with MHL in which they need to learn language.
In the Netherlands, early intervention (EI) groups support speech and language development for young children with HL, providing intervention in small groups by qualified professionals. This study aims to provide an insight into the acoustics characteristics of the EI groups, by using measurements at the Dutch EI groups, since these groups are an important place for language development for children with MHL.
Methods: A total of 23 young children within the sensitive language learning period (2-4 years) with MHL using Phonak HAs are included in this study. Data collection was performed during one EI group day on 6

different locations in the northern half of the Netherlands. Acoustic environment was characterized by two measurements; 1) HAs data logging, analyzing information from the environment to classify the scene. For example, they plot the amount of time a HA user is in a silent or noise situation, or the time the HA was connected to a remote microphone system. 2) Language Environment Analysis (LENA) recorder, worn by the child, classifying the auditory environment in its own categories.
Results: The average of 16 childrens HA measurements classified 72% of the time a calm situation, 25% speech in noise, 3% comfort in noise, 1% music, 0% remote microphone program. 6 other children connected to a remote microphone system, resulting in the HA scene classification 43% calm situation, 22% speech in noise, 3% comfort in noise, 1% music, 33% remote microphone program. 23 LENAs classified 42% as meaningful speech, 40% distant speech, 15% silence and background, 2% noise and 1% TV and electronic sounds.
Conclusions: The biggest HA scene classification at the EI groups was 'calm situation', the LENA scene most classified was 'meaningful speech'. We will discuss these results, as well as the variety in remote microphone use at these EI groups.

Hyperacusis: Results of a Novel Treatment Approach

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Background: Abnormal sensitivity to the loudness of ordinary sounds, or hyperacusis, often is comorbid with other disorders including autism, migraine headaches, fragile x syndrome, Williams syndrome, and other conditions. Such a condition can have a profound impact on daily life. This includes behaviors to compensate for abnormal sound sensitivity, such as avoiding many acoustic environments and the use of earplugs or ear muffs to limit offending sound exposures. Such sound avoidance, however, can exacerbate the hyperacusis condition. This counterproductive management strategy also poses a major barrier for transitioning patients into best-practices treatments.
The scientific bases for sound sensitivity disorders are not fully understood but a growing body of literature based on data from human subjects and animal models point to involvement of the central nervous system, including auditory and non-auditory regions of the brain, and to mechanisms that alter central gain through various interactions of excitatory and inhibitory processes. The use of sound therapy is thought to counter those processes and result in an expanded dynamic range.
Methods: A novel treatment protocol was designed to transition the patient away from hearing protection devices and towards health sound exposure. It includes structured counseling with script and visual aids focused on understanding the condition, current prevailing theories regarding mechanisms and manifestations with the central auditory system, and the value of healthy sound exposure. The protocol includes the use of a behind-the-ear sound therapy device with multiple features custom fit to the patient. A custom ear piece designed to function as a custom earplug with maximum attenuation. Mild amplification is adjusted to provide unity gain within the passband of the device, allowing comfortable exposure to low level sounds. Output limiting limits exposure to high-level, offending sound levels. A sound generator provides sound therapy in the form of low-level sea-shell like noise. To encourage the use of sound therapy as much as practical, thin-tube coupling of the speaker to the ear piece can be swapped in the field between the custom occluding ear piece and an open-dome configuration. The latter is for use in environments with low risk for offending sound levels. As sound therapy expands the dynamic range, there is a corresponding release of output limiting.
Results: Despite a wide range of severities at baseline, patients demonstrated benefit on multiple indices. Most dramatic was their loudness tolerance for running speech, which increased by 34 dB on average over six months, or 5.6 dB per month. Subjective indices of hyperacusis changed accordingly, with significant improvements on the Hyperacusis Questionnaire, the Tampa Scale of Hyperacusis, the Noise Avoidance Questionnaire, and Questionnaire on Hypersensitivity to Sound.
Conclusions: Complimentary subjective feedback indicated a return to many daily activities posttreatment and high satisfaction with the intervention. [Work Supported by NIDCD R21 DC015054].

A Comparison Between Stimulus-Reconstruction Algorithms for Decoding EEG Responses to the Speech Envelope

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Background: Normal-hearing listeners possess the remarkable ability to comprehend a target speaker whilst ignoring background sounds. In competing-speakers scenarios, a listener's electroencephalography (EEG) tracks the temporal envelope of an attended speaker more strongly than that of an unattended speaker. The strength of this tracking, quantified by the ability of a backward model to reconstruct each speech envelope from a listener's EEG recordings, serves as a marker of auditory attention. Smart hearing aids may one day be able to use this attention marker to selectively amplify desired sounds and suppress unwanted sounds in the acoustic mixture, leading to improved comprehension levels and lower cognitive workloads for hearing-impaired wearers.
Methods: We compare the performances of two state-of-the-art algorithms at decoding the envelope of speech in noiseless conditions from EEG recordings: the canonical correlation analysis (CCA) approach, and the deep neural networks (DNN) approach. We characterise both the mean reconstruction performance of the two approaches, and the noisiness of the reconstruction. We then apply these algorithms to a competing-speakers dataset. The algorithms are evaluated based on two metrics: the decoding accuracy, and the expected latency of a hearing device in detecting changes in auditory attention. We also test whether these stimulus-reconstruction algorithms generalise between listening conditions and speakers. Finally, we enhance the attention decoding accuracy of both algorithms by employing state-space models to reduce the noisiness of the observed attention markers.
Results: We replicate the finding that the CCA method yields high correlation scores compared with other stimulus-reconstruction algorithms. However, the derived attention markers are extremely noisy, which limits the attention decoding performance of the CCA approach. However, when the CCA approach is combined with a simple classifier, its attention decoding performance exceeds that of the DNN-based approach. We verify that the performance of both approaches remains high, even when the listening conditions and voices are changed. By employing state-space models such as the Kalman filter or hidden Markov model, we were able to considerably improve on the state-of-the-art, both in terms of the attention decoding accuracy, as well as the ability of a decoder to detect attention switches with a low latency.
Conclusions: Methods of stimulus-reconstruction show promise for real-world auditory attention decoding, since they are insensitive to the listening conditions and speakers present within a given auditory scene. Amongst these, the CCA approach combined with a simple classifier achieves the best auditory attention decoding performance. The auditory attention decoding performance of the stimulus-reconstruction approach can be greatly enhanced by employing models of a listener's attentional dynamics. Such enhancement will be necessary for real-world auditory attention decoders that are based on miniaturised EEG montages.
This project was supported by UK Research and Innovation
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Auditory Electrophysiology Reveals HIV-Related Central Nervous System Dysfunction in Children

Christin Ealer*[1], Christopher Niemczak[1], Silvia Bonacina[2], Trent Nicol[2], Jonathan Lichtenstein[3], Albert Magohe[4], Sam Leigh[1], Abigail Fellows[1], Enica Massawe[4], Nina Kraus[2], Jay Buckey[1]
[1]Geisel School of Medicine at Dartmouth, [2]Northwestern University, [3]Dartmouth-Hitchcock Department of Psychology, [4]Muhimbili University of Health and Allied Sciences
Background: Diffuse central nervous system damage occurs in people living with HIV (PLWH) even with effective anti-retroviral therapy. Our previous work (White-Schwoch et al. (2020)) shows that the frequency following response (FFR), an electrophysiologic test focused on central auditory pathways, reveals differences between adult PLWH and HIV-negative controls. In this study, we investigate whether the FFR shows central nervous system dysfunction in children living with HIV (CLWH).
Methods: Subjects were recruited from an ongoing longitudinal study in Dar es Salaam, Tanzania. Data were analyzed from 165 CLWH and 158 HIV-negative children (ages 3 to 9 years). All data were from the child's first visit. The groups were matched on age, years of education, gender, socioeconomic status, left and right Pure Tone Averages (PTAs), and pre-stimulus noise. The speech-evoked frequency following response (FFR), using "ba", "ga", and "da" syllables, was administered to all subjects. Response outcomes included stimulus-to-response correlation (a measure of neural precision), inter-response correlation (a measure of response consistency), fundamental frequency, first formants, and peak latencies. Analysis included the calculation of CLWH and HIV- group means, t-tests to compare group means, and calculation

of Cohen's d to assess the effect size of each significant result. A p-value of 0.0025 was used to assess significance.
Results: CLWH and HIV-negative children significantly differed on several key FFR measures. CLWH had significantly worse stimulus-to-response correlations than their HIV-negative counterparts across all three speech stimuli (all $p < 0.0025$, d ranged from 0.35 to 0.49). The CLWH had significantly lower inter-response correlations (all $p < 0.0001$, d ranged from 0.39 to 0.67). The "ba", "ga" and "da" first formants were significantly lower in the CLWH (all $p < 0.0015$, d ranged from 0.35 to 0.64). The fundamental frequency for "ba", "ga" and "da" did not statistically differ between the HIV groups. The CLWH and HIV-negative groups did not significantly differ on any "ba", "ga" and "da" latency.
Conclusions: These results are consistent with HIV-related central nervous system dysfunction observed in those with HIV. FFRs from CLWH were 1) selectively less robust (smaller first formants, but equivalent fundamental frequency), 2) less consistent (less inter-response similarity) and 3) less precise (weaker stimulus-to-response correlations). These findings are consistent with our previous findings, which documented less robust first formants in HIV-infected adults, and expand upon the characterization of central nervous system dysfunction by considering a broader set of FFR variables, including measures of consistency and precision.

Priorities in Hearing: Towards a Common Goal

Robert MacKinnon*[1], Antje Heinrich[2], Christian Sumner[3], Christina Newman[3], Frederick Gallun[4]
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Background: Otolaryngology research is of wide relevance to health and society. Nevertheless, it can be challenging for those seeking funds to provide clear evidence of the importance of specific topics that merit funding. Funders also face a challenge in making strategic decisions about which areas to prioritise. Citable evidence of expert consensus on the value of different topics and questions can be of value to both groups. We believe that there is currently no contemporary published collection of priorities in hearing research based on a broad engagement of international expert stakeholders from academia, industry and clinical practitioners from all areas of hearing research.
Our objective is to consult the international field of hearing researchers about what they think the priorities in hearing research should be for the next 5-10 years, with a view to developing and publishing an agreed set of priorities. Such a resource could increase the competitiveness of hearing research projects seeking funding, and hopefully increase the capacity of the field overall.
Methods: This study is using mixed methods (qualitative/quantitative). A pilot set of priorities was generated by sending an open response questionnaire to 40 UK and European experts. The responses underwent independent thematic analysis, which yielded a wide range of fields and sub-fields. Based on this pilot, we generated a new structured open response questionnaire, widely soliciting opinions from senior experts internationally. Data collection is on-going. Based on themes emerging from this larger, international dataset, we plan to canvas the entire international field of hearing research, junior and senior, to rate and rank the themes. This will provide quantitative data on the importance of the different themes and sub-themes, where significant progress is possible, and whether themes and sub-themes were currently receiving the appropriate amount of attention. It is intended that these data will be of significant benefit those applying for funding in all areas of the field.
Results: The open response questionnaire can be accessed here: https://bit.ly/arohearingsurvey
We encourage contributions from all senior (>5-10 years post PhD) research active members of all areas of the community (whether in academia, with industrial partners or in the clinic). We have in total 68 valid responses so far. This is a rich dataset, with some detailed and in-depth entries. We are currently seeking to balance up contributions from around the globe, with more contributions from outside Europe. We cannot present any analysis of themes until this stage of data collection is complete.
Conclusions: The present work aims to benefit all hearing researchers by providing a large-scale canvas of opinion on the state of hearing research and current priorities. The goal is to provide clarity and evidence of mandate to grant funding bodies, and to increase competitiveness in a challenging funding landscape.

Rapid Perceptual Learning in Adult Cochlear Implant Recipients: What Does It Mean for Speech Recognition

Ranin Khayr*[1], Riyad Khnifes[1], Talma Shpak[2], Karen Banai[3]

[1]*University of Haifa and Bnai-Zion Medical Center* [2]*Bnai-Zion Medical Center,* [3]*University of Haifa*
Background: Speech recognition in cochlear implant (CI) recipients are quite variable, particularly in challenging listening conditions. Demographic, audiological, and cognitive factors explain some, but not all, of this variance. The literature suggests that rapid auditory perceptual learning explains unique variance in speech recognition in listeners with normal hearing and those with hearing loss. We now ask if rapid auditory learning can be observed in adult CI recipients and if so if it also accounts for portions of the variance in fast speech and speech in noise recognition in CI recipients.
Methods: 36 adult CI recipients (ages 35-77, M=55) completed a comprehensive battery of challenging speech recognition tests (sentences in speech shaped noise, four-talker babble noise and natural-fast speech), cognitive measures (vocabulary, working memory, attention, and verbal processing speed), and a rapid perceptual learning task (time-compressed speech). Accuracy in the speech tasks was modeled with a series of generalized mixed linear models that accounted for demographic, cognitive and speech-related factors before accounting for the contribution of the perceptual learning task.
Results: Learning (indicated by a positive slope) was present in 27 of the 36 participants. Mean recognition accuracies in the learning task improved from 0.28 to 0.49 over the course of 40 sentences. Perceptual learning had unique contribution to the recognition of both natural fast speech and speech in noise. For individuals with similar demographic and cognitive characteristics, an increase of 1 SD in the perceptual learning slope was associated with ~59% and ~25% increase in the odds of correctly recognizing natural fast speech and speech in noise (respectively). Of the cognitive measures assessed, working memory and attention each had a unique positive contribution to speech recognition in both tasks.
Conclusions: The majority of CI recipients exhibited learning, suggesting that rapid auditory perceptual learning can be restored with the use of the implant even in adult recipients. This learning accounts for some of the individual differences in the recognition of speech in noise and natural fast speech, consistent with findings from age-related hearing loss. Thus, across populations, rapid auditory perceptual learning might serve as a skill that supports speech recognition in various adverse conditions. In CI users, the ability to rapidly adjust to on-going acoustical challenges is one of the factors associated with good CI outcomes. Overall, CI recipients with better working memory and attention and with faster learning rates had better speech recognition.

Podium #35 - Vestibular Periphery: From Molecules to Neurons

10:30 a.m. - 12:30 p.m.
Oceans Ballroom 1-4

Moderators: Anna Lysakowski and Larry Hoffman

Six2 Regulates Morphogenesis and Planar Cell Polarity (PCP) of the Vestibular Epithelia

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Background: The transcription factor Sine Oculis Homeobox Homolog (Six)2 has been shown to be critical for development and patterning of a number of tissues including heart, kidney, and eyes. Recent RNA-seq data suggest that Six2 is widely expressed in the embryonic and early postnatal vestibular epithelium. However, little is known about its function in this context. In this study we sought to validate Six2 expression in the developing otic vesicle by in-situ hybridization and investigate its function using a Six2 germline deletion mouse model.
Methods: We performed RNAScope in-situ hybridization in cryosections of CD-1 timed mating embryos at embryonic days (E) 10.5, 12.5, 14.5, 17.5 and at postnatal day 0 (P0). The samples were co-immunolabelled with Sox2, HuD, NF-H and Myosin7A to identify prosensory progenitors, supporting cells, neurons and type I and type II hair cells. To investigate the role of Six2 in inner ear morphogenesis, we labelled whole-mounted vestibular epithelia for Myo7a, Sox2, Ki67, phalloidin and espin. Whole mounted utricles and cristae were also immunostained for oncomodulin (OCM) and Myo7A to determine the effect of Six2 deletion on striolar area, and for beta spectrin to determine hair bundle orientation. Six2 homozygous knockouts (Six2-/-) and wild type (Six2+/+) littermates were collected and compared at E17.5. The angles of hair bundle orientation with respect to reference frame, were measured by Image J and rose diagrams were

generated using Oriana software. The samples were also labelled with VANGL2 and Gαi3 to investigate the localization and distribution of core and intracellular PCP proteins.
Results: Our data suggests that Six2 is expressed earlier than hair cell differentiation, at least as early as E10.5 in both dorsal and ventral regions of the otic vesicle. At E12.5, Six2 is expressed in both sensory and non-sensory cells and expression in both sensory and nonsensory regions persists throughout development. In the cochlea, Six2 becomes enriched in the outer hair cells perinatally, whereas in the utricle it is expressed in both type I and type II sensory hair cells. Germline deletion of Six2 increases the overall area of utricular macula and the number of immature hair cells in the lateral extrastriolar region. Consistent with changes in overall area, the OCM positive striola areas were also increased, however the numbers of OCM+ cells were reduced. Additionally, Six2 deletion also results in misorientation of hair bundles in the utricle of homozygous knockout animals compared to the control littermate, thus affecting planar cell polarity.
Conclusions: Altogether, our data suggest that Six2 is required for proper development of the inner ear sensory epithelia. Future directions are aimed at uncovering the mechanisms of vestibular phenotypes elicited by Six2 deletion and further characterization of the role for Six2 in the sensory and nonsensory cells of the auditory system

The C-Terminus of Met Component Tmie Contributes to High Frequency Sensitivity of the Vestibular System

Peng Sun*[1], Teresa Nicolson[2]
[1]Stanford University School of Medicine, [2]Stanford University
Background: Mechanotransduction in sensory hair cells requires several membrane components including Transmembrane inner ear protein (Tmie). Recent evidence has shown that Tmie interacts with the Transmembrane Channel like subunits Tmc1 and Tmc2 of the complex in several species and peptides of the positively charged C-terminal domain of Tmie can bind to phospholipids, particularly PIP2. Deletions within this intracellular domain decrease the targeting of Tmie to the site of mechanotransduction in zebrafish and cause the attenuation of mechanotransduction currents in mouse cochlear explants. The in vivo consequences of C-terminal deletions in Tmie have not been fully explored.
Methods: Transcripts for tmie were detected at higher levels in the striolar region of the zebrafish larval utricle. As this region is predicted to be more sensitive to high frequency stimuli, we developed a simple, noninvasive method to assess eye movements in zebrafish larvae in response to high frequencies. We based our assay on previous reports demonstrating that bone conducted vibration or air-conducted sounds at 500 Hz selectively activate central zone afferents. Our customized device was constructed to measure the eye movements of zebrafish larvae in a dorsal up position induced by a controlled vertical vibration. In response to either pulse or step stimuli, we observed that the eyes rapidly rotated forwards about a right-left axis, and more slowly rolled backwards after reaching the maximum rotation. We tracked rotation of the eye by measuring the movement of centroids marking individual pigment cells within the outer layer of the retina using a custom script and plotted responses to various frequencies and intensities.
Results: Testing frequencies between 50 and 2000 Hz, we found that wild-type larvae displayed eye movements that were most sensitive to 300 Hz with a threshold of 0.02 g, which is similar to the sensitivity and threshold reported for responses to bone conducted vibration in mammals. Mutants expressing either a null allele of tmie or otogelin had dramatically attenuated responses. To assess the role of the C terminus of Tmie in high frequency sensitivity, we characterized the effects of a weak allele, tmiet26171, that encodes a C-terminal deletion in Tmie. Although latency of the response was unaffected, tmiet26171 larvae exhibited decreased sensitivity at all frequencies tested, with the strongest defects seen at frequencies above 750 Hz.
Conclusions: Our results demonstrate that high frequency vestibular responses are a conserved feature among vertebrates and are mediated by the utricle with negligible input from the saccule in zebrafish larvae. The data obtained with tmiet26171 larvae suggest that binding of the positively charged C-terminus of Tmie to PIP2 is important for robust sensitivity of vestibular hair cells.

Understanding How Efferent Signaling Impacts Spike-Timing Patterns of Vestibular Neurons by Modulating Multiple Types of Ion Channels

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Background: The vestibular system encodes a wide range of head movements through afferents that are defined by their spike-timing, which ranges from highly irregularly spaced intervals to highly regular intervals. Variability in afferent spike-timing reflects, in part, diversity in the expression of ion channels such as low-voltage activated potassium channels (Kv1 and KCNQ) and hyperpolarization-activated cyclic nucleotide gated (HCN) channels. We recently showed that both KCNQ and HCN channels in vestibular ganglion neurons are sensitive to efferent modulation via muscarinic acetylcholine receptor pathways. Activating muscarinic receptors enhances the activation of HCN channels and closes the low-voltage gated potassium channel (KCNQ). Although vestibular efferents have recently been shown to modulate spike rate, we hypothesize that by modulating both groups of ion channels, vestibular efferent signaling may also be important for modulating spike-timing regularity.
Methods: To test this hypothesis, we injected currents and measured firing patterns from vestibular ganglion neurons before and after administering the muscarinic acetylcholine receptor agonist Oxo-M. Disassociated neurons were extracted from Long-Evans rat at post-natal day 15.
Results: Our preliminary results suggest that the effect of the muscarinic acetylcholine receptor agonist Oxo-M on spike-timing regularity is highly variable. Some vestibular ganglion neurons generate more spikes at increasingly regular intervals while other cells have firing patterns that remain largely unchanged despite observable changes in KCNQ and HCN channels. We hypothesize that efferents impact spike-timing regularity differently in cells depending on the ion channel composition of low-voltage gated potassium currents. To understand this possibility, we implemented a conductance-based model of vestibular ganglion neurons in which the proportions of KCNQ (efferent-sensitive), Kv1 (efferent-insensitive), and HCN channels are defined and subject to modulation by simulated efferent input. Model simulations combined with patch-clamping data suggest that spike number and spike-timing regularity will only increase in cells whose low-voltage-gated potassium current (IKL) is conducted almost entirely through KCNQ-channels, which are susceptible to closure by muscarinic acetylcholine receptors. In contrast, cells, in which IKL is a mixture of currents flowing through Kv1 and KCNQ channels, are less likely to experience dramatic, if any, changes in firing pattern.
Conclusions: This work demonstrates how the complex regulation of cell-intrinsic properties such as the composition of KCNQ and HCN channels may, in principle, impact the nature of efferent control over afferent firing patterns.

Stimulus-Response Coherence Represents a Measure of Response Fdelity Among Vestibular Afferent Neurons

Larry Hoffman*[1], Kevin Wright[1], Michael Paulin[2]
[1]Geffen School of Medicine at UCLA, [2]University of Otago
Background: The dynamic response characteristics of vestibular afferent neurons are generally characterized by measures of sensitivity and phase relative to an applied stimulus. However, these measures do not incorporate more contemporary views of sensory spike trains exhibiting intrinsic, or spontaneous, discharge. Metrics that incorporate the heterogeneity in spontaneous discharge may provide alternative perspectives of how this component of the spiketrain contributes to signal transmission along sensory pathways. An alternative approach involves measures of how well the response of an afferent reflects qualities of the input stimulus, relative to components of the spiketrain that are not associated with the stimulus. The present study was undertaken to explore the metric of stimulus-response coherence, how it is associated with conventional measures of afferent spontaneous and evoked discharge, and its relationship with various qualities of head movement stimuli.
Methods: Electrophysiologic recordings from individual semicircular canal afferent neurons were obtained from barbiturate-anesthetized adult chinchillas. Epochs of spontaneous discharge were recorded, from which estimates of interspike interval coefficient of variation (CV*) were computed. Response discharge during application of discrete sine and complex (band-limited Gaussian; BLG) rotational stimuli was also recorded. The response spiketrains were analyzed to determine sensitivity and phase. Stimulus•response coherence was computed following multitaper spectral analyses. Response parameter distributions were compared using the Kullback-Leibler divergence in a bootstrap resampling paradigm.
Results: Conventional measures of semicircular canal afferent response dynamics (i.e. sensitivity and phase at discrete sine stimulus frequencies) exhibit broad heterogeneities, which were positively associated with spontaneous discharge CV. Stimulus-response coherence at 1.6Hz (peak velocity: 15 or 30°/s) ranged 0.30 – 0.99 (median=0.94), though associations with sensitivity or CV* were not found. Rather, distributions of

coherence measures were similar for 2 afferent groups parsed by CV* <0.1 or ≥0.1. BLG stimuli with intensity of 4°/s rms evoked responses exhibiting coherence values (median=0.57) that were lower than response coherence evoked by BLG stimuli with intensity of 8°/s rms (median=0.82; p=0.02). These results suggest a tendency for afferents to exhibit greater response fidelity with modest increases in complex "naturalistic" stimulus magnitude. BLG stimuli of 8°/s rms were associated with coherence values among low-CV afferents that were similar to those of high CV afferents (p=0.57). This indicates that some low-CV* afferents exhibit high-fidelity responses to higher stimulus amplitudes similar to high-CV* afferents.
Conclusions: Coherence represents an important metric of response fidelity relative to stimulus features. The present results indicate that afferent response fidelity was enhanced with modest increases in stimulus magnitude. Additionally, the results indicated that response coherence of low-CV* afferents is not compromised by their lower sensitivities, demonstrating their capability for conveying high-fidelity information about naturalistic head movements.

Loss of Pou4f3 From Adult Vestibular Hair Cells Leads to Reduced Vestibular Function and Hair Cell Death

Kendra Stansak*[1], Betty Chen[2], Tianwen Chen[1], Tian Wang[3], Caroline Nall[1], Nnenna Ezeilo[2], Kaley Graves[4], Tierah Macon[1], Wu Zhou[5], Hong Zhu[5], Alan Cheng[3], Brandon Cox[2], Bradley Walters[5]
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Background: The vestibular sensory epithelia of the inner ear contribute to balance, proprioception, and motion. In humans, age-related vestibular dysfunction represents a major health issue that affects much of the population and is the leading cause of fatal falls among aged individuals. Despite the prevalence of vestibular dysfunction and its impact on mortality and morbidity, the causes of age-related vestibular decline are poorly understood. The transcription factor Pou4f3 plays a critical role in the development and innervation of inner ear hair cells, and POU4F3 mutations are known to cause hearing loss. However, significantly less is known about Pou4f3's role in vestibular function, particularly whether it is necessary for maintenance of vestibular hair cell health during adulthood and aging.
Methods: To test whether POU4F3 changes during aging, vestibular end organs from mice and from human donors were immunolabeled with antibodies against POU4F3, SOX2, and MYO7A. To test whether Pou4f3 is necessary for adult vestibular hair cell survival and function, we conditionally deleted Pou4f3 from ~50% of type II hair cells in male and female mice at 8 weeks of age using an Atoh1-CreER:Pou4f3loxP/loxP mouse model. Mice were trained and tested on rotarod prior to tamoxifen induction. Six weeks post-induction, mice were again tested on rotarod performance, then vestibuloocular reflexes (VOR) were recorded. Tissues were collected and hair cell survival and POU4F3 expression were examined in utricles and horizontal cristae.
Results: In human utricles, a number of MYO7A+ vestibular hair cells lacked POU4F3 immunoreactivity at all ages investigated. Quantification of POU4F3-; MYO7A+ hair cells across age in wildtype mice suggested moderate loss of POU4F3 from hair cells with age. VOR assessments showed that conditional deletion of Pou4f3 led to decreased gain and increased phase during rotational acceleration, while linear VORs were largely unaffected. In rotarod experiments, conditional deletion of Pou4f3 caused reduced performance as compared to wild-type controls. Phalloidin labeling and MYO7A immunolabeling revealed decreased numbers of stereociliary bundles and hair cells in both the central and peripheral zones of the horizontal cristae. Consistent with the preferential activity of Atoh1-CreER in type II hair cells, cell death in the cristae was most notable for SOX2 and MYO7A double positive hair cells, however, there did not appear to be significant type II hair cell death in the utricles despite confirmed loss of POU4F3 immunoreactivity.
Conclusions: The data suggest that Pou4f3 plays a role in vestibular hair cell survival in adult mammals, and that loss of Pou4f3 from type II hair cells can lead to bundle degeneration and impaired survival in the cristae. Studies are ongoing to examine potential sex differences and to test whether Pou4f3 is necessary for type I hair cell survival.

Bulk and Single-Cell Transcriptome Reveal the Cellular Heterogeneity and Damage Response of the Adult Human Utricle

Emilia Luca*[1], Neke Ibeh[2], Ryosuke Yamamoto[1], Dallas Bennett[1], Vincent Lin[3], Joseph Chen[3], Michael Lovett[4], Alain Dabdoub[5]
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Background: The utricle, an inner ear balance organ, is comprised of a mosaic of cells that can be divided into two major types: sensory hair cells and non-sensory supporting cells. Hair cells are responsible for detecting movement and supporting cells for maintaining homeostatic functions. Loss of hair cells due to aging, genetic factors, infections, and ototoxic drugs, leads to permanent balance deficits affecting millions of people worldwide. Since supporting cells survive after damage, they represent an excellent target for endogenous regeneration. However, there is no information about their heterogeneity and damage response in humans.
Methods: We used single-cell RNA-sequencing (scRNA-seq) to examine hair cell and supporting cell heterogeneity in utricles from vestibular schwannoma patients. We also used a gentamicin hair cell damage paradigm to assess human utricle's genes and transcription factors involved in the damage and potential regenerative response. We damaged, in culture, utricles from the same cohort of patients and evaluated the early response to injury, as this might represent a critical time window to set the stage for hair cell regeneration. Therefore, after 24 h, we isolated the RNA from the sensory epithelia and performed bulk and scRNA-seq in control and gentamicin-treated samples. Following bioinformatic analyses, we validated candidate genes via RNAscope.
Results: We identified six putative types of supporting cells for the first time, including novel supporting cell-like cells marked by ANKRD1 and CRYAB. Characterizing the different types of supporting cells is a first step towards identifying which subtype(s) represent 'stem' cells for hair cell regeneration. Bioinformatic analyses of the bulk dataset identified 47 genes with significant transcriptional changes between control and damage conditions highlighting an immediate cellular response of the adult sensory epithelium to damage. In addition, we identified three transcription factors among up-regulated genes: JUN, TCERG1, and the deafness gene COCH. We also profiled gene expression changes at higher resolution, performing bioinformatic analyses on scRNA-seq datasets from control and gentamicin-treated samples. We identified 365 differentially expressed genes, including five transcription factors. Among the down-regulated transcription factors, we found NFIB known to be a regulator of cell proliferation, differentiation and promoter of cell survival.
Conclusions: We successfully performed bulk and scRNA-seq on adult human utricles, discovered human-specific genes, including novel hair cell genes like SCGB2A1, and identified the earliest response to gentamicin damage. This data suggests that the adult utricle has the capacity to initiate a regenerative response. Overall, these discoveries will advance fundamental knowledge in inner ear regenerative medicine and pave the way for developing therapeutics to treat balance dysfunction.

Single Cell RNA-Seq of Labyrinthine Tissue Identifies Innate and Adaptive Immune Mechanisms in Meniere's Disease

Umberto Donato[1], Daniela Renado[2], Nofrat Schwartz[3], Doug Hildrew[3], Joseph Santos-Sacchi[4], Jennifer Molitano-Gunel[2], John Kveton[3], Dhasakumar Navaratnam*[5]
[1]University of Southern Florida, [2]Department of Neurosurgery, Yale School of Medicine, [3]Department of Surgery, Division of Otolaryngology, Yale School of Medicine, [4]Yale University School of Medicine, Surgery, Neuroscience, Cellular and Molecular Physiology, [5]Yale University School of Medicine
Background: Meniere's disease is a significant cause of morbidity affecting older individuals in particular. Common manifestations of the disease include dizziness, tinnitus ear fullness and hearing loss. The cause of the disease has remained elusive with disordered fluid balance in the labyrinth and autoimmunity being the chief hypothesized etiologies. Importantly, therapeutic options are limited to steroids and diuretics.
Methods: Single cell RNA sequencing of labyrinthine tissue of patients with severe Mennieres disease was performed using the 10X genomics platform and compared to labyrinthine tissue from patients undergoing resection of Schwannomas. Data was analyzed using a number of R and Python based suites and algorithms.

Results: In this preliminary work, we uncovered a surprising enrichment and diversity of leukocyte populations within the labyrinth including members of the innate and adaptive immune system. These include macrophages, T cells, B cells and natural killer (NK) cells. We determined that the cellular constituents and their transcriptional signatures differed when compared to labyrinthine tissue obtained from patients undergoing removal of schwanomas of the 8th nerve (controls). For example, we found that labyrinthine tissue from patients with MD contained NK cells that was conspicuously absent in patients with schwanomas. Moreover, we see clear differences in populations and signaling of monocyte-macrophages, endothelial cells, CD4 and CD8 T cells, and in B cells in MD labyrinthine tissue. Pathway analysis of differentially expressed genes in patients with MD suggest a number of common pathways and drug targets in different cell populations, many of which presently are in clinical use for other diseases.
Conclusions: Our data indicate that both innate and adaptive immunity plays a significant role in Meniere's disease. The discovery of a number of potential drug targets suggest potential treatments for this disabling disorder with limited therapeutic options at present. These data will require further validation in a larger cohort of patients.

Symposium #36 - The Eyes as a Window to Auditory Processing and Perception

10:30 a.m. - 12:30 p.m.
Crystal Ballroom D-E

The Eyes as a Window to Auditory Processing and Perception

Maria Chait, *UCL Ear Institute*

Over the last decade, Pupillometry (the measurement of pupil responsiveness and size) has attracted considerable attention in hearing research - both in the context of basic investigation into hearing function, and as a non-invasive, cheap and portable means for assessing listening challenges in patient populations.

Capitalizing on the well-known link between pupil responses and the brain networks that control vigilance and arousal, 'classic' work has focused on using pupil measures to quantify listening effort. However, recent developments in technology and understanding of the neural circuitry that controls pupil responses have prompted the expansion of pupillometry to multiple different domains of hearing research.

This symposium will highlight the range of questions that are currently being pursued with this technology. It will include work in both human and animal models, from 'low-level' effects of arousal on neural responses in auditory cortex to 'global' effects linked to perception and attention.

Presentations will span the domains of listening effort, learning, distractability and auditory scene analysis. We will discuss the relation between pupil-linked arousal and neural excitability as measured via electrophysiology in animal models; how pupil dilation responses can be used as a metric of auditory detection, discrimination and distraction in animals and humans; how tracking pupil dynamics reveals listeners' sensitivity to the statistical structure of rapidly unfolding auditory signals; and how pupil responses provide unique insight into the factors that affect listening effort, speech communication and learning.

This symposium touches on key issues in systems and cognitive neuroscience and audiology and should therefore be of interest to the broad ARO community. We expect to attract those who are already using pupillometry in their work, and those who might be interested to add it to their toolkit.

** this is a re-submission (with some modification; 2 new presenters) of a symposium previously scheduled to take place during ARO2022. Unfortunately, we had to withdraw due to covid-induced impact on the availability of a large proportion of the original presenters.

What Can Pupillometry Tell Us About Voice Perception
Thomas Koelewijn, *University of Groningen, University Medical Center Groningen, Department of Otorhinolaryngology/Head and Neck Surgery*

Speech perception in multiple-talker listening conditions can be challenging and effortful especially in people with hearing impairment. Perceiving differences in voice cues like fundamental frequency (F0) and vocal-tract length (VTL) can help listeners segregate competing talkers, which improves speech understanding. Research showed that cochlear implant (CI) hearing and vocoding (simulating CI-hearing) reduce sensitivity to F0 and VTL voice cues, potentially contributing to difficulties in understanding speech in adverse listening conditions. Pupillometry has shown to be an objective measure for cognitive processing load in adverse listening conditions, also referred to as listening effort. Using a variety of listening tasks, studies have shown different types of speech degradation, by using different types of maskers (e.g., noise vs. speech) or vocoding, to affect the pupil dilation response. It is relatively unknown how voice perception processes, that make use of voice cue information, affect listening effort in adverse (e.g., vocoded and/or masked by speech) listening situations. This presentation will focus on the effect of voice discriminability on listening effort. In the presented studies, F0 and VTL voice cues were systematically manipulated while participants performed voice cue discrimination tasks (CVC-triplets) or speech-on-speech listening tasks (sentences), while stimuli were either clear or vocoded. The impact of voice training and vocoding on listening effort during voice cue discrimination and speech-on-speech listening was investigated by means of pupillometry. Our main hypothesis was that improvement in voice cue discrimination would lower listening effort. Together with performance outcomes, conventional Peak Pupil Dilation outcomes as well as more advanced time-based GAMM analysis outcomes will be discussed. These outcomes provide insight on the impact that voice discriminability and voice familiarity have on listening effort in normal and CI-listening.

Pupil Dynamics Underlying the Subjective Experiences of Effort and Tiredness From Listening
Ronan McGarrigle, *University of Bradford*

Pupillometry has recently emerged as a potential tool for estimating the mental effort associated with listening in adverse conditions. However, the absence of associations in the literature between the task-evoked pupil response (TEPR) and perceived effort cast some doubt over this interpretation. We present findings from two experiments showing that changes over time in TEPR during a taxing speech recognition task are associated with subjective tiredness, but not effort, ratings. These findings suggest that pupillometry is sensitive not just to varying levels of acoustic or attentional demand, but also to changes in perceived tiredness from listening that unfold over time.

Interactions Between Pre-Stimulus and Stimulus-Evoked Pupil Dilation Indices of Listening Engagement
Stefanie Kuchinsky, *Walter Reed National Military Medical Center*

Understanding speech in adverse conditions often requires substantial effort not only to listen to auditory stimuli. Listeners must also prepare and maintain the mental processes necessary to carry out listening task goals within and across trials, often termed a “task set.” With greater time-on-task, it can become particularly challenging to sustain task-set and stimulus-evoked mental processes, potentially leading to poorer performance and fatigue.

Changes in pupil dilation within pre-stimulus (baseline) and stimulus-evoked (time-locked to stimulus onset) epochs have been used to index the mental resources engaged by task-set preparation and stimulus processing, respectively, due to their close relationship with tonic and phasic activity in the locus coeruleus norepinephrine (LC-NE) system. Studies of listening effort have historically focused on the latter: examining the engagement of cognitive resources for processing an auditory stimulus. However, studies have also observed that pre-stimulus pupil size (PSPS) is sensitive to the difficulty of preparing for an upcoming listening trial and can be impacted by time spent performing a task.

In this talk, I will describe two studies with younger, normal-hearing listeners that investigated interactions between baseline PSPS and the stimulus-evoked pupil response within and across listening trials of varying lengths. In a study of Mandarin lexical tone learning, we observed significant reductions in pupil size across the trials of the experiment (indicative of word learning). Trials on which PSPS was larger were associated with even smaller word-evoked pupil responses. In a separate study that involved listening to 60-second stories, story-evoked pupil responses decreased over time within each story and across repetitions of each

story within a block. PSPS reflected expectations about the upcoming signal-to-noise ratio (SNR) difficulty, which was observed to mediate SNR effects during the subsequent story-evoked pupil response.

Together, these studies demonstrate the importance of characterizing listening engagement across moment-to-moment and trial-by-trial changes in the processes that support speech processing and task-set maintenance. I will discuss my ongoing translational work that aims to collect pupillary measures of listening engagement via a head mounted display in the audiology clinic.

Disclaimer: The views expressed in this abstract are those of the author and do not reflect the official policy of the Department of Army/ Navy/Air Force, Department of Defense, or U.S. Government.

Eyes Have Ears: Using Pupillometry to Index Attentional Capture by Irrelevant Sound
Francois Vachon, *Université Laval*

The presence of task-irrelevant sound is known to impede cognitive functioning. For instance, the occurrence of unexpected irregularities in the auditory background tends to capture attention, hence disrupting performance. There is growing evidence that violations of acoustic regularities can also trigger a pupil dilation response (PDR). Here, we propose a systematic assessment of the PDR as a potential psychophysiological proxy for this form of distraction. Our strategy consisted in examining whether the PDR and attention capture share the same functional properties. Through a series of studies, we established that the PDR mimics an orienting response: the PDR showed a habituation/dishabituation pattern, was influenced by the size of the deviation but was not reliant on acoustic novelty, and was amenable to top-down cognitive control. Taken together, these findings indicate that the PDR to deviant sounds is a valid biomarker of attentional capture in the auditory domain.

Evoked Pupil Response to Investigate the Effect of Salient Distractors on Attentional Effort Allocation
Dorothea Wendt, *Eriksholm Research Centre*

Listeners with normal hearing can direct their attention towards a particular talker of interested and are usually able to ignore another talkers, which is referred to as top-down – or intentional attention. However, bottom-up or automatic attentional processes might capture the attentional focus through salient interruptions. The potential strength of a sound to capture attention is referred to as auditory saliency. In particular people with hearing impairment frequently report environmental sounds to be disturbing and annoying especially when they are of a transient type. This talk will present findings of ongoing investigations about the impact of salient sounds (distractors) on the attentional resource allocation during speech understanding. Specifically, we were interested in the impact of hearing impairment as well as the timing of a distractor (i.e., when distractors is presented before, while or after the target speech) on attentional resource allocation using pupillometry. We hypothesized that the distractibility can be measured from the distractor-evoked pupil response, which suggests a disruption of intentional attention and the effort to restore attention to the target. It is hypothesized that hearing impairment increases this distractibility and that the timing of the distractors affects the degree of distractibility as indicated by the evoked-pupil response. The presented results will help to better understand the impact of distractors on attentional effort during speech processing. This may help to develop experimental paradigms that go beyond the question of how well a target stimulus can be attended, by asking how well a distracting stimulus can be ignored.

Pupillometry as a Reliable Metric of Auditory Detection and Discrimination in Animal Models
Srivatsun Sadagopan, *University of Pittsburgh*

Pupillometry shows great promise as a non-invasive method of measuring auditory thresholds in animal models. Previous studies have largely used simple stimuli such as pure tones. Here, in a guinea pig animal model, we used pupillometry in the context of an auditory oddball paradigm to estimate detection and discrimination thresholds to stimuli spanning a range of complexities (tones, vocalizations and tone clouds), across different stimulus contingencies (acoustic changes and categorical changes), and with or without reinforcement. To do so, we first obtained pupil dilation responses to a range of oddball stimuli that parametrically differed from standard stimuli. We then used growth curve analysis to fit these responses and evaluate whether oddball responses significantly differed from standard stimuli. Using this technique, we

could obtain pupillometric discrimination thresholds across the wide variety of stimuli described above, which allowed us to characterize basic features of auditory detection and discrimination in guinea pigs. We further ascertained that pupillometric categorization-in-noise thresholds broadly agreed with thresholds obtained using operant behavioral training, underscoring the robustness of using the pupil dilation response as a metric of auditory detection and discrimination. In ongoing work, we are using pupillometry to probe some complex auditory deficits in a noise-induced temporary threshold shift model. These results lay the foundation for using pupillometry as a reliable method for comparing broad similarities between human and animal auditory processing.

Impact of Pupil-Indexed Arousal on Correlated Variability and Sound Representation in Neural Populations

Stephen David, *Oregon Health and Science University*

Correlated variability within neural populations, sometimes called noise correlation, substantially impacts the accuracy with which information about sensory stimuli can be extracted from neural activity. Previous studies have shown that changes in behavioral state, reflecting phenomena such as attention and/or arousal, can change correlated variability. However, the degree to which these changes impact neural encoding of sensory information remains poorly understood, particularly in the auditory system. To study this problem, we used linear arrays to record populations of single neurons in ferret auditory cortex while monitoring arousal via pupillometry. Spontaneous increases in arousal tended to decrease the overall degree of correlation. However, the decreased correlation did not consistently improve the accuracy of neural coding. Instead, changes in correlation occurred in a low-dimensional population subspace, and the alignment this space with sound-evoked responses determined its effect on sensory coding. These results establish a clear link between behavioral state and correlated neuronal variability. However, the changes appear to reflect processes that are not simply related to the accuracy of sensory coding.

Sustained Pupil Responses Track the Statistics of Rapidly Unfolding Sounds

Maria Chait, *UCL Ear Institute*

The auditory system continuously analyses rapidly unfolding probabilistic information, even when this information is not immediately relevant to behaviour. Accumulating work has demonstrated that sensitivity to auditory regularities plays an important role in auditory scene analysis, speech perception and attention. What sound statistics does the auditory system automatically monitor?

Non-luminance mediated changes in pupil size index changes in instantaneous arousal. It has been suggested that these responses reflect the amount of processing resources drawn by a given task or stimulus and that stimulus uncertainty (which is associated with a draw on processing capacity) can therefore be manifested in pupillary dynamics.

I will present a series of studies in which we investigate pupil responses to statistically shaped auditory signals. Naïve participants performed an incidental task that did not require monitoring sequence structure. The stimulus sequences were rapid, preventing conscious tracking of sequence statistics thus allowing us to focus on the automatic tracking of different types of regularities, and transitions between them. We ask (1) how the fast-paced and automatic mechanisms that detect changes in statistics within rapid sensory signals interface with pupil-linked arousal, (2) how pupil responses compare to other aspects of brain dynamics (EEG measures).

We demonstrate that, broadly, pupil responses show reduced sustained amplitude for predictable relative to unpredictable auditory patterns, consistent with the notion that regularity facilitates processing by reducing processing demands. However the specific pattern of pupil size modulation (pupil size decrease, relative to unstructured sequences) as a function of sequence statistics suggests a difference between EEG and pupillometry. EEG responses track the precision (predictability) of unfolding sequences. In contrast, the pattern of pupil responses is more consistent with monitoring for environmental change probability. The implications of these findings to our understanding of how the brain monitors for environmental statistics will be discussed.

SATURDAY, FEBRUARY 11, 2023
POSTER SESSION 1

SA1. Open Board

SA2. The Impact of Age-Related Cochlear Synaptopathy on Electrophysiological Recordings and Speech Recognition Performance-Intensity Functions

Bruna Mussoi*[1], Caitlyn Menolasino[1], Kaitlyn Mazzola[1]
[1]Kent State University

Category: Aging

Background: While the mechanisms underlying age-related changes in speech recognition are thought to be multifactorial, cochlear synaptopathy has been proposed as a possible peripheral auditory contributor. Although the evidence from animal studies supporting the presence of cochlear synaptopathy appears compelling, findings from prospective studies in human listeners have been less conclusive. In this study, we sought to better characterize the effect of aging on speech understanding, by obtaining performance-intensity functions in quiet and in noise and examining their association with auditory brainstem responses evoked by high level stimuli.

Methods: Fifteen younger (19-36 years) and fifteen older participants (65-81 years) with normal hearing through 4 kHz took part in this study. Distortion product otoacoustic emissions were obtained at f2 frequencies ranging from 2000 to 8000 Hz, with participants largely meeting criteria for normal outer hair cell function. NU-6 word lists were used to obtain performance-intensity functions in quiet at six levels ranging from 10 to 90 dB HL in quiet, and in the presence of multitalker babble, at six signal-to-noise ratios ranging from -5 to +20 dB SNR (noise fixed at 50 dB HL). Click-evoked ABRs were obtained at 116 peSPL at two presentation rates, 11.3 and 41.3 clicks / second. Tiptrodes were used as the reference electrode. All testing was administered through insert earphones to the right ear.

Results: Preliminary analyses indicate that ABR V/I ratios were significantly larger and wave I and V latencies were significantly longer in older adults, when compared to their younger counterparts. NU-6 scores on performance-intensity functions in quiet and in noise did not differ across the age groups after controlling for hearing thresholds. Finally, ABR V/I ratios were not associated with the slopes of performance-intensity functions or with estimated sound levels (dB HL, dB SNR) corresponding to 50% correct speech recognition.

Conclusions: The electrophysiological findings in this study are consistent with an interpretation of cochlear synaptopathy in older adults. However, ABR measures were not associated with speech recognition abilities – even when full performance-intensity functions were obtained, using low-context monosyllabic words that depend more heavily on peripheral processing. Thus, cochlear synaptopathy did not appear to contribute to speech recognition performance in quiet or in noise. It is possible that the clinical manifestation of cochlear synaptopathy in humans differs from that of animal models.

SA3. Frequency-Dependent Distribution of Different Subtypes of Spiral Ganglion Neurons in the Cochlea and the Changes During Aging

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Category: Aging

Background: Sound information is transmitted from the cochlea to the brain by type I spiral ganglion neurons (SGNs), which can be further classified into three subtypes based on their physiological properties and selective expression of molecular markers. It remains unclear how the distribution of these subtypes varies across different frequency regions in the cochlea, and whether this distribution changes during aging. We investigated these questions using reported molecular markers, including calretinin (CR), calbindin (CB), and TuJ-1, which are selectively expressed in subtype Ia, Ib, and all type I SGNs, respectively.

Methods: CBA/CaJ mice of either sex were used in three age groups, including 2-5 months (young), 17-19 months (middle age), and 28-32 months (aged). Mouse cochleae were cryo-sectioned and immunostained against CR, CB, and TuJ-1 following standard protocols. SGNs were classified into four groups based on the expression pattern of the stained markers, including CR+ (type Ia), CB+ (type Ib), CR-CB- (type Ic), and CR+CB+ (overlapped Ia/Ib) neurons. The distribution of these SGN groups was analyzed across different frequency regions at the base, middle, and apex of the cochlea.

Results: We found a gradient distribution of type Ia and Ib SGNs along the frequency axis, in which both have relatively low prevalence at the base and high prevalence at the apex. In contrast, the gradient was the opposite for type Ic SGNs. Such frequency-dependent distribution was largely maintained during aging, except a significant reduction of type Ic SGNs was observed at the base.
Conclusions: The findings suggest that three subtypes of type I SGNs contribute differently in processing sounds at different frequencies. During aging, the prevalence of type Ic SGNs is remarkably reduced at the high-frequency region, which may uniquely contribute to age-related high-frequency hearing loss.

SA4. Impacts of Aging on Binaural and Spatial Auditory Functioning

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Category: Aging
Background: Aging is often accompanied by a decrease in peripheral hearing sensitivity caused by loss of hair cells. However, older adults may also experience central processing deficits. These listeners have, even with a normal audiogram, difficulties in listening situations where there are multiple active sound sources and/or background noise, e.g., a busy restaurant or cocktail party. We hypothesize that these suprathreshold sound processing deficits may be partly related to decreased temporal precision of neural activity in the sound localization pathway, and/or other central auditory system deficits involving processing more complex stimuli such as speech. These alterations are in addition to changes in cognitive abilities with age, which may further decrease performance in adverse listening conditions.
Methods: We are conducting a large, combined human-subjects and animal-model study examining the impact of aging on auditory functioning, and binaural processing in particular. Inclusion criteria include human subjects ranging in age from 21 to 89 years, all with normal hearing thresholds or only a mild sensorineural loss for frequencies from 250 to 4000 Hz inclusively. Excluded are neurodegenerative diseases, non-native English speakers, and those showing significant cognitive deficits upon screening. A variety of assessments measuring auditory system integrity and behavioral performance are being evaluated across multiple sessions. Behavioral outcome measures include adaptive tests of speech-in-noise understanding and spatial acuity in a hemi-anechoic sound chamber, temporal fine structure (TFS) and spectro-temporal modulation (STM) sensitivity tests under headphones, measurements of extended-high-frequency (EHF) hearing thresholds to 16 kHz, and a working memory assessment. Physiological measures include auditory brainstem responses (ABRs) and the calculated binaural interaction component (BIC) with a midline electrode montage, electrocochleography, and otoacoustic emissions. In addition, subjective questionnaires of hearing handicap and noise exposure are completed. Presented in this poster are selected preliminary human subject findings from this ongoing study.
Results: Early results indicate significant relationships between age and several measurements including working memory capacity, speech understanding in noise, spatial acuity, and TFS and STM sensitivity. Greatest aging effects are seen in the more adverse listening conditions such as decreased signal-to-noise ratios for speech-in-noise and spatial acuity tasks, and an off-axis listening condition for spatial acuity. Additionally, EHF hearing loss is more often observed with advanced age.
Conclusions: The results point to additional potential contributions to age-related loss in auditory functioning that are important to consider in a clinical setting. These may include central deficits in processing binaural stimuli, changes in cognitive function, and hearing loss in the EHF range. Future analyses will examine interactions between the various measures, and examination of electrophysiologic data to determine if there are significant differences in peak latencies and amplitudes, or in presence of a measurable BIC, across ages. Support: NIH-NIDCD R01 DC017924 (PIs: Tollin and Klug)

SA5. Rapamycin Added to Diet Delays Noise-Induced Acceleration of Age-Related Hearing Loss in UMHET4 Mice

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Category: Aging
Background: While effects of noise on age-related hearing loss (ARHL) had long been considered to be cumulative, a study by Fernandez et al. (2015) cast the influence of noise in a new light. A single noise exposure given to young mice, from which there was full recovery of auditory brain stem response (ABR)

thresholds, increased later occurring age-related hearing loss (ARHL) at 20 and 24 months of age compared to no-noise littermates. Our studies (Altschuler et al., 2022) found that a small arms fire (SAF)-like impulse noise, which caused only temporary ABR threshold shifts (TS), produced an even earlier acceleration of ARHL. Increased TS appeared by 12 months of age at low frequency (4 kHz), further increasing at 18 months. There was elevation / acceleration of ABR TS at high frequency (48 kHz) at 18, 21 and 24 months of age. The present study tested a treatment, if adding rapamycin to diet would reduce/delay impulse noise acceleration of ARHL. Rapamycin has many neuroprotective effects and we previously found rapamycin reduced / delayed ARHL (Altschuler et al, 2018, 2021).
Methods: Six months old male and female UMHET4 mice received baseline 4, 12, 24 and 48 kHz ABR measures. They then received unilateral impulse noise exposure (fifty biphasic impulses over 2.5 minutes at 160 dB SPL) and were randomly divided into a group getting rapamycin added to diet or a group with no rapamycin. ABR thresholds were re-tested 1 month later and only mice with full recovery of ABR thresholds remained in study. These mice were re-tested at 12, 18, 21 and 24 months of age as were age-matched no-noise controls on normal diet. Mice were then euthanized, and cochleae assessed for hair cell loss.
Results: The SAF-like impulse noise exposed mice without rapamycin treatment confirmed our previous results showing a significantly elevated (compared to no-noise controls) ABR TS at 4 kHz appearing at 12 months of age. Elevation of ABR TS at higher frequencies became significant at 18 months. The noise exposed mice with rapamycin treatment had significantly less elevation of 4 kHz ABR TS at 12 months of age compared to the noise exposed mice without rapamycin, even though elevation still occurred. At 48 kHz. rapamycin treated impulse noise mice had ABR TS close to that of no-noise, untreated controls at 18, 21 and 24 months of age, with significantly less TS than untreated impulse noise mice.
Conclusions: Results show that adding rapamycin to diet within a day after an impulse noise exposure significantly reduces but does not eliminate noise-induced acceleration of ARHL. It will be interesting to test if a later start of treatment retains efficacy

SA6. Increased Central Auditory Gain in 5xFAD Alzheimer's Disease Mice

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Category: Aging
Background: The Alzheimer's Disease (AD) is a neurodegenerative disease without any cure. All current therapies require accurate diagnosis and staging of AD to ensure proper strategy. The increasing prevalence of the disease has caused a great burden to the health care system worldwide. Thus, it has become an urgency to find efficient approaches for AD diagnosis and progression. Auditory dysfunction has been suggested as a promising biomarker for AD diagnosis. However, it remains unclear how auditory dysfunction and Alzheimer's disease progression are correlated.
Methods: In the present study, we compared the auditory dysfunctions in transgenic AD mouse models 5xFAD and APP/PS1. Those animals were bred in CBA/B6 hybrid background to compensate the hearing loss deficits in C57/B6 strain. We further performed longitudinal analysis of auditory dysfunctions in 5xFAD at different stages. The auditory features were monitored by Auditory Brainstem Response (ABR) tests with quiet background or noise, and Distortion Product Otoacoustic Emission (DPOAE) test.
Results: 5xFAD mice appear to show more severe auditory dysfunctions, in comparison with APP/PS1 mice. ABR waveform analysis revealed increased central gain in 5xFAD. Longitudinal analysis further showed that the increased central gain was manifested prior to hearing loss and wave I amplitude reduction, suggesting a central origin of this phenotype. Plaque distribution analysis showed significant deposition in central auditory structures, with time course coinciding with age-related central gain increase observed in 5xFAD. Donepezil treatment reversed the central gain increase, suggesting that cholinergic deficit is involved in this AD-related phenotype. Despite central gain, deficit for hearing in noise, which has been reported in AD patients, was also observed in 5xFAD.
Conclusions: With those results, we conclude that auditory dysfunction is a possible outcome of AD, and the ABR abnormalities could serve as a biomarker for AD progression. Overall, this study suggests auditory measurement, a low-cost, non-invasive test, as a possible approach for AD diagnosis and the estimation of disease stage.

SA7. Increased Expression of Group II MGluRs in the Inferior Colliculus, Medial Geniculate Body, and Auditory Cortex of Aged Compared to Young Adult Mice

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Category: Aging

Background: Ligands targeting metabotropic glutamate receptors (mGluRs) have a potential for clinical development in several psychiatric and neurological disorders. These receptors are expressed throughout the auditory system but knowledge regarding their distribution is limited.

MGluRs are classified into three groups: group I includes receptor subtypes 1 and 5, group II – subtypes 2 and 3, and group III – subtypes 4, 6, 7, and 8. The objective of this project is to characterize group II mGluR (mGluR2/3) expression in the mouse inferior colliculus (IC), medial geniculate body (MG), and auditory cortex (AC). This interest arises from our recent results showing that behavioral signs of tinnitus in mice were suppressed by intraperitoneal administration of mGluR2/3 agonist LY354740 (Galazyuk et al., 2019). Furthermore, LY354740 reduced spontaneous activity in the IC. On the other hand, local iontophoretic and topical mGluR2/3 pharmacological activation in the IC enhanced sound-evoked and spontaneous firing (Kristaponyte et al., 2020).

Work from other brain areas demonstrated that group II mGluR expression is age dependent. Thus, we compared mGluR2/3 expression between young adult and aged mice. We investigated receptor expression levels across four IC regions -- dorsal (ICd) and lateral (IClc) cortices, and dorsal and ventral parts of the central nucleus (ICcd and ICcv, respectively), three MG subdivisions -- dorsal (MGd), ventral (MGv), and medial (MGm), and three AC subregions -- primary auditory cortex (A1), dorsal area (AuD), and ventral area (AuV).

Methods: Brains from six young adult (84-85 days old) and six aged (20-21 months old) CBA/CaJ mice were used. Anti-mGluR2/3 antibody (Millipore AB1553) was labeled with a biotinylated anti-rabbit antibody and visualized via Ni-DAB reaction. Optical density was measured in images from each region, and neuropil-only or cell body-only optical density measures were collected by specifying an ROI within each image.

Results: mGluR2/3 staining varied within and between areas. In the IC, staining was darker dorsally than ventrally. In the MG, the darkest staining was present in the MGv, followed by MGd and MGm. In cerebral cortex, the general pattern was similar across areas (A1, AuD, AuV) with staining darker near the surface and diminishing toward white matter. Despite the variations across different auditory regions, every region tested showed stronger DAB staining, i.e., increased mGluR2/3 levels, in the aged animals.

Conclusions: Group II mGluR staining varies within and across auditory nuclei, suggesting that mGluR-mediated modulation is more prominent in some parts of the auditory midbrain, thalamus and cortex than in others. Significantly, receptor staining in each of these areas changes over the lifespan, with higher expression levels suggesting that mGluR2/3 modulation may play an increasingly important role as the auditory system ages.

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SA8. Transient and Sustained Evoked Potentials as Measures of Age-Related Cochlear Neuropathy in Humans: Evidence From ABR and FFR

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Category: Aging

Background: Healthy aging is thought to cause auditory-nerve (AN) degeneration and may be associated with auditory processing deficits beyond what is captured by the clinical audiogram. Although it is assumed that AN damage can lead to suprathreshold deficits, reliable and robust diagnostic measures are missing. The first wave of the auditory brainstem response (ABR wave-I) has been used both in animal studies and in human studies as a measure of peripheral neural integrity. However, wave-I of the scalp ABR relies on high-level stimulation and is often difficult to acquire within the limited time window offered in most clinical settings. It is known that the amplitude of the brainstem frequency-following responses (FFR) elicited by tones decreases with age. The source of this decrease may reflect upstream consequences of age-related cochlear neural degeneration. In recent modelling work, we proposed that age-related loss of AN fibers

leads to a reduction of synchronized population activity, but further experimental support for a peripheral source of age-related FFR reductions is missing.
Methods: In a large cohort of listeners (n=104), across a wide age-span (18 to 77 years of age), with normal to near-normal hearing thresholds (<= 20 dB HL up to 2 kHz), we measured suprathreshold (115 dB ppeSPL) click-evoked ABRs and FFRs to 250-ms low-frequency (326 Hz) tones presented at 85 dB SPL using scalp EEG electrodes and ear-canal tiptrodes. Additionally, we measured extended high-frequency audiometric thresholds (from 8 to 16 kHz), as well as the middle-ear muscle reflex, self-reported hearing status (SSQ12) and estimated life-span accumulated noise exposure. Cognitive performance was assessed using a reverse digit-span test.
Results: We observed correlated age-related reductions in pure-tone FFR and ABR wave-I amplitude in listeners with normal or near-normal audiometric thresholds. Later waves of the click-evoked ABR, in particular the brainstem generated ABR wave-V, did not show a similar age-related reduction.
Conclusions: In the present study, we provided evidence that the FFR to low-frequency tones is correlated both with age and ABR wave-I. It is known that age is correlated with progressive cochlear neural degeneration, and our results suggest that the FFR may be a potential biomarker of AN degeneration. Although we found correlations between wave-I amplitude and the FFR, other age-related effects are also likely to contribute to the observed decline in the FFR. In particular, the FFR may be sensitive to age-related declines in the precision of temporal coding of sustained periodic stimuli. More direct electrophysiological measures of temporal coding generated at the level of the cochlea are needed to further verify whether these effects are peripheral in origin.

SA9. Mental Health Before and During the COVID-19 Pandemic Among Older Adults With Disabling Hearing Loss in the United States

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Category: Aging
Background: When the World Health Organization declared the COVID-19 pandemic in March 2020, most individuals and communities in the U.S. were unprepared for this global public health emergency. Disruption of healthcare service delivery, social isolation, and financial hardship can increase the likelihood of experiencing anxiety and depression. The objective of this study was to examine mental health among older Americans with hearing impairment (HI) from 2019 to 2021, which represents the time before and during the COVID-19 pandemic.
Methods: We analyzed data from the National Health and Aging Trends Study (NHATS), a nationally representative survey of Medicare beneficiaries. Among the total of 3,753 older adults (aged from 70 to 90+ years; female 56.0%) who participated three rounds from 2019 to 2021, 2,680 completed pure-tone audiometry in 2021. The respondent was categorized as having disabling HI if the four-frequency pure-tone average (PTA) for the better ear was ≥ 35 dB HL. Depression and anxiety were assessed using the Patient Health Questionnaire for Depression and Generalized Anxiety Disorder Screener with a score of three being the criterion. Sampling weights were applied in statistical analyses to account for the complex survey design.
Results: The prevalence of disabling HI was 32.7% [95% confidence interval (CI): 30.2 - 35.3%], or 10.7 million older individuals in 2021. 24.4% of individuals with HI were hearing aid or cochlear implant users (95% CI: 21.2 - 27.5%).
In 2019, 10.5% (95% CI: 8.5 - 12.6%) of older adults with disabling HI had depression; in 2020, 13.2% (95% CI: 10.8 - 15.6%); and in 2021, 10.9% (95% CI: 8.6 - 13.1%). In 2019, 7.5% (95% CI: 5.6 - 9.4%) of older adults with disabling HI had anxiety; in 2020, 9.5% (95% CI: 7.2 - 11.8%); and in 2021, 9.6% (95% CI: 7.2 - 11.8%).
The prevalence ratio (PR), or relative risk, in 2019 was 30% greater (PR = 1.30, 95% CI: 1.12-1.51) for older adults with depression if an individual had disabling HI and 35% higher in 2020 (PR = 1.35, 95% CI: 1.17-1.54). The risk dropped to 17% higher in 2021 (PR = 1.17, 95% CI: 0.96-1.43). Older adults who had disabling HI were 23% more likely to have anxiety at the beginning of the pandemic (PR = 1.23, 95% CI: 1.02-1.48). However, this link between anxiety and HI was not statistically significant before the pandemic (PR = 1.18, 95% CI: 0.99-1.41) or during the second year of the pandemic (PR = 1.14, 95% CI: 0.91-1.42).

Conclusions: The findings suggest that the unexpected COVID-19 pandemic was associated with additional social and emotional stress for those with disabling HI, particularly during the onset of the pandemic, resulting in a higher level of depression and anxiety.

SA10. Neural Representation of Selective Auditory Attention in fMRI

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Category: Auditory Cortex and Thalamus: Human Studies

Background: Selective auditory attention, which allows a listener to actively focus on an acoustic target despite competing sounds, can be deployed to focus on either spatial or nonspatial sound features, recruiting different brain networks. While past studies have used representational similarity analysis (RSA) to explore how different stimulus features (categories) are represented in different brain regions of interest (ROIs), here we used RSA to understand how different ROIs reflect differences in executive function. Specifically, we explored representational patterns for selective auditory attention using functional magnetic resonance imaging (fMRI) collected while subjects focused different forms of attention on a target syllable presented amidst competing distractor syllables.

Methods: fMRI data from a rich (21 conditions) auditory experiment were collected from 19 listeners performing spatial attention, nonspatial attention, or passive listening. After pre-processing the fMRI data, a general linear model (GLM) was fitted to generate a whole-brain activation map for each subject on each trial. For each subject, we defined anatomical regions of interest (ROIs) using the Destrieux brain atlas, then used the spatial pattern of activation within each ROI as features for neural decoding. For each subject and each ROI, we trained support vector machines (SVMs) to classify each pair of conditions using leave-one-run-out cross-validation. We used SVM classification accuracies as the measure of dissimilarity of brain activity between condition pairs to construct representational dissimilarity matrices (RDMs). The RDM patterns represent the information about cognitive state consistently encoded in each brain region, generalized across subjects and the rich stimulus conditions. We built several conceptual ideal models, each representing a specific attentional state, to compare to the RDMs for each ROI.

Results: Information about attentional task appears across a bilateral set of brain regions including large portions of the frontal and parietal lobes and regions in superior temporal gyrus. Superior parietal lobule contained the strongest information for each of three separate contrasts that we tested: differentiating spatial vs. non-spatial attention and both attention types vs. passive listening. Within spatial attention conditions, calcarine sulcus most strongly encoded information about target direction (left or the right): within non-spatial conditions, inferior frontal sulcus and superior temporal gyrus encoded information about the target talker pitch (high or low).

Conclusions: A widely distributed brain network comprising regions in temporal, parietal, and frontal lobe carries information about brain states during auditory attention tasks. These results show that fMRI-based RSA can reveal neural patterns related to executive control processes (here, auditory selective attention).

SA11. Effect of Attentional Modulation on Cortical and Subcortical Responses to Competing Streams

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Category: Auditory Cortex and Thalamus: Human Studies

Background: Organisms often need to attend to one signal within a crowded acoustic scene. While attentional effects are well-documented in the auditory cortex, it is still unclear how the function of auditory subcortical nuclei is affected by attention. Here, electroencephalography (EEG) was collected while subjects attended to a melody in one pitch range while ignoring an interleaved, competing melody in a different pitch range. We measured whether and how attention modulates subcortical and cortical responses to identical stimuli.

Methods: To measure attentional effects on simultaneously recorded cortical and auditory brainstem responses, we adapted an established dual-stream selective auditory attention paradigm. Here, temporally interleaved higher- and lower-pitch melodies were created by concatenating pitch-evoking 'pseudotones' generated by convolving a periodic impulse train (low pitch: 40-56 Hz; high pitch: 64-96 Hz) with a brief tone pip at a carrier frequency (low: 3500 Hz; high: 4500 Hz). This results in streams that are perceptually

segregated by their pitch height and that do not interfere with one another's peripheral representation (as the 3500 Hz and 4500 Hz carriers are well separated in the cochlea). The stimuli were designed to allow us to measure the auditory brainstem response to each note in each stream.
Each trial's resulting interleaved melodies comprised six notes (3 low and 3 high), with a within-band presentation rate of 2Hz and an across-band presentation rate of 4Hz. Participants were instructed to attend to the high or low pitch melodies, and responded whenever they heard a repeated 3-note motif in the attended stream while we simultaneously measured EEG. We extracted both subcortical ABRs and cortical event-related potentials (ERPs) to each note, and average inter-trial phase coherence (ITPC) at 2Hz and 4Hz; behavioral performance was measured.
Results: We observed robust ABRs evoked by each tone pip, one per pitch period, within each note, especially waves I and V. Each note onset also evoked cortical activity; however, because the notes from the competing melodies are separated by only 250 ms, the ERPs from each note overlap. For this reason, we analyzed the inter-trial coherence magnitude and phase, which show peaks at 2 Hz and 4 Hz. Once we gather data from a full cohort of subjects, we will analyze the effects of attention by comparing responses when listeners attend to the high-and low-pitched melody, and compare ABRs evoked by low- and high-pitched tone pips.
Conclusions: By combining two previous paradigms, we are able to simultaneously evaluate effects of top-down attention on brainstem and cortical physiological responses. Specifically, by segregating brainstem responses both perceptually (in pitch) and in the cochlea (because of differences in the carrier frequencies used), this paradigm holds promise for addressing the question of whether top-down attention affects brainstem responses.

SA12. Aging and Noise Exposure Yield Different Patterns of Low- vs High-SR Compromise in Gerbil Auditory Nerve

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Category: Auditory Nerve
Background: Aging and noise exposure are two primary etiologies associated with declines in hearing function. Animal studies suggest that neural losses play a role in these functional declines and that outcomes may differ based on the constellation of fibers targeted. Relevant to our investigations of age and noise vulnerability of low- vs high-spontaneous rate (SR) auditory nerve fibers (ANF), we characterized cochlear dysfunction and underlying histopathology arising from these etiologies in Mongolian gerbils, a species with well-characterized fiber distributions by SR subtype.
Methods: Animals were held unexposed in an age-graded series or noise-exposed and held for acute or chronic post-exposure times. We recorded sound-evoked cochlear responses: outer hair cell (OHC)-based distortion product otoacoustic emissions (DPOAE) and round-window recorded compound action potentials (CAP), peri-stimulus time responses (PSTR) and spontaneous neural 'noise.' We quantified hair cells and inner hair cell (IHC) synapses by immunolabeling and confocal imaging. Strial condition was assessed in subsets of animals.
Results: In unexposed gerbils, response thresholds were generally well preserved for both DPOAEs and all neural responses over the age range studied. Suprathreshold DPOAEs also were relatively unchanged in animals 96 wks or younger, whereas neural response magnitudes showed progressive declines. By 144 wks, all responses were mildly reduced at lowest and highest frequencies, with mild OHC losses evident in corresponding cochlear regions. IHC synapse loss with age was a ~flat function of frequency/place from 2-45 kHz, reaching ~30% in oldest animals. Strial area and length were slightly reduced in apical and basal regions in oldest animals.
Noise exposure produced acute threshold elevations and reduced suprathreshold amplitudes for OHC- and neural-based responses. Response declines were maximum in the mid- and basal-cochlear regions targeted by the noise band. Magnitudes of the declines were dose-responsive, as were rate and extent of recovery, which for some exposures were total. In contrast, post-noise synapse counts remained subtotal at extended post-exposure times.

Conclusions: Taken together, results suggest different patterns of ANF compromise with aging and noise exposure: 1) Onset activity-sensitive gross neural responses (CAP and PSTR peaks) and spontaneous neural noise, all dominated by high-SR activity, 2) steady-state PSTR plateaus, reflecting contributions across fiber subtypes, and 3) synapse counts across cochlear regions having differing distributions of fiber subtypes; all showed gradual declines with age, suggesting mixed-SR involvement. In contrast, noise exposure targeting a cochlear region with mixed-SR populations appeared to acutely injure both low- and high-SR fibers, but high-SR-dominated responses, including spontaneous neural noise, showed earlier, more complete recovery, consistent with greater vulnerability of low-SR neurons, for exposures tested here. Findings inform cochlear models and translational efforts to better diagnose and more selectively treat cochlear injury from noise and aging.
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SA13. Higher-Dimensional Neural Representations Predict Better Speech Category Learning and Native Language Experience

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Category: Auditory Cortex and Thalamus: Human Studies
Background: Recent neuroscientific work [1-3] in the visual and auditory domain has shown that neural populations in the visual and auditory cortex encode information more efficiently when their responses are high-dimensional and uncorrelated (i.e., less redundant). Building upon these findings, we investigated the dimensionality of speech representations in a fronto-tempo-parietal network that supports speech sound processing in native listeners and speech learners [4]. Because channel bandwidth increases with dimensionality, we hypothesized higher dimensionality for native listeners (vs. non-native listeners) and successful learners (vs. poor learners).
Methods: We used Principal Component Analysis to assess the dimensionality of fMRI-BOLD responses to 40 Mandarin tones in 33 native speakers of Mandarin and 53 native English speakers trained with feedback to categorize these tones. We measured the dimensionality of voxel-wise representations of tones in the Heschl gyrus (HG), superior temporal gyrus (STG), left inferior parietal, inferior frontal gyrus–pars opercularis, and inferior frontal gyrus–pars triangularis. To assess the effects of language experience on representational dimensionality, we compared the dimensionality of voxel-wise representations of sounds between native listeners and learners. To assess the effects of training on representational dimensionality, we calculated the Pearson correlation coefficient between dimensionality and accuracy improvement across learners.
Results: We found higher dimensionality in the left hemisphere of native speakers (linear mixed-effects model, $ps<0.001$) and successful learners (Pearson's $rs>0.3$; $ps<0.02$). Additionally, we found a gradual reduction of dimensionality along the dorsal stream (temporal>parietal>frontal; $ps<0.001$).
Conclusions: Our findings indicate that language experience and auditory training can increase channel capacity by decorrelating neural activity across neural populations. They also show that the neural coding of speech features is more sparse (i.e., more granular) in primary (HG) and secondary (STG) auditory regions and more redundant in frontal regions. This frontal redundancy may reflect a low dimensional coding of invariant (i.e., less granular) representations of tone categories. This interpretation is consistent with two-stage hierarchical models of speech categorization [5-6], which postulate a higher degree of stimulus selectivity in temporal relative to frontal regions.
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SA14. Human Intracranial Responses to Speech: Latency and Attention Effects

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Category: Auditory Cortex and Thalamus: Human Studies

Background: Speech is generally assumed to be processed hierarchically in human cortex, with low-level areas focused on representation and with task and attention effects increasingly apparent as we ascend the hierarchy of processing. Here we ask whether responses recorded intracranially in humans provide direct evidence of this hierarchical processing.

Methods: Intracranial stereo-electroencephalography (sEEG) responses to /bi/ and /pi/ speech syllables were measured from patients undergoing invasive recordings for epilepsy surgery. The sEEG electrodes were implanted intracerebrally in the supratemporal plane and recorded local field potentials from cortical regions including Heschl's gyrus (HG), superior temporal gyrus (STG), superior temporal sulcus (STS), temporal-to-parietal junction, inferior frontal gyrus (IFG), and other parietal and frontal sites. Neural responses were measured across two different attentional conditions: (i) passive listening to the /bi/-/pi/ syllables, and (ii) active listening that involved subjects making a /b/ versus /p/ category-identity decision.

Results: Preliminary intracranial data (N=4) provide evidence consistent with hierarchical processing of speech in cortex. Specifically, latency of right-hemisphere sEEG high-gamma-band (75-150 Hz) envelope responses to speech input monotonically increases from HG and STG (a few ms) to STS and parietal areas (of the order of tens of ms), and frontal cortex (of the order of hundreds of ms). Latency results also reveal brain regions (in particular, in frontal cortex and subcortical areas) that are not responsive to speech in the passive condition, but are highly responsive in the attended condition. Furthermore, we observe that the attention effect (quantified by contrasting neural response amplitudes in the active and passive listening conditions) increases as we ascend along the auditory processing hierarchy in cortex, with HG showing a minimal attention effect (in line with prior reports; Nourski et al., Front Hum Neurosci, 2017) and STG, STS, and other downstream regions (e.g., in parietal and frontal cortex) showing progressively bigger attention effect sizes.

Conclusions: This study was supported by National Institutes of Health grants R21DC019217 (to LLH and TJA) and T32DC11499 (to VV).

SA15. Role of Inhibitory Neuron Types of the TRN in Auditory Processing

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Category: Auditory Cortex and Thalamus: Structure and Function

Background: Interactions between inhibitory and excitatory neurons along the auditory pathway shape how acoustic information is processed in the brain. The thalamic reticular nucleus (TRN) is the main source of inhibition in the thalamus and sends inhibitory projections to the medial geniculate body (MGB). Although the TRN is necessary for transfer of important acoustic information to structures in the auditory pathway, TRN's function at the cellular level remains poorly characterized. The TRN is comprised entirely of two dominant sub-classes of inhibitory neurons: parvalbumin- (PV) and somatostatin- (SOM) neurons. Their function in modulating the MGB has yet to be characterized; therefore, this study tests whether and how SOMs and PVs of the TRN modulate auditory responses in MGB. We first hypothesize that PV neurons of the TRN project to the ventral MGB, or first-order auditory thalamus, whereas SOM neurons of TRN project to the dorsal/medial MGB, or higher-order auditory thalamus. Secondly, we hypothesis that PV and SOM neurons differentially control sound responses in MGB and that they have distinct tuning properties.

Methods: We used viral tracing methods and a combination of optogenetics and in-vivo electrophysiology.

Results: These experiments show that SOM neurons of the TRN project to the medial and dorsal subdivisions of the MGB (n = 4). Whereas the projections of the PV neurons of the TRN target the ventral subdivision of the MGB (n = 4). Optogenetic inhibition of PV neurons of the TRN show a significant increase in the tone-evoked responses in 29% of MGB neurons (N = 237, n = 4) and a significant decrease of tone-evoked responses in 53% of MGB neurons, while 18% of significantly tuned neurons remain unaffected. This manipulation also increases responses to best frequency and side frequencies of MGB neurons in the neurons with facilitated responses and a decrease to best frequency and side frequencies of

those with suppressed responses. Silencing of SOM neurons of the TRN show a significant increase in the tone-evoked responses in 5% of MGB neurons (N = 328, n = 4) and a significant decrease of tone-evoked responses in 62% of MGB neurons, while 33% of significantly tuned neurons remain unaffected. This manipulation also increases responses to best frequency and side frequencies of MGB neurons in the neurons with facilitated responses and a decrease to best frequency and side frequencies of those with suppressed responses.
Conclusions: Together these results suggest that PV and SOM neurons of the TRN anatomically project to distinct sub-nuclei of the MGB and have differential modulation of tuning properties of the MGB. Inhibition of PV and SOM neurons of the TRN have differential effects across a population of MGB neurons. This study will establish a novel role of PVs and SOMs of the TRN in auditory processing.

SA16. Hypomyelination Reduces Parvalbumin Interneuron Density and Auditory Cortex Inhibitory Function

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Category: Auditory Cortex and Thalamus: Structure and Function
Background: It has long been known that myelination increases conduction velocity of action potentials and provides metabolic support to axons. This process is critical for the normal patterns of neural circuits' activation and synchronization, and its disruption can cause severe information processing defects. For a long time, myelin was thought to be restricted to excitatory neurons, and studies on dysmyelination focused primarily on excitatory cells. However, it has recently been appreciated that in the neocortex, myelin is also present on inhibitory neurons, but the impact of myelin defects on inhibitory circuits and neurons remains unknown.
Methods: To explore this question, we analyzed the primary auditory cortex (A1) in mice with mild hypomyelination caused by loss of oligodendrocyte ErbB receptor (ErbBR) signaling, a pathway critical for myelin maturation. NRG1, a ligand for ErbB receptors, is expressed by neurons and activates oligodendrocyte ErbBRs, leading to myelin gene expression and increased myelin thickness. For this studies we used mice with oligodendrocyte specific expression of a dominant-negative ErbB4 (CNP-DN-ErbB4), and mice with oligodendrocyte-specific inducible ErbB3 knock-out (PLP1-creERT: ErbB3 flox).
Results: MBP mRNA, MBP protein levels, and the amount t of MBP immunoreactivity around axons in A1 was reduced in CNP-DN-ErbB4 mice, indicative of a mild hypomyelination. Laser-scanning photostimulation (LSPS) analysis showed that that CNP-DN-ErbB4 mice have reduced functional inhibitory connections to A1 L2/3 neurons without changes in excitatory connections, resulting in altered excitatory/inhibitory balance. Molecular analysis of multiple synaptic markers demonstrated that these effects are not associated with altered expression of GABAergic and glutamatergic synaptic components. In contrast, analysis of mRNA expressed by inhibitory interneurons uncovered a reduction in parvalbumin (PV) expression without changes in somatostatin and VIP. Accordingly, immunostaining showed a reduction in A1 PV+ neuron density. Importantly, lineage tracing using PV-Cre showed that the number cells that expressed PV during brain formation was normal, indicating that there was a reduced PV expression without loss of PV neurons. Immunostaining showed that hypomyelination occurs in both PV+ and PV- axons, and MBP and PV mRNA levels, are highly correlated, suggesting that myelination influences PV expression. Findings in CNP-DN-ErbB4 mice were corroborated in PLP1-creERT: ErbB3 flox mice.
Conclusions: Our results show that subtle defects in myelination can lead to large changes in gene expression and function of PV interneurons which result in large-scale changes in network function in the neocortex, and shift networks towards excitation.

SA17. Brief Co-Activation of Cortical Inhibitory Neurons and Basal Forebrain Cholinergic Neurons Produces a Long-Term Suppression of Sound-Evoked Activity

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Category: Auditory Cortex and Thalamus: Structure and Function
Background: A precise balance between excitation (E) and inhibition (I) is essential for the normal operation of cortical circuits. Hearing loss or aging tips the central auditory E:I balance towards hyperactivity and disinhibition. Many lines of evidence have identified hypofunction of parvalbumin-

expressing (PV) inhibitory interneurons as a critical locus of dysregulation in tinnitus, hyperacusis, and other age-related hearing disorders. Here, we explore novel strategies to rekindle PV-mediated inhibition in the mouse auditory cortex (ACtx) with the ultimate aim of reversing associated perceptual disorders associated with disinhibition.
Enhancing inhibition using GABA-acting drugs or genetically targeted stimulation of PV neurons can reverse neural hyperactivity and restore normal sound processing, but these changes do not persist after GABAergic activation is discontinued. Basal forebrain cholinergic neurons (BFCNs) can exert rapid, specific, and lasting changes in cortical sound processing for stimuli co-occurring with BFCN stimulation. Here, we asked whether activating PV neurons during BFCN stimulation could produce a rapid and enduring reduction in sound-evoked ACtx activity.
Methods: To independently activate genetically targeted BFCNs and ACtx PV neurons, we expressed a Cre-dependent, soma-targeted, red-shifted opsin in the basal forebrain and ChR2 in ACtx PV cells using a virus with a PV-selective regulatory element (S5E2) in Chat-Cre mice. Our initial experiments used acute high-density single-unit recordings to characterize the tone-evoked receptive fields of neurons before and up to 60 minutes after a brief 15-minute PV and BFCN co-activation (N = 5 mice, n = 157 units). We then used 2-photon calcium imaging to assess the persistence of plasticity in chronically tracked layer 2/3 pyramidal neurons for three days following PV and BFCN co-activation (N = 3, n = 280 neurons).
Results: In acute single-unit recordings, pairing BFCN and ACtx PV cell stimulation resulted in substantial (>30%) suppression of sound-evoked spiking in ACtx regular-spiking units for up to 60 minutes post pairing, while spontaneous firing rates were largely unchanged. Activating PV neurons along or asynchronously with BFCN stimulation produced no such effect (0% suppression). Two-photon pyramidal neuron imaging confirmed this short-term plasticity and revealed persistent suppression out to 3-days post-pairing, the longest time point tested thus far.
Conclusions: Hyperactivity in ACtx circuits is thought to underlie debilitating suprathreshold hearing disorders such as hyperacusis and tinnitus. There are no widely effective methods to maintain suppressed neural activity and those that can work require continuous dosing. Our results suggest that just 15 minutes of combined PV and BFCN activation can produce sustained reductions in sound-evoked activity that last for several days. Ongoing work seeks to determine whether PV and BFCN co-activation can reverse hyperactivity and hypersensitivity in mice with cochlear neural degeneration.

SA18. Function of Auditory Cortex and Sound Sensitivity Affected by FOXG1 Gene Mutation

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Category: Auditory Cortex and Thalamus: Structure and Function
Background: Forkhead box G1 (FOXG1) gene is an important nuclear transcriptional factor regulates cell differentiation, proliferation and development in the brain. FOXG1 syndrome (FS) is rare and devastating neurodevelopmental disorder resulting from mutation of a single allele of the FOXG1 gene mutation. FS is characterized by a wide variety of symptoms, including severe intellectual disability, involuntary and continuous jerky movements, feeding problems, sleep disturbances, seizures, irritability and excessive crying. Some of the patients also report their children with FS show intolerance to loud sound, a character of hyperacusis. Although hyperacusis was commonly reported in children with neurological diseases, including Autisms and Williams syndrome, the neural mechanism of hyperacusis in these developmental disorders is still largely unknow. In this study, we will study sound behavioral sensitivity and auditory cortex function in order to understand the cause of sound sensitivity in FS.
Methods: CBA mice, with a single amino acid change in Foxg1 gene (G216S) identified in children with FS, have been used in this study. Sound sensitivity and tolerance behaviors were evaluated using acoustic startle response and behavior in open field with/without loud sound. Local field potentials of the auditory cortex were recorded in mouse with/without FOXG1 mutation to evaluate the function affected by Foxg1 mutation. Cortical layer development affected by FOXG1 mutation was examined using Nissl staining.
Results: In our preliminary studies, we found increased freezing behavior in the G216S mutant mice compared to the WT mice under loud sound (80-90 dB SPL). Enhanced amplitude of local field potential and firing rates of auditory cortex were recorded in the G216S heterozygous mice. Layer function of the cortex response was analyzed to identify it functional development.

Conclusions: FOXG1 gene mutation impairs layer development of the auditory cortex and causes hyperexcitability of cortical response. The hyperexcitability of the cortical response may increase sound sensitivity and aversive response to environmental stimuli.

SA19. Is There an Error Signal in Auditory Cortex During Vocalization?

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Category: Auditory Cortex and Thalamus: Structure and Function

Background: Vocalization is a sensory-motor process requiring auditory self-monitoring to detect and correct errors in vocal production. This process is thought to be mediated through an error signal encoding the difference between vocal motor predictions and sensory feedback, but direct evidence for the existence of such an error signal is lacking. In the auditory cortex, there is a is a well described vocalization-induced suppression seen both during human speech and non-human primate vocal production. Despite this suppression, the auditory cortex remains sensitive to perturbations in sensory feedback, a sensitivity that has been shown to be important in feedback-dependent vocal control. Although the mechanisms of suppression and vocal feedback encoding are unclear, auditory cortex activity during vocalization has been suggested to represent an error signal. However, past studies have been limited to a single perturbation of vocal feedback and therefore have been unable to fully test this hypothesis.

Methods: In this study, we investigated the vocal responses of auditory cortical neurons in marmoset monkeys, testing frequency-shifted feedback of varying magnitude and direction. Using implanted electrode arrays, we recorded multiple single-units from freely moving animals during vocal production, altered vocal feedback in real-time and testing individual units with several different feedback shifts.

Results: Consistent with an error signal hypothesis, we found that population-level neural activity scaled with the magnitude of feedback shifts, but were symmetric between positive and negative frequency changes. Feedback sensitivity was greatest in units suppressed during vocalization, and in units with frequency tuning overlapping vocal acoustic ranges. When individual units were tested with multiple feedback shifts, they often exhibited preferences for either positive or negative frequency changes, with only a minority sensitive to shifts in both directions. Units with feedback direction preferences also often exhibited different spectral tuning properties.

Conclusions: These results suggest that vocal responses and feedback sensitivity in the auditory cortex are consistent with an error signal calculation during vocal production, seen at both the individual unit and population level. The existence of a vocal error signal has potentially important implications for our understanding of the interplay between hearing, vocal production, and speech.

SA20. Automated Segmentation of Brainstem, Midbrain, Thalamus, and Auditory Cortex: Test-Retest Reliability and Comparison to Manual Segmentation

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Category: Auditory Cortex and Thalamus: Structure and Function

Background: Studying human auditory system structure at the resolution needed to capture both group- and individual-level differences is challenging, especially for the small brainstem nuclei and the complex gyrification patterns of Heschl's Gyrus (HG). In vivo investigations of the human subcortical auditory systems are currently rarely performed because of the lack of reliable, standardized means for automatically detecting such structures from 3-Tesla magnetic resonance images. While studies of HG are more common, many investigations rely on manual labeling, which is time intensive, prone to biases, and potentially misses subtle anatomical variations. Recently-published brainstem atlases, derived from 7T functional data, and toolboxes for automatic segmentation of HG, have opened the door to the automated measurement of in vivo auditory system structure. We parametrically evaluated these new tools in a retrospective analysis of T1 structural scans. The volume of the cochlear nucleus, superior olive, inferior colliculus, medial geniculate body, and HG were extracted for each hemisphere. The validity of these measurements was assessed by evaluating test-retest reliability and consistency with manual segmentation.

Methods: Automated and manual segmentation was performed on T1 magnetic resonance images (MRI) from 12 participants (mean = 29.7 ±6.7; 22 – 43 years) scanned twice over 3.8 (±3.7) days on average. A 3-Tesla Siemens MRI scanner (64-channel receive coil-array) was used to maximize MRI detection sensitivity, using the following parameters: 0.7mm isotropic sagittal magnetization-prepared rapid gradient-

echo acquisition, FA, 8°; TE, 2.22 ms; TR, 2,400 ms; voxel size (0.8, 0.8, 0.8); image dimensions (300, 320, 208), 208 slices total. We used three atlases developed from human histology (Big Brain), post-mortem, or functional in vivo MRI data (Sitek et al. eLife, 2019). For each atlas, three different transformation degrees of freedom (nonlinear, 6, 12) were applied to transform and analyze the auditory system in individual subject space. For HG, the TASH toolbox (Rocha et al., Scientific Reports, 2020) was utilized. Automated volumetric measurements were validated against manual segmentation. We used the ITK-SNAP software, version 3.8.0 for the manual segmentation and SimNIBS (which in turn utilizes CAT12 and SPM) for subcortical automated segmentation.
Results: For auditory brainstem structures, we found high levels of test-retest reliability between test sessions (correlation coefficients r>=0.8) using the Big Brain atlas and in vivo atlas when the algorithm used 12 degrees of freedom or a nonlinear approach. A comparison between automated and manually selected volumes for inferior colliculus showed moderate to high levels of correlation. An analysis of HG is underway.
Conclusions: Preliminary results the feasibility of objectively identifying brainstem areas with high reproducibility and measurements comparable to those of manual segmentation. This work lays the groundwork for more wide scale investigations linking human pathologies, behaviors, and experiences to the anatomical properties of the subcortical and cortical auditory system.

SA21. Effect of Suppressive Masking on the Dynamic Range of Auditory-Nerve Responses: Characterization With Forward-Masked Compound Action Potentials

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Category: Auditory Nerve
Background: A frequency-specific cochlear response can be suppressed or reduced by other frequency components of an incoming sound, even though these elicit little or no response at that cochlear location. This phenomenon, known as suppressive masking (or two-tone suppression in the simplest case of two tones), is a consequence of compressive nonlinearities and different mechanical couplings in the cochlea. Electrocochleography combined with forward-masking paradigms has recently been used to study basic properties of the inner ear (e.g., frequency selectivity, phase coding), and could also be used to assess suppressive masking with the advantage that it directly reflects the activity of auditory nerve (AN) fibers while limiting the invasiveness of recordings.
Methods: We investigated the effects of suppressive masking on the dynamic range of AN fibers using forward-masked click-evoked Compound Action Potentials (CAPs) recorded at the round window of anesthetized chinchillas. For the analysis, we relied on a recently developed model that was accurate in fitting CAP waveforms associated with notched-noise forward-maskers of different notch widths and notch attenuations. The main component of this model is a sigmoid-like input-output (I/O) function controlling the growth-of-masking as a function of cochlear-filter output intensity. For this specific study, we added suppression as a constant dB/dB rate shifting the I/O function to higher intensities when the energy passing through a second filter bank is increased. We also adapted the experimental paradigm, including presenting the forward-maskers at different sound levels. The parametric I/O function and the suppression rate were estimated by minimizing the prediction error of differences in the CAP amplitude between pairs of maskers using gradient descent.
Results: Suppression masking was investigated at CF (center frequency) of 5 kHz using maskers with varying notches or frequency bands around that frequency. 24 maskers were presented at 6 sound levels, totaling 144 masking conditions. The model provided a less accurate prediction of the CAP waveforms compared to our previous study with a single sound level, presumably because of cochlear nonlinearities. However, the addition of the suppression rate decreased the prediction error by 10% and described well the overall trend of growth-of-masking. We found a suppression rate of 0.35 dB/dB, consistent with above-CF suppression rates found in the literature. Future development of the model will separate suppression below and above CF, the former being characterized by both higher thresholds and higher suppression rates.
Conclusions: Suppressive masking is perceptually relevant for normal hearing and altered with sensorineural hearing loss. Electrocochleography offers the opportunity to investigate suppression of AN responses in a less invasive manner, and could help refine our understanding of nonlinearities in the human peripheral auditory system.

SA22. Distortion Product Electrocochleography From the Ear Canal

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Category: Auditory Nerve

Background: In animal studies the difference-frequency distortion product of two tones (F2-F1) measured using electrocochleography (ECochG) is of largely neural origin when within the phase-locking range of auditory nerve fibers (<1500 Hz). At moderate and low intensities, the spectral peak of the F2-F1 frequency can be larger than those produced by the primaries and is removed by neurotoxins. This neural contribution thus differs from distortion product otoacoustic emissions which rely on the function of outer hair cells, and instead closely resembles the auditory nerve neurophonic (ANN) to low frequencies. Importantly, the source of this distortion ANN is phase-locking to the envelope that originates from the characteristic frequency region of the primaries, and can be generated by primary frequencies across the hearing range. Thus, their detection could in principle provide a sensitive test of neural function across the frequency range of the cochlea.

Methods: EcochG recordings were made from the ear canals of young, normal hearing subjects (n=19). Stimuli were tonal complexes comprised of two or more primary frequencies, delivered through Etymotic 3b transducers coupled to the ear canal with foam inserts sheathed with gold foil that served as the active ear canal electrode. The reference electrode was on the contralateral mastoid and the ground on the forehead. Responses were recorded to 1000-2000 repetitions of bursts (53-160 ms duration) presented in condensation starting phase, typically at 80 dB SPL. Measurements were the spectral peaks at the F2-F1 frequencies using Fourier methods. Peaks two standard deviations above the noise floor were considered significant.

Results: Ear canal recordings have a challenging signal to noise ratio (SNR), and low frequencies have the additional challenge of increasing noise levels. However, the tissue between the cochlea and ear canal produces a low-pass environment, so low frequencies are also the spectral region where the signal can be relatively large. All subjects had responses to some or all of the F2-F1 frequencies tested across a range of primary frequencies (500-4000 Hz). The largest responses were generally to the lowest F2-F1 frequencies (200 or 250 Hz), yet because of the increased noise level the SNR over the range of 200-900 Hz was relatively constant. For F1 frequencies of 2000 and 4000 Hz, the lowest thresholds were to F2-F1 frequencies within the range 400-700 Hz. Preliminary data with simultaneous masking reduced the F2-F1 magnitude, indicating a neural component even at the high intensities used.

Conclusions: Ear canal ECochG of F2-F1 frequencies are possible with high frequency carriers, indicating the ability to monitor this component across the entire audible frequency range. An indication that they are of cochlear origin is the extended frequency range, which is higher than the frequency-following response recorded from central levels. A neural component is suggested from the masking results.

SA23. Molecular Signatures Define Subtypes of Auditory Afferent Neurons With Distinct Peripheral Projection Patterns and Physiological Properties

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Category: Auditory Nerve

Background: Sensory afferent neurons within single sense organs have diverse properties that are crucial for information coding. Type I spiral ganglion neurons (SGNs) are the sensory afferents of the auditory system that innervate inner hair cells (IHCs). Type I SGNs have been proposed to consist of three subtypes that differ in their gene expression profile, spontaneous rate (SR) of action potential firing, sound sensitivity and dynamic range.

Methods: To further characterize the properties of type I SGNs, we have generated mouse lines expressing CreERT2 in molecularly defined SGN subtypes. Using these mice lines, we assessed connectivity, as well as molecular and physiological features of these defined groups of SGNs.

Results: One group of genetically marked type I SGNs that expresses CreERT2 from the Lypd1 genetic locus preferentially innervates the modiolar side of IHCs and has low SRs. A subset of the remaining type I SGNs that expresses Cre-ERT2 from the Calb2 genetic locus labels neurons that preferentially innervate the pillar side of IHCs and has a wider range of SRs.
Conclusions: We conclude that murine type I SGNs consist of genetically defined subtypes with distinct properties, but even neurons within subtype classes differ in their electrophysiological characteristics. Gradual changes in the properties of type I SGNs might be critical to increase the dynamic range of the auditory system to contribute to its tremendous signaling capability.

SA24. Open Board

SA25. A Comparison of Rigid Versus Conformable Auditory Brainstem Implant Position Analysis Using Different Imaging Techniques: From Nonhuman Primate to Human

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Category: Auditory Prostheses
Background: Variable perceptual outcomes in auditory brainstem implant (ABI) patients can be explained by several factors that include electrode array position. Our group has studied the relationship between perception and ABI array position using quantitative three-dimensional (3D) image analyses of postoperative computed tomography (CT) (Barber, 2018). ABI users with the best speech perception have arrays tilted superiorly and closer to the midline and have a higher number of active electrodes (Egra-Dagan, 2021). Previously, our group developed a novel conformable ABI array design that overcomes the biomechanical mismatch inherent in the rigid clinical ABI (Vachicouras, 2019), but its delineation by image analysis has not been fully investigated. Here, we evaluate three-dimensional (3D) rigid and conformable ABI position using computed tomography, X-ray, and 7 Tesla magnetic resonance imaging (MRI).
Methods: Two different devices were included in this study: 1) rigid "clinical" ABI device (Cochlear Corporation) and 2) "conformable" ABI for use in nonhuman primate (NHP) and cadaveric studies. Experiments were conducted at Massachusetts Eye and Ear (MEE) and the École Polytechnique Fédérale de Lausanne (EPFL). 3D ABI position on imaging was compared across subjects by measuring the ABI lateral angle and the posterior angle. We also quantified cerebellopontine (CPA) angle displacement.
Results: Most subjects underwent a suboccipital craniotomy for placement of the ABI. The clinical ABI was used in patients and cadaveric specimens. The conformable ABI was placed in cadaveric specimens and NHP. Postoperative CT analysis in NHP demonstrated conformable ABI position with an angle of 61.24˚ in the lateral view and an angle of 83.20˚ in the posterior view. In the cadaveric specimens, one subject had a posterior angle of 94.57˚ and a lateral angle of 37.97˚, while the second had a conformable ABI with an angle of 83.66˚ and 61.55˚, respectively. The CPA displacement was 138.03˚ with the clinical ABI and 112.56˚ with the conformable ABI. Post-operative x-ray vs CT scan comparison in patients demonstrated a lateral angle of 25.66˚ vs 64.0˚ and a posterior angle of 10.65˚ vs 29.0˚ for subject 1 and 9.28˚ vs 8.66˚ and 57.52˚ vs 59.03˚ for subject 2, respectively.
Conclusions: Clinical ABI users and studies with cadaveric specimens and NHP subjects shared similar 3D array orientations on X-ray and CT. These findings support the utility of 3D image analysis of ABI array position for future intraoperative image guidance approaches to improve surgical accuracy and clinical outcomes.

SA26. Light-Tissue Interaction in the Cochlea

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Category: Auditory Prostheses
Background: In the advent of optical protheses, it has been argued that the benefit of optical stimulation over electrical stimulation is the possibility of direct neural excitation, without contact, and with high spatial precision. The increase in spatial precision enables the activation of more discrete, independent populations of neurons, providing finer control of the neural firing patterns that result from stimulation.

Light propagation in the tissue is determined by optical properties of absorption and scattering, both being wavelength-dependent. Because most biological tissues are complex turbid media (multiple scattering events), the spot size of the optical radiation at the target structure and the energy delivered to the target, can be affected by these properties. As a result, the decrease in spatial precision may nullify the advantage of optical stimulation over electrical stimulation.
To better predict the neural population in the cochlea that will be stimulated using certain optical parameters (wavelength, optical power and beam profile), it is important to characterize the optical transmission profile in the human cochlear bone, in the visible (for optogenetics) and near-infrared (for INS) wavelengths, which are poorly explored in literature.
Methods: We measured light scattering in cadaveric human cochlea bone at several wavelengths in the visible and near-infrared range (450-1860 nm). The measurement probe was a cochlear electrode having bare die photodiodes (size 350x550 µm2, PD2214HP1, MTG, Germany) sensitive in the spectrum 400-1100 nm. All sensors' cathodes were soldered using epoxy in one uncoated silver wire (core 76 µm, A-M Systems, USA), also denoted as backbone. The distance between sensors was 2 mm. Then, we soldered a coated platinum-iridium wire (core 25 µm, A-M Systems, USA) in each anode. The output current of the photodiode was converted to voltage using a load resistor.
Using a precision wire saw (SXJ2, MTI Corp., USA), we performed cutlines perpendicular to the cochlea, with a thickness 250 µm and spaced of 2 mm. To determine the light scattering profile, two strategies were chosen. First, we introduced in the cutlines an optical fiber (core 220 µm) to measure the scattered light. For every cutline, we scanned light at six depths (15 points per depth, 1 mm separation). Second, we introduced the electrodes with the sensors facing the oval window.
Results: The study is ongoing. We obtained the scattering profile for a green laser (power 15 mW). Light is considerably scattered in all directions. Further profiles will be obtained using other wavelengths and positioning the light source at different orientations with respect to the target.
Conclusions: Computed tomography images of the same cochlea will be obtained to overlap a 3D model with the light profiles obtained. These results provide valuable insights on light distribution in the cochlea bone, of importance in optical neurostimulation and neuromodulation.

SA27. UltraHearing: Encoding Complex Auditory Information in Body-Coupled Ultrasound for a New Hearing Technology

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Category: Auditory Prostheses
Background: Body-coupled ultrasound, which consists of acoustic waves above 20 kHz, has been shown to activate the auditory system via a cochlear pathway. Studies have shown that at low ultrasound frequencies (50-100 kHz), the activation mechanism is through bone conduction. However, newer studies have demonstrated that at ultrasound frequencies in the hundreds of kilohertz the activation relies on a fluid pathway, in which the ultrasound induces vibrations in the cerebrospinal fluid (CSF) of the cranial cavity, which travel into the cochlea via the cochlear and vestibular aqueducts. Our previous work showed amplitude modulation of the ultrasound signal (e.g., 220 kHz) with tones (1-40 kHz) successfully evokes a frequency specific activity particularly at the low and middle frequencies (<20 kHz). This work expands upon those findings by characterizing the extent to which complex speech-like signals (in the form of guinea pig vocalizations) are able to be encoded in the ultrasound stimuli.
Methods: In this study, we investigated the neural activity in response to complex signals transmitted via air- and fluid-conduction (ultrasound). Neural activity was recorded using a two-shank 32-channel NeuroNexus electrode array placed in the central nucleus of the inferior colliculus (ICC) of ketamine-anesthetized guinea pigs. Air-conducted stimulation consisted of guinea pig vocalizations presented through a closed-field speaker coupled to the ear via a hollow metal bar. For fluid-stimulation, we extracted the envelope of up-sampled versions of the vocalizations using the Hilbert transform, and then modulated a 220 kHz or 520 kHz sinusoid. The signal was presented via two different ultrasound transducers coupled to the brain with agarose, depending on the chosen center frequency. To compare the neural activity, we created post-stimulus time histograms (PSTHs) and utilized the Earth Mover’s Distance (EMD) metric to compare the evoked activity by the stimuli.

Results: Ultrasound evoked responses showed some similarity in temporal patterns as those evoked by air-conduction for most vocalizations. EMD analysis demonstrates that there is a higher degree of similarity when comparing the same vocalization across ultrasound versus air-conducted stimulation rather than with random vocalizations compared to each other. Control experiments with the transducer separated from the guinea pig demonstrate that these effects require the direct coupling with the animal's head, and that the signal is not transmitted via air-conduction.
Conclusions: Body-coupled ultrasound can encode complex signals such as guinea pig vocalizations when amplitude-modulated. Varying center frequencies can alter the extent of encoding similarity between ultrasound and air-conducted stimuli. Future research will investigate whether these differences can be explained due to nonlinear transmission properties through the fluid pathway and/or the transducer's frequency response characteristics in which development of better ultrasound stimulation patterns could improve hearing performance.

SA28. Using Interleaved Stimulation to Explore Selectivity and Nonlinearity in the Electrically Stimulated Auditory System

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Category: Auditory Prostheses
Background: The ALFIES (ALternating Frequency Interleaved Electrical Stimulation; Carlyon et al 2021) method measures the sustained phase-locked neural response to stimulation by a cochlear implant (CI). It does so by interleaving two pulse trains, each amplitude modulated by the 2nd and 3rd harmonics (F1 and F2) of a fundamental frequency F0, where F0~40 Hz. Because the pulse trains modulated at F1 and F2 Hz are interleaved, the EEG response does not contain a distortion product at F2-F1=F0 Hz arising from any instantaneous nonlinearity, such as may be produced by the CI itself. However temporal dependencies introduced by the auditory system can partially undo the interleaving, and subsequent neural non-linearities produce a "Neural Distortion Response (NDR)" at F0 Hz. The NDR has a group delay of about 40 ms and is of similar size for recording electrodes close to and contralateral to the CI, thereby distinguishing it from electrical artefact. Here we probe the nature of the temporal dependency necessary for the NDR, report amplitude growth functions (AGFs), and compare the NDR to the Electrically Evoked Auditory Steady State Response (EASSR).
Methods: NDRs and EASSRs were measured using a custom-made BioSemi EEG system with a very high (>262 kHz) sampling rate and for an F0 (NDR) and AM rate (EASSR) close to 40 Hz. Experiment 1 measured NDRs for interleaved 480-pps pulse trains for 5 users of the Cochlear CI and 2 Advanced Bionics users. The inter-pulse interval (IPI) between the F1 and F2 pulses ranged from 0- 984 µs. Experiment 2 measured NDR as a function of stimulus amplitude up to a maximum value of 1.5 dB above the listener's most-comfortable loudness level (MCL) for 3 Cochlear and 5 AB users. Experiment 3 measured AGFs for the EASSR in 1.5-dB steps up to MCL and for carrier rates of 480 or 500 pps.
Results: NDRs were roughly constant for IPIs up to 200-400µs, dropped by 6 dB at an average IPI of 411 µs, and were in the noise floor by 984 µs. NDR AGFs were steep and monotonic for all CI users except for a small decrease at high levels observed for two participants. SSR AGFs were also steep; no monotonicities were observed. At MCL the EASSR was slightly but significantly larger than the NDR. Group delays for NDRs and EASSRs were ~40 ms.
Conclusions: The time course of the IPI effect is consistent with temporal dependencies (facilitation, accommodation) known to occur at the level of the auditory-nerve (AN) membrane; neural nonlinearities responsible for the NDR may occur at any point from the AN up to auditory cortex. The steep and largely monotonic AGFs for both the NDR and SSR are encouraging for their use as fast objective measures of MCL.

SA29. Progress and Challenges With Implantable Microphones for Cochlear Implants

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Category: Auditory Prostheses
Background: We have developed implantable microphones that could be used in a fully implantable cochlear implant, or in other implanted hearing prostheses. Our microphones operate by picking up the

sound signal via the motion of the umbo or via intracochlear pressure. Hearing assistive devices using implantable microphones can enable enhanced and directional hearing by making use of the natural filtering of the external ear. Implantable microphones would enable the use of hearing assistive devices in all environments, day and night.
Methods: The transduction mechanism of our implantable microphones is based on the piezoelectric properties of polyvinylidene fluoride (PVDF), a common engineering plastic that can be manufactured as a piezoelectric film. PVDF is biologically compatible and has been used in medical devices, for example surgical sutures in combination with permanent implants. A custom low-noise charge amplifier with the ability for differential input is used with our microphones to maximize signal and reduce noise and susceptibility to electromagnetic interference.
Results: The cantilever microphone is two layers of PVDF separated by a backing and detects the sound signal via bending. The output from each PVDF layer is connected to the differential amplifier where the output is the amplified difference between the two signals from the PVDF layers thus providing good common mode rejection. The signal-to-noise characteristics of the cantilever microphone are comparable to those of hearing aid microphones.
The intracochlear microphone design remains under basic development. These microphones work through compression of the PVDF material. Based on theoretical considerations, the sensitivity due to compression is not large enough to achieve comparable signal-to-noise characteristics to hearing aid microphones. However, previous experimental work with the intracochlear microphone and the possibility of direct integration with cochlear implant electrode keeps us interested in this design. We are exploring new designs that seek to improve signal-to-noise by using new materials and geometries.
Conclusions: For all our microphone designs, future challenges include making the devices biocompatible, ensuring robustness and durability, ensuring low pick-up of environmental EMI, minimizing cross-sensitivity, and designing and testing bracing mechanisms and integration strategies for surgical feasibility.

SA30. Open Board

SA31. Characterizing Musically Evoked Emotions in Cochlear Implant Users
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Category: Auditory Prostheses
Background: Advancements in cochlear implant (CI) technology have allowed the profoundly deaf to achieve excellent speech comprehension; however, perception and appreciation of music still remains a challenge. Specifically, CI users have difficulties differentiating pitch, harmony, and timbre. In Western music, major and minor keys are often associated with happy and sad emotions, respectively. Previous work suggests that CI users, compared to normal-hearing (NH) listeners, have diminished perception of certain musically evoked emotions due to deficits in hearing pitch-related musical elements. It is still unclear, however, whether pitch perception is important in conveying music-based emotions. The purpose of this study is to investigate how musical pitch affects the perception of emotions in music in CI users.
Methods: Eight CI users and 8 NH listeners matched on age, sex, and musical experience completed a set of online perceptual recognition tasks (www.teamhearing.org). These tasks included identification of major and minor melodies and arpeggios, melodic contour identification, and rating of musical valence and arousal on happy, sad, peaceful, and scary songs materials from the Ambert-Dahan et al. (2015) library. In a second task, participants were asked to rate tempo-controlled sad and peaceful songs whose keys were inversely manipulated: sad songs were transposed into major keys to sound like peaceful songs and peaceful songs were transposed into minor keys to sound like sad songs. Due to their reduced perception of pitch, we hypothesized that CI listeners would have difficulties hearing differences between happy, sad, peaceful, and scary music compared to NH listeners. Furthermore, given that musical keys are determined by the distance between pitches (i.e., musical intervals), we predicted that for key adjusted excerpts (i.e., major converted to minor and minor converted to major), CI listeners would have diminished recognition of the altered emotions driven by major/minor key adjustments compared to NH listeners with decreased recognition of key manipulation correlated with reduced major and minor key and melodic contour identification.

Results: Preliminary results suggest that CI users are less able to hear and rate differences in arousal, valence, and emotion scary, peaceful, and sad excerpts. For happy excerpts, CI users performed similarly to NH users on ratings of arousal, valence, and emotion. Data collection is still ongoing.
Conclusions: The emotional aspect of music is often cited as a source of enjoyment for listeners. Understanding why CI users have difficulty interpreting the emotional meaning within songs could lead to greater music appreciation and improvements in their quality of life. Results from this research may aid in creation of auditory rehabilitation programs that use techniques, such as musical interval training, to improve music perception in CI participants so they can live more emotionally fulfilling lives.

SA32. Effects of Cochlear Implant Electrode Location on Electrophysiological Measures of Neural Health

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Category: Auditory Prostheses
Background: Previous work in cochlear implanted animals has identified measures of the electrically-evoked compound action potential (ECAP) that relate to the density of surviving spiral ganglion neurons (SGNs). Related work in cats and humans suggest that these measures also reflect the positioning of the electrode array within the cochlea. Specifically, in human subjects, we found that the distance between the electrode and the modiolus ('medial-lateral distance') influenced some, but not all, ECAP measures. Due to the narrowing of the scala tympani from base-to-apex, the medial-lateral distance might covary with SGN density which tends to be greater in the apex. Therefore, the specific relationship between medial-lateral distance and ECAP-based neural health measures requires additional analysis.
Methods: Participants included 25 adult (>18 years old), peri- or post-lingually deafened cochlear implant recipients. All were users of Cochlear ™ implant systems, and had at least 3 months' cochlear implant experience. We measured ECAP amplitude-growth functions (AGFs) on each electrode using two interphase gaps (IPGs) of 7 and 30 μs, and calculated the slope of the AGF for a fixed IPG as well as the difference in slope between the two IPGs (IPG effect) for each electrode. Post-operative CT images were completed using well-established methods, which provided estimates of electrode medial-lateral distance, scalar location (scala tympani vs vestibuli), and insertion angle. To better parse contributions of electrode location on ECAP measures, we examined how ECAP slopes change for 1) fixed insertion angles across a range of medial lateral distances and 2) fixed medial lateral distance across a range of insertion angles.
Results: Results showed that, consistent with previous findings, the slope of the ECAP AGF for a fixed IPG, but not the IPG effect, decreased with increasing medial-lateral distance. For a fixed insertion angle, there was not always a consistent relationship with medial-lateral distance. For a fixed medial lateral distance, the ECAP AGF slope tended to increase with increasing insertion angle. Scalar location did not seem to significantly affect these results.
Conclusions: Results agree with previous findings that suggest electrode location, specifically medial-lateral distance, influence ECAP measures using a fixed IPG, when measured across the entire electrode array within each subject. When medial lateral distance is fixed, increases in ECAP AGF slope results are consistent with better neural survival in apical compared to basal cochlear regions.

SA33. Do Bilateral Ci Users Perceive the Same Loudness Growth From Their Left and Right Ear?

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Category: Auditory Prostheses
Background: Bilateral Cochlear Implants (BiCI) can achieve improvements over unilateral implantation in many tasks, including speech perception in noise. However, BiCI users can perform worse in terms of controlling vocal intensity when using both CIs compared to when they use only one of their CIs. This may be because CI users do not perceive matched loudness growth functions from each ear, and this perceptual mismatch may negatively affect their vocal control abilities. The purpose of this study was to see if BiCI users perceive different loudness growth with their left and right ears.
Methods: Three post-lingually deaf adults with bilateral cochlear implants were included in this study. Participants were asked to listen to speech-shaped noises ranging in presentation level from 35 dBA to 85 dBA in 1 dB increments, presented in a randomized order in the sound field. Participants were tested using their left and right ears separately in a randomized order. They rated the loudness of the sounds using a

visual analog scale, with the left side of the scale indicating the softest sound and the right side indicating the loudest sound they could perceive.
Results: The preliminary findings revealed that the loudness growth function in the participants had a sigmoidal pattern. Contrary to our hypothesis, the slopes of the loudness growth functions for the left ear versus the right ear were similar.
Conclusions: Preliminary results found similar loudness growth functions for both ears. This suggests that bilateral CI users receive a matched loudness perception across ears. Future research will investigate what other factors underlie poor vocal intensity control when using both CIs together.

SA34. Quantifying Neural Synchrony at the Level of the Auditory Nerve in Cochlear Implant Users With Recordings of the Electrically Evoked Compound Action Potential

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Category: Auditory Prostheses
Background: While cochlear implant (CI) users can achieve excellent listening outcomes in quiet, speech recognition in background noise remains a significant challenge (Zaltz et al., 2020). Studies in acoustic hearing have demonstrated that human listeners with declined neural synchrony at the level of the auditory nerve (AN), such as patients with auditory neuropathy spectrum disorder, experience excessive difficulties in understanding speech in background noise (e.g., Rance, 2005; Zeng et al., 2005). Additionally, a recent study in listeners with acoustic hearing showed that neural synchrony at the level of the AN is a strong predictor of speech recognition in noise for both young and elderly listeners (Harris et al., 2021). The present study developed a method for assessing neural synchrony at the level of the AN in CI users with recordings of the electrically evoked compound action potential (eCAP).
Methods: Thus far, ten post-lingually deafened adult CI users have participated in the study. In each participant, neural synchrony at the level of the AN was quantified using two measures assessing trial-to-trial variability: the variability in inter-peak latency (VIL) and the phase-locking value (PLV). The VIL was calculated as the standard deviation of inter-peak latencies (i.e., P2 – N1) across trials. Larger values of the VIL indicate more variability in the eCAP response across trials and therefore less neural synchrony. The PLV is unitless and ranges from 0 to 1, indicating no synchronization and perfect synchronization across trials, respectively. Therefore, larger PLVs indicate more neural synchrony. The correlation of the two measures of neural synchrony was evaluated. Additionally, both measures were compared with psychophysical gap detection thresholds (GDTs) because neural dyssynchrony at the level of the AN is associated with larger GDTs in acoustic hearing (Zheng et al., 2005; Starr et al., 2008).
Results: Preliminary results indicate that VIL and PLV are strongly correlated ($r = -0.89$, $p < 0.001$). Both measures of neural synchrony were also highly correlated with psychophysical gap detection thresholds (VIL: $r = 0.74$, $p = 0.015$; PLV: $r = -0.73$, $p = 0.016$).
Conclusions: Preliminary results suggest that both measures successfully characterize neural synchrony at the level of the AN. Like in acoustic hearing, the perception of silent gaps in electric hearing is affected by the degree of neural synchrony at the level of the AN.

SA35. Visual Analog of the Acoustic Amplitude Envelope Benefits Cochlear Implant Recipients in Speech Perception in Noise

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Category: Auditory Prostheses
Background: For many cochlear implant (CI) recipients, visual cues are beneficial for improving speech perception performance. However, the audiovisual integrative process in CI users is not well understood in general. The audiovisual benefit in suprasegmental feature perception (duration, pitch, emotion, etc.) has not been consistently observed in individual CI users (Algefords, 1996; Most et al., 2009). Results of our recent study showed that the critical prosodic feature conveyed by the visual channel was the amplitude envelope information, which could be effectively integrated and processed online with auditory signals to enhance speech perception in noise for normal hearing listeners (Yuan et al., 2020). This study built upon this result and evaluated the effectiveness of the visually presented acoustic amplitude envelope that is independent of articulatory gestures in supplementing speech perception in noise in postlingually deafened adult CI users.

Methods: To date, six study participants, ranging in age between 41.87 and 72.76 years, have been recruited and tested. All subjects were implanted with a CochlearTM Nucleus® device in the test ear(s). Their sentence-level speech perception performance was measured using Harvard sentences (IEEE, 1969) presented in auditory-only or audiovisual mode in both quiet and multi-talker babble noises with signal-to-noise ratio (SNR) at +5 dB and +10 dB. Auditory signals were directly streamed from the computer to the CI on tested ears using the CochlearTM Wireless Mini Microphone 2+. The extracted target envelopes were synchronized with the amplitude of a spherical-shaped ball and presented as visual stimuli on a desktop monitor. Sentence recognition accuracy was compared between results measured in audio-only and audiovisual modalities.
Results: Our preliminary results suggested an improvement in speech intelligibility in the +5 dB SNR noise condition for audiovisual mode compared to audio-only mode ($t(5) = -2.982$, $p=.015$). No apparent difference was observed between results measured in the +10 dB SNR noise condition using these two modes. Speech perception performance measured in the quiet condition using the audiovisual mode appeared to be worse than those measured using the audio-only mode.
Conclusions: The amplitude envelope, as a reliable source of temporal cues, can be delivered through different sensory modalities (e.g., visual) to enhance speech intelligibility when auditory perception ability is compromised in CI users, especially in challenging hearing environments.

SA36. A Computational Model for Predicting Objective Measures of Electrode-Neuron Interface Inside the Implanted Cochlea

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Category: Auditory Prostheses
Background: Functionality of the electrode-neuron interface inside the cochlea affects the hearing outcomes of a cochlear implant (CI) user. That functionality can be assessed directly by measuring electrically evoked compound action potentials (eCAPs) – eCAP threshold and the slope of the eCAP amplitude growth function (AGF) being commonly measured in clinical practice. Animal studies have showed how especially changes in eCAP metrics following a change in stimulus characteristic can be indicative of cochlear health (Ramekers et al., 2014). On the other hand, studies on human CI users have shown how eCAP metrics are affected by non-neural aspects (Schvartz-Leyzac et al., 2020). These somewhat conflicting findings hinder the interpretation of results in clinical practice and in optimization of coding strategies. Here, we aim to provide more insight into the topic by means of computational modeling.
Methods: A simplified 2-D computational model was designed to simulate how electrical signals from the stimulating electrode reach the auditory nerve fibers (ANFs) distributed along the cochlea, evoking them to release action potentials that can be recorded as compound responses at the recording electrodes. The geometrical constrains were derived from post-humous and post-operative neurophysiological data (Avci et al., 2016; Yoshimura et al, 2020). The spiking activities of the independent ANFs were predicted with a phenomenological model (Takanen and Seeber, 2022) and convolved with a unitary response function (Vernsel et al., 1992) to derive the compound response at the recording electrode. Both the electrical input to the neurons and the neural responses were attenuated by 1.2 dB/mm (Nelson et al., 2008).
The model was applied to investigate effects of neural survival and non-neural aspects on eCAP AGFs. The inter-phase gap (IPG) effect of cochlear health was evaluated by varying the IPG (2.1 and 30 µs) in the stimulus while varying the neural survival from 250 to 2000 ANFs. Four recording electrodes and two electrode-neuron distance models were used to evaluate influences of non-neural aspects.
Results: Model predictions were found to agree with data from literature: The eCAP AGF slope and the IPG effect on the slope – when computed as the difference between the slopes of the two AGFs expressed on a linear input-output scale – depended on the neural survival as found in (Ramekers et al., 2014, 2015; Pfingst et al., 2017). The effect of the stimulating-recording electrode distance on the maximum eCAP amplitude was reproduced (Gärtner et al., 2015). The predicted dependency of the eCAP threshold on the electrode-neuron distance was similar to the one reported by Schvartz-Leyzac et al. (2020).
Conclusions: The model predictions demonstrate previously found dependencies of eCAP metrics on neural and non-neural aspects, providing supportive evidence for findings from animal studies. This also bolsters the use of the model as a clinical research tool.

SA37. Electrophysiological Evaluation of Cochlear Function in Chd7Gt/+ Mice With Chronic Cochlear Implants

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Category: Auditory Prostheses

Background: Chd7Gt/+ mice are a model of human CHARGE syndrome (Coloboma, Heart defects, Atresia choanae, Genital abnormalities and Ear malformations), an autosomal dominant disorder that often includes mixed sensorineural/conductive hearing deficits. Previous studies in Chd7Gt/+ mice reported severe hearing loss at 4 and 16 kHz, but no differences in ABR Wave I peak latency or amplitude, inner hair cell synapses, or spiral ganglion neuron density compared to wild type mice. Interestingly, histological analysis revealed hyper-myelinated Type I spiral ganglion axons. Individuals with CHARGE syndrome typically perform well with cochlear implants (CIs), yet challenges exist in designing fully restorative therapies and in understanding the etiologies of the sensorineural hearing loss in CHARGE. As a first step toward addressing these challenges, we analyzed middle ear structures and tested whether auditory-nerve function in Chd7Gt/+ mice with a CI was comparable to wild type controls.

Methods: Chd7Gt/+ mice (6 – 8 weeks old) (N = 8) with severe hearing loss at 4 kHz and partial hearing loss at 16 kHz were implanted in the scala tympani with a platinum/iridium two-ball-electrode CI. Electrically-evoked compound action potentials (ECAPs) were measured on the day of implantation and then every other day until euthanasia. ECAP amplitude growth function (AGF) slopes and shapes were compared to data collected previously in wild type mice implanted (two-ball N = 3, and multi-ring N = 3 electrode implants) at varying ages (7 – 18 weeks).

Results: Anomalies of the bulla, middle ear, and round window were observed in 7 of the 8 Chd7Gt/+ mice. Defects included thickened bullae, abnormal tissue in middle ears, abnormal stapedial arteries, and tissue/bone covered round windows. Mice with middle ear and round window anomalies exhibited the most severe hearing loss at both frequencies. One Chd7Gt/+ mouse without visible anomalies had severe hearing loss only at the 4 kHz frequency. Four Chd7Gt/+ mice did not recover from the CI surgery and the remaining four were tested for 9 to 33 days post-implantation. ECAP AGF shapes, slopes, and peak amplitudes were similar between the Chd7Gt/+ and wild type mice on the day of implantation and over the course of several weeks.

Conclusions: In this small cohort of animals, middle ear defects were common, yet cochlear function was similar after CI between Chd7Gt/+ and wild type mice. A larger sample of animals with comparable ages and measurement points, as well as further in-depth analysis of latencies and histology will be needed to tease out any subtle differences in wild type versus Chd7Gt/+ CI mice. The ability to implant these mice and obtain ECAP recordings demonstrates the feasibility of the mouse CI model for assessing auditory-nerve function in mutant mice.

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SA38. Neural Network Models of Hearing Through a Cochlear Implant

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Category: Auditory Prostheses

Background: Cochlear implants (CIs) are one of the great success stories in biomedical engineering, but nonetheless fail to restore fully normal hearing in individuals with sensorineural deafness. One plausible limitation on current CIs is suboptimal algorithms for converting sound into electrical stimulation. Models that can predict what a person hears with CI input could help develop better stimulation strategies for CIs. Here, we investigate models of CI-mediated hearing based on deep artificial neural networks, which have recently been shown to reproduce aspects of normal hearing behavior and replicate hierarchical organization in the auditory system.

Methods: We modeled normal hearing by training a deep artificial neural network to recognize speech using simulated auditory nerve input from an intact cochlea. We modeled CI hearing by testing this same trained network on simulated auditory nerve input from a CI. To simulate possible consequences of learning to hear through a CI, we retrained this network on CI input. Further, to model the possibility that only part of the auditory system exhibits this plasticity, in some models we retrained only the late stages of the network.

Results: When the entire network was reoptimized for CI input, the model exhibited near-normal speech intelligibility scores. Performance on par with CI users was achieved only when just the late stages of the models were reoptimized (keeping the weights of the early stages unmodified).
Conclusions: Our results are consistent with the possibility that limitations on CI-mediated speech perception relate to incomplete plasticity that prevents the rest of the auditory system from optimally decoding CI input. Overall, our work validates deep neural networks with altered peripheral input as a candidate model of auditory perception in CI users and suggests that the difficulties of CI users could partly reflect plasticity limitations in the human brain, rather than being entirely due to impoverished auditory nerve representations from CI stimulation.

SA39. Speech Lateralization in Quiet and Noise With "Channel Specific" Mixed Rate Stimulation Strategy Using the CCi-Mobile Research Processor

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Category: Auditory Prostheses
Background: Bilateral cochlear implants (BiCIs) do not restore sound localization abilities to the full extent exhibited by typical hearing (TH) listeners, especially in noisy settings. This is partly because most clinical strategies adopt high-rate stimulation across all electrodes and do not encode interaural time differences (ITD) well. While high-rate stimulation is important for speech intelligibility, low-rate stimulation is needed for ITD sensitivity. Studies have shown that "mixed rate" strategies, combining low- and high-rate stimulations, could restore ITD sensitivity to a certain extent while maintaining good speech understanding. However, not all listeners receive the same benefits from using a "mixed rate" strategy, especially for listening in noise. We hypothesize that, for the "mixed rate" strategy to be effective, the channel selection needs to be customized for each individual such that the low-rate stimulation is delivered to the "best" site(s) with good ITD sensitivity in order to perceive a well-lateralized auditory object in noise.
Methods: BiCI listeners were tested using a bilaterally-synchronized portable research processor–the CCi-MOBILE. Within each individual, two "mixed rate" strategies were designed with a total of 10 electrode pairs in each. Initially, ITD sensitivity was determined for all electrode pairs along the electrode arrays. Then, the "best" and "worst" pair of electrodes were selected to receive the low-rate stimulation, while the remaining electrode pairs received high-rate stimulation, thus forming two "mixed rate" strategies. Using these two "mixed rate" strategies, as well as their clinical strategy, listeners were then presented with CNC words in quiet, or in speech-shaped noise. Each CNC word had a non-zero ITD while the noise always had an ITD of zero. Using a lateralization task, listeners were asked to indicate the intracranial position of the word.
Results: Preliminary data suggest that listeners may lateralize speech with better precision with the low-rate ITDs provided by the "mixed rate" stimulation (especially when the "best" electrode pair was used), as compared to when using the high-rate-only clinical strategy, regardless of background noise.
Conclusions: Results will be discussed in the context of whether participants could better lateralize speech both in quiet and noise using a "mixed rate" strategy, due to the addition of low-rate pulse-timing ITD. Additionally, it will reveal the possible impact of channel specificity on the effectiveness of the "mixed rate" strategy.

SA40. Detection of Cell Occupation on Cochlear Implants and Implant Position for Atraumatic Insertion and Long-Term Monitoring of Stimulation Efficiency

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Category: Auditory Prostheses
Background: Implant functionality can be significantly affected by cell occupation (fibrosis). For cochlear implants (CI), fibrocyte growth on the stimulation electrodes can lead to reduced electrical stimulation of the spiral ganglion cells, and thus loss of implant functionality (Foggia et al., 2019). Therefore, the objective of this project is the development of an impedance spectrometric method with commercially available CI electrode arrays for the recognition and characterization of cell occupation on CI stimulation electrodes. However, cochlear implants are particularly challenging, as each inserted CI has an individual connection

and electrode geometry to be considered when detecting cell occupation. Therefore, the development of a new method for predicting the CI electrode geometry, and thus CI position, is required. This method will also allow for monitoring of the CI position during insertion and long-term use to understand the possible loss of stimulation efficiency.
Methods: We measured impedances of commercially available CI electrode arrays immersed in electrolytes and particle suspensions of known electric properties, varying particle size, concentration, conductivity, and surface charge in a controlled manner (Blum et al., 1995; Maier, 1997). For comprehensive experimental investigations, both enlarged and full-scale physical electrode models are used together with corresponding physical cochlear models. The experimental data is used to validate finite element model simulations required to define in-silico model-based correlations between the CI electrode impedances and the CI position, cell occupation, and frequency. Finally, the concept of monitoring cell occupation of CI electrodes and implant position is validated with commercially available CI electrode arrays using full-scale physical cochlear models and cochlear explants.
Results: The very first in-silico model describing the electrode impedance in dependence of CI curvature and cell occupation is developed using FEM simulations. Experimental data, with enlarged CI electrode arrays on flexible printed circuit boards (used as enlarged physical CI models), were used to validate the FEM simulations. The instrumentation for impedance measurement of the commercially available CI electrodes immersed in particle suspensions was set up. Preliminary data of these commercially available CI electrodes (MED-EL, AB, Cochlea Ltd) are available.
Conclusions: More data will be collected will allow definite interpretation of the data.

SA41. Effects of Bone-Anchored Hearing Aids on Auditory Evoked Responses in Individuals With Single-Sided Deafness

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Category: Auditory Prostheses
Background: In individuals with single-sided deafness (SSD), the effectiveness of air-conduction hearing aids in overcoming asymmetrical hearing loss has been limited. Recently, as non-invasive bone-anchored hearing aids (BAHAs) have been developed, there is interest in evaluating the applicability of this amplification for SSD patients using cortical auditory evoked responses. The objective of this study was to determine if BAHA use alters the pattern and strength of cortical activities in SSD patients.
Methods: To see whether short-term BAHA use can yield neural and behavioral changes, 8 SSD subjects including 5 left-sided deafness and 3 right-sided deafness had used their BAHAs for 3 months. Electrophysiological and behavioral tests were administered 3 times at baseline, (pre-BAHA), the first day of BAHA fitting (1st-BAHA), and 3 months after BAHA use (2nd BAHA). Cortical activities were recorded during an active sound localization task. N1 and P2 responses were measured to discern the effect of BAHA use on the cortical activities at both sensor and source levels.
Results: Behaviorally, the reaction time during sound localization was shorter for 1st- and 2nd-BAHA compared to the pre-BAHA sessions. The use of BAHA substantially improved N1 amplitude; however, the improvement was diminished during 2nd-BAHA. The BAHA-induced changes in cortical activity were greater for the P2 than the N1 in that the P2 amplitudes increased gradually with BAHA use. At the source level, similar P2 enhancements with BAHA use were shown. Moreover, for pre-BAHA ipsilateral hemispheric dominance for the hearing side was found, while the P2 activity was altered more symmetrically in the brain after BAHA use. Brain-behavior relations showed that N1 source activation was positively correlated with better sound localization performances in individuals with SSD.
Conclusions: The results of this study suggest that BAHA use enables to induce the brain plasticity to reorganize the cortical mechanisms of spatial processing for sound localization in individuals with SSD. In addition, the P2 would be more responsive to BAHA-related neurophysiological changes than N1.

SA42. Characterizing Localization of Stationary and Moving Sound in Children Using Bimodal and Bilateral Cochlear Implant Devices

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Category: Binaural Hearing and Sound Localization
Background: The aims of this work were to determine whether: 1) children using bilateral devices have better perception of moving sound direction than localization of stationary sound and 2) children using bilateral cochlear implants (BCI) have better spatial hearing than bimodal device users. Children with hearing loss have poor sound localization and poor access to binaural cues. Hearing devices might be mismatched and could be provided too late to promote binaural hearing. Children with bimodal devices experience more device mismatch than peers with BCI. Hypotheses were two-fold: 1) binaural and spatial hearing is more compromised in children using bimodal devices than children using BCIs and 2) children with BCIs use head and eye movements more accurately than children with bimodal devices to find location of stationary and moving sounds
Methods: Localization of stationary and moving stimuli was measured using a 2-m x 2-m x 2-m soundbooth with a speaker fixed to the end of a moving arm (1-m in length) and concealed by a black curtain. Stimulus was band-pass filtered white-noise. Stationary stimuli were presented anywhere along a pseudorandom range within a 120° arc in the azimuthal plane and moving stimuli were presented with different angular distances (0°, ±20°, ±40°, where "+" indicates rightward and "–" indicates leftward). Root-mean-square error (RMSE) between stationary stimulus location over the 120° arc were calculated and response and logistic regression quantified perception of moving sound direction. Concurrent head and eye movements were collected in real time using wearable technology (camera-based eye-tracking and head-motion sensing mini-tracker). Movements were later binned by stimulus location or movement condition and analyzed relative to onset of stimulus presentation and stimulus offset to create displacement waveforms. These waveforms were quantified by area under the curve which is a measure of overall movement as a product of time, relative to each hemifield. Participants were 24 children with BCI [MAge(SD)=13.0(3.3)years], 34 children with bimodal hearing [MAge(SD)=12.3(3.3)years] and 6 children with typical hearing [MAge(SD)=12.8(1.3)years].
Results: Children with bimodal hearing and BCIs struggled to perceive location of stationary sound [$F(2,61)=18.2, p<0.001$] and direction of moving sound [$F(2,84)=273.8, p<0.001$]. Concurrent head movements revealed an interaction between group and speaker position for stationary sound presentation ($F(22,1319.7)=7.30, p<0.001$) and moving sounds ($F(8,543.9)=6.30, p<0.001$). Sensitivity to interaural level and timing differences in a task of lateralizing sounds was found to be greater in children with BCI (ILD: MSlope=0.77; ITD: MSlope=1.1) compared to bimodal peers (ILD: MSlope=0.06; ITD: MSlope=0.02) however this was not predictive of localization accuracy.
Conclusions: Results indicate that early hearing loss disrupts development of spatial hearing which is not resolved by hearing devices, bimodal devices cause mismatches which disrupt access to binaural cues relative to bilateral CIs and impaired perception of binaural cues carry over into spatial hearing.

SA43. Clinical Programming Can Reduce Access to Binaural Cues in Children With Bilateral Cochlear Implants: Evidence From Cortical and Behavioral Measures

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Category: Binaural Hearing and Sound Localization
Background: We aimed to measure access to binaural cues in children through their bilateral cochlear implants (CIs) using the cortical evoked potential acoustic change complex (ACC) response and behavioral lateralization. Bilateral CIs (BCIs) provide hearing advantages to children but spatial hearing remains impaired. Children with BCIs are sensitive to interaural level differences (ILDs) but these important binaural cues may be disrupted by mismatched dynamic ranges (DR) created by separate programming of left and right devices. We hypothesized that ILD sensitivity provided through children's bilateral CI processors will: 1) be detected by ACC amplitude, 2) be lateralized to one side, and 3) will be poorer for children programmed to have asymmetric levels between CI processors.
Methods: Participants were children who use BCIs (n=21, aged mean(SD)=14.0(3.4) years) and typically developing peers (n=6, aged mean(SD)=14.6(2.7) years). The BCI group were simultaneously implanted (n=15, aged mean(SD)=6.2(4.6) years) or sequentially implanted (n=6, aged mean(SD)=3.1(3.6) years at CI-1 with an inter-implant delay of mean(SD)=2.1(1.7) years). Electroencephalography (EEG) was recorded at

64-channels across the surface of the head, evoked by a 1 kHz pure tone with 100% amplitude-modulation at 40Hz presented at 60 dB SPL bilaterally through research processors at user settings in the BCI group and through insert earphones in the control group. EEG stimuli were first presented at ILD=0 for 1 s and then with an ILD (6 conditions presented in random order: ±4-, 10-, and 20-dB SPL, where + is right ear weighted and – is left ear weighted) for another 1 s. Participants completed a behavioral lateralization task using the same stimuli. Sensitivity to ILD was measured by ACC amplitude and rate of change in lateralization from left to right responses (gaussian slope).
Results: Clear cortical responses were obtained in both groups to the onset, change in ILD, and offset of sound at a central-midline electrode (CZ) referenced to the back of the head. Behavioral sensitivity to ILDs ranged from poor to good (slope range=0.1 to 5.0 (proportion of responses by ILD)). Difference in DR between right vs. left CI maps ranged from 0 to 25.4 (mean(SD) = 8.6(6.9)) current units (CUs). Preliminary analyses suggest increasing ACC amplitude to large ILD changes in both groups and reduced behavioral ILD sensitivity in children with large asymmetries in map levels. The relationship between ACC amplitude changes and map asymmetries are being assessed.
Conclusions: The ACC can quantify cortical detection of ILD cues in children with bilateral CIs. Further analyses will assess whether mapping strategies limit cortical sensitivity to ILDs and behavioral access to these important binaural cues.

SA44. An Indirect Method of Measuring Binaural Fusion in Humans and Animals

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Category: Binaural Hearing and Sound Localization
Background: The ability of a listener to understand speech in noisy listening environments can be impaired by abnormal binaural fusion. Binaural fusion is the perceptual integration of two or more sounds of different frequencies, presented to the two ears, into a single perceived sound. Listeners with hearing loss experience abnormally broad binaural fusion of sounds differing by 1-4 octaves in pitch, which is associated with difficulties understanding speech in noise (Reiss et al., 2017; Oh et al., 2019). To better understand the processes underlying binaural fusion, this project seeks to develop a method of measuring binaural fusion in an animal model. Currently, binaural fusion is measured in humans by asking if one or two sounds were heard. To study fusion in animals, however, an indirect measurement must be developed. One option is to utilize binaural masking level difference (BMLD, Deatherage et al., 1969), a phenomenon in which a tone is more audible under binaural than ipsilateral presentation of masking noise. We hypothesize that this phenomenon requires binaural fusion of the noise maskers since typically the same noise must be presented to both ears for masking release to occur. Here we pilot this method of measuring binaural fusion based on BMLD in humans.
Methods: Binaural fusion of noise bands was measured both directly and indirectly in normal-hearing (NH) listeners (N=5). First, binaural fusion range was measured directly, using a 1-interval 3-alternative forced choice (1I-3AFC) task. On each trial, a 1/2-octave noise band centered at 1000 Hz was presented in a reference ear and paired with a simultaneous 1/2-octave noise band of variable center frequency in the contralateral, comparison ear. Subjects were asked to indicate whether they heard one or two pitches, and, if they heard two pitches, which ear had the higher pitch. Second, binaural fusion range was measured indirectly using a BMLD paradigm, with a 4I-1AFC task. Again, 1/2-octave noise bands were presented on each trial, with the contralateral center frequency varied. A 1000 Hz tone was presented in the reference ear during one interval. Subjects were asked to report the interval they believed contained the tone.
Results: Preliminary findings suggest similar fusion ranges estimated using the two methods, with most participants having the greatest fusion and greatest BMLD at or near the reference frequency. However, there was some variability and asymmetries among listeners. In particular, BMLDs were small, and future work will investigate ways to optimize BMLD for fusion measurements.
Conclusions: Validation of a method of measuring binaural fusion in animal models will allow for observation of neural activity in areas such as the auditory cortex for stimuli known to be fused versus non-fused, with the potential to provide insights into the neural processes and the underlying mechanisms of binaural fusion.

SA45. Does Inappropriate Bilateral CI Stimulation Impair Binaural Hearing, and If So, Can It Be Rehabilitated?

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Category: Binaural Hearing and Sound Localization
Background: Early deafened patients with bilateral cochlear implants (biCI) experience difficulties in spatial hearing. This is mainly due to poor sensitivity to interaural time differences (ITD), one of two essential cues for spatial hearing. It has been suggested that a lack of early auditory experience may be responsible for this poor ITD sensitivity. However, we suspect the inability of today's speech processors to encode ITDs in the timing of their stimulus pulses to be responsible. In fact, most of clinical CI processors encode little or no temporal information in the timing of the electric stimulus pulses during normal use binaurally, resulting in inconsistent and uninformative delivery of ITD cues to the auditory system of these CI listeners. This motivated us to investigate whether uninformative pulse timing ITDs could prevent the development of normal ITD sensitivity in biCI recipients and, if so, whether this could be rehabilitated by training with synchronized stimulation delivering informative ITDs.
Methods: We implanted neonatally deafened rats with biCIs and trained them on a sound discrimination task meant to simulate demands placed on patients learning to use CIs for everyday tasks. The animals were required to discriminate amplitude modulated from unmodulated pulse trains at 900 pulses per second, but in addition received binaural cues. For one cohort, interaural level differences (ILDs), ITDs and amplitude modulated (AM) cues all systematically co-varied, providing a consistent and redundant set of auditory cues. The second cohort received the same consistent AM and ILD cues, but the ITDs on pulse timing were randomly selected from a ±500 µs range. After five weeks of training, the animals were tested for their ITD sensitivity and relative perceptual weighting of each cue to determine which of these cues they actively used to perform the task. This was followed by a four-week rehabilitation period in which all animals were exclusively exposed to informative, co-varying ILDs and ITDs before sensitivity and usability of ITDs were retested.
Results: Our results of these behavioral studies showed that ITDs were only used for sound lateralization if the biCI rats were presented with informative ITDs from the outset. In contrast, animals exposed to stimulation with random, uninformative pulse timing ITDs, mimicking the stimulation that patients fitted with clinical CI processors would experience, showed a much-reduced ITD sensitivity. Importantly, this could be restored through rehabilitation training.
Conclusions: Overall, our results suggest that ITD sensitivity may decline, or even be lost, if binaural CIs are fitted which do not routinely provide informative pulse timing ITD cues. When presented with CI stimulation containing only useful, coherent ITDs, all rats achieved good ITD sensitivities at a clinical pulse rate.

SA46. Development and Validation of a French Screening Speech-In-Noise Self-Test, in Synthetic Voice and Antiphasic Presentation

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Category: Binaural Hearing and Sound Localization
Background: Conventional pure tone audiometry is currently the gold standard for evaluating and screening hearing impaired people. In order to perform global screening in the population, certain constraints such as the use of a soundproof booth, qualified personnel, or calibrated equipment slow down its implementation. Nevertheless, difficulties in understanding in noise are considered by most people to be the greatest handicap associated with hearing loss. Speech-in-noise test has the advantage that supraliminal measurement of the speech recognition threshold (SRT50) does not necessarily require calibration of speech and noise levels. This is therefore easier to use for mass hearing screening.
Methods: SoNoise is a tablet based speech-in-noise self-test, using calibrated bluetooth headphones. The sentences presented to the subject consist of a combination of three words (number-noun-color). Various

SoNoise tests were developed with natural and synthetic female voices, and in diotic and dichotic (antiphasic) versions in order to benefit from binaural masking. The words used were equalized in intensity with 83 normal hearing listeners in order to harmonize the probability of recognition of each word. In the first study, we compared SRT50 with natural and synthetic voices with 38 normal hearing subjects. In the second, we measured binaural masking level difference with 46 normal hearing. In the third, we standardized the SoNoise synthetic antiphasic version with 216 normal and hearing impaired subjects. None of the participants had ever done any SoNoise test before.
Results: For the diotic version, the results show that there is no significant difference between the SRT50 measured in natural speech and synthetic speech, with -9.96 (SD 1.32) and -10.07 dB SNR (SD 1.34) respectively. For the dichotic antiphasic version, a standard of -17.56 dB SNR (SD 1.49) is determined. The binaural masking level difference measured was 7.5 dB with normal hearing. With normal and hearing impaired, we found a strong correlation between the pure tone average (PTA) and the SoNoise synthetic antiphasic version. ROC curves were then calculated. For a PTA of 20 dB HL, the threshold value is -14.5 dB SNR and corresponds to a sensitivity of 87% and a specificity of 87%. For a PTA of 30 dB HL, the threshold value is -13.7 dB SNR and corresponds to a sensitivity of 89% and a specificity of 90%. The test lasts around 3 minutes.
Conclusions: The SoNoise test in its synthetic and antiphasic version is a fast, playful and reliable tool to screen hearing impaired people at 20 and 30 dB HL cut-offs.

SA47. Sound Localization Cues in Single-Sided Listeners

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Category: Binaural Hearing and Sound Localization
Background: In single-sided deafness (SSD), binaural hearing cues are disrupted, leaving listeners only to rely on monaural pinna and head-shadow cues. Despite lack of binaural input, some single-sided listeners are able to use monaural cues to improve their localization abilities. Evidence suggests this is due to remapping and/or reweighting of monaural cues, although the contributing factors and the extent to which this can be leveraged in real world listening remains unclear. Cochlear implants (CI) for SSD provide increased opportunity to provide binaural input, but also remain limited in their ability to fully restore timing and level difference cues associated with true binaural hearing.
This study examines the localization abilities and use of localization cues in congenitally deafened SSD listeners, acquired SSD listeners, and SSD listeners utilizing a CI.
Methods: Sound localization was evaluated in three listening groups: congenital/early-onset SSD, acquired/adult-onset SSD, and adult-onset SSD with CI. Localization was evaluated for +/- 90˚ azimuth and +/- 30˚ in elevation using 150 ms gaussian white noise bursts which were broadband (0.2 – 20 kHz; 50, 60, 70 dBA), high-pass (3 – 20 kHz; 60 dBA), and low-pass (0.2 – 1.5 kHz; 60 dBA) filtered. Sound localization accuracy and its associated reaction time were obtained through natural head-orientation.
Results: Localization abilities were significantly poorer in the monaural listening conditions than for normal hearing. SSD listeners preserved their localization abilities in elevation at the normal hearing side. Mean absolute error improved significantly when using a CI, although remained poorer that that observed in normal binaural hearing. Congenitally unilateral deafened listeners showed improved localization performance compared to acquired SSD, but poorer than those using a CI. Reaction times decreased towards the impaired or CI side.
Conclusions: SSD listeners take advantage of monaural spectral and head-shadow cues to improve spatial hearing abilities in the absence of treatment. At the group level, improved outcomes are observed in the CI listening condition, although significant inter subject variability in performance is observed.

SA48. The Interaural Phase Modulation Response in Typical Hearing 7-Month-Olds

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Category: Binaural Hearing and Sound Localization
Background: Binaural hearing, the ability to integrate information between the two ears, allows us to hear in complex acoustic environments and is critical for sound localization. To acquire language, infants must distinguish between noise and meaningful auditory objects such as speech in noisy, real-world

environments. However, the development of binaural processing is not well understood. In this study, we investigate the neural encoding of interaural timing differences (ITDs) in 7-month-olds using electroencephalography (EEG). By 7 months, infants show sophisticated auditory skills, including speech discrimination abilities; thus, we hypothesize that we will see robust neural encoding of ITDs at this age.
Methods: Participants in this study were 22 typical-hearing 7-month-olds. Using a 32-electode Biosemi Active Two EEG system, an auditory steady-state response to periodic interaural phase modulations (IPM) in an ongoing amplitude modulated (AM) tone, called the interaural phase modulation following response (IPM-FR) was recorded. The stimuli consisted of a 520-Hz carrier tone that was sinusoidally amplitude-modulated at a rate of 81.6 Hz and a depth of 100%. To generate an IPM, the sign of the carrier is periodically modulated between leading in the right and left ear at a rate of 6.8 Hz. Infants passively listened to two IPM depth conditions (±90°, 0/180°) with tones presented at 65 dB SPL via Etymotic ER-2 insert ear tips in a sound attenuated booth. The stimuli were presented continuously for about 5 minutes in each condition for a total recording time of about 10 minutes. A subset of these participants (n=9) was also tested at 75 dB SPL to investigate the effect of stimulus level. EEG data processing included the removal of poor electrode contacts, de-noising using spatial filtering, then transformation into the frequency-domain via a fast Fourier transform to track the IPM rate of 6.8 Hz.
Results: Preliminary analyses show that statistically significant (Hotelling's T-squared test, $p<0.05$) IPM-FRs were recorded in at least one IPM depth in the majority of infants tested (20 of 22). Similarly, at 75 dB SPL, statistically significant IPM-FRs were recorded in 7 of 9 infants in at least one IPM depth condition.
Conclusions: These preliminary findings show IPM-FRs recorded in the majority of infants tested, suggesting that the neural encoding of ITDs at these two ITDs (±90°, 180°) is functional by 7 months. Future work should include younger infants to characterize earlier stages of binaural processing. Ultimately, understanding the neural mechanisms that support binaural hearing and their developmental trajectory may improve our ability to identify children at risk for listening and learning difficulties, especially in noisy, real-world environments.

SA49. Binaural Processing – Fast, Sluggish, or Both?

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Category: Binaural Hearing and Sound Localization
Background: Mammals are remarkably sensitive to interaural time differences (ITD) with just-noticable differences as small as 20 μs. This enables them to unmask signals that differ in their interaural configuration from the masker. However, in multiple binaural tone-in-noise detection experiments, detection thresholds have been observed to be elevated if short tones are presented within about 100 ms temporal proximity to a change in the interaural configuration of the masker. Thus, binaural processing in general has been termed "sluggish". On the contrary, a similar number of more recent other studies can only be explained when assuming fast binaural processing. Examples are (1) accurate localization or lateralization based on single source-dominated glimpses of less than 10 ms duration embedded in longer segments of uninformative ITDs, and (2) detecting modulation of interaural correlation or phase at rates of 100 Hz and beyond.
In this study, we investigate whether or not those apparently contrasting observations about the binaural processing speed can be explained with a single "intermediately long" temporal integration window.
Methods: For the relevant experiments of both the "fast" and the "sluggish" categories, we computed the interaural correlation coefficient of the bandpass-filtered signals as resulting from double-sided exponential temporal integration windows from 0 to 200 ms. To infer human sensitivity from the resulting changes in interaural correlation, we compared them to reference data concerning correlation discrimination.
Results: To obtain interaural correlation changes of discriminative magnitude as a response to rapidly alternating ITDs, a time constant smaller than 10 ms is needed. This is not in line with the time constant of at least 30 ms required to explain a binaural unmasking threshold elevation for a target presented 80 ms apart from the a change in masker correlation.
Conclusions: Binaural cues appear to be available with a high temporal resolution. As in other, non-binaural detection experiments, the apparent sluggishness in some binaural experiments is thought to origin from rebuilding of auditory object representations due to a change in the interaural statistics.This could potentially be simulated as a varying interaural correlation sensitivity, adapting with a time constant of about 30 ms.

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SA50. Proofreading Workflow for the Semantic Segmentation of Mitochondria in Serial Section Electron Microscopy Image Volumes

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Category: Brainstem: Structure and Function

Background: The acquisition of volume electron microscopy images provides an opportunity to study at high-resolution, in unbiased manner, the numbers, geometries and placement of subcellular organelles within cells. Manual segmentation of the resultant large populations of organelles is intractable, so auto-segmentation using machine learning (ML) approaches have been applied to the task. ML approaches are not error-free and require efficient proofreading. We describe here a method for rapid proofreading for segmentations of mitochondria.

Methods: Anisotropic serial blockface scanning EM (SBEM) image volumes of 98 µm x 78 µm x 68 µm of the medial nucleus of the trapezoid body (MNTB) of a 6-day old mouse were collected. Mitochondria probability maps were then obtained using CDeep3M2, a containerized U-Net, trained to segment voxels of 3D SBEM volumes containing mitochondria. The probability maps were thresholded, with connected components determined on the resulting binary 3D image volume. The connected components represent objects properly classified mitochondria, misclassified as mitochondria, or multiple mitochondria (as many as 20) grouped into a single object. The latter two errors require correction prior to additional analysis. We determined a proofreading process on binary images (a voxel is classified as mitochondrion or not mitochondrion), by raising the probability threshold for prediction that a voxel belonged to the class mitochondrion, and removing voxels that erroneously merged objects. Each connected component was subjected to marker assisted binary watershed across a set of increasing probability thresholds from 50% to 94%. This set of results created a tree for semantic segmentations.

Results: We implemented procedures for human proofreading by creating a custom widget add-on to Napari, an open source tool for image handling. The user interface presented a series of decisions about which branch of the tree was correct and/or if the resulting single-branch segmentation was indeed a valid mitochondrion. Since our interest is in scalability of a task to volumes that contain 10^5 - 10^6 mitochondria, we measured task and decision times as well as segmentation accuracy. Ten trained human proofreaders assessed segmentation accuracy on 1,520 connected components of varying complexity, and in the process separated grouped mitochondria into individual elements. The time to complete individual evaluations was as low as 0.86s. For merged mitochondria, the mean time per user to indicate thresholds for separation into smaller objects varied from 13.58 to 30.81s with complexity. Concurrence between users evaluating the same test set ranged from 88.8% to 93.4%.

Conclusions: These data indicate that simpler objects need not be proofread, which will significantly reduce total time for and scalability of proofreading.

SA51. How Small Frequency and Level Shifts in Background Stimulation Induce Prepulse Inhibition of the Acoustic Startle Response

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Category: Brainstem: Structure and Function

Background: Testing animals for the ability to discriminate between stimulus levels or frequencies at the behavioral level mostly requires time-consuming training. Here we explore the possibilities of applying a reflex-based behavioral method for a more time-efficient measurement of discrimination abilities. The acoustic startle response is elicited in response to sudden loud acoustic stimuli with reflexive contraction of skeletal muscles. A preceding weaker acoustic or non-acoustic stimulus can reduce the startle response amplitude, a phenomenon called prepulse inhibition (PPI). This procedure does not require training of animals.

Methods: We tested rats in a modified behavioral paradigm combining a continuous background stimulus with a shift in frequency or stimulus level acting as a startle-modifying prepulse (= shift-prepulse). A thorough characterization of the effects of shift-prepulses in twelve rats was performed by systematically changing the following three stimulation parameters: i) frequency of background stimulation, ii) step size

and direction of shifts and iii) timing of the shift-prepulse. For investigating level changes, step sizes up to ± 15 dB were tested, starting from background levels of 65 and 75 dB SPL. Frequency shifts were tested in the range of ± 1% up to ± 30% around background frequencies of 8 and 16 kHz.
Results: Change-induced PPI increased with change size for both, frequency and stimulus level shifts. Maximal inhibition depended in both cases on background frequency, with strongest inhibition occurring at the lowest frequency tested (8 kHz). Level shifts starting from a background of 65 dB SPL yielded significantly lower inhibitions than shifts from 75 dB SPL. Level increases led to significantly higher inhibition values than level decreases. Frequency shifts were tested with different timings of the shift-prepulse. Highest inhibition values were found for shift-prepulses clearly separated from the startle stimulus (80 ms shift duration, starting 130 ms before startle pulse) compared to shift-prepulses lasting until the start of the startle pulse. Dependent on timing and background frequency, different thresholds for eliciting significant inhibition were found with lowest thresholds as small as ± 2% around 8 kHz for shift-prepulses starting 130 ms before the startle pulse, lasting 130 ms or 80 ms. Contrary to the findings for level shifts, no effect of the shift direction was found in the frequency shift paradigm.
Conclusions: In summary, the prepulse inhibition paradigm optimized this way allows for a fast and reliable characterization of auditory discrimination and detection thresholds. Even small changes in background stimulation parameters can induce strong PPI. This can be applied to determine suprathreshold hearing deficits after acoustic trauma. In addition, these paradigms are well suited to investigate the neuronal basis of prepulse processing by combining behavioral with electrophysiological measurements.

SA52. Gap Junctions' Effect on Excitability and Firing Behavior of a Biophysically Detailed Neural Network Models of Bushy Cells

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Category: Brainstem: Structure and Function
Background: Bushy cells (BCs), one of the main cells of the ventral cochlear nucleus (VCN), enhance the synchronization behavior seen in auditory nerve fibers (ANFs). BCs reside in clusters where, in addition to receiving excitatory input from ANFs, they also receive excitation from other bushy cells within the cluster via gap junctions. These gap junctions have been proposed as a potential mechanism for the synchrony enhancement behavior of spherical BCs that receive relatively few ANF inputs.
Methods: In this study, principal cell models of VCN are implemented based on Xie-Manis (2013) as modified version of Hodgkin-Huxley type models. The spread of the characteristic frequency of incoming ANF inputs and the excitatory post synaptic current (EPSC) amplitude and time constants are estimated from the literature. Gap junctions are implemented as conductances (g_gap) that directly connect the intracellular spaces of the bushy cell models. Structures such as fully connected networks and clusters linked by a common neuron are built to inspect the gap junctions' effect on the excitability of individual cells and the spread of excitation between the BCs. Current injection inputs are used to understand how excitation spreads through the cells and how the fundamental dynamics of the network depend on the value of g_gap. ANF inputs are provided to evaluate the network's response to more physiologically relevant stimuli.
Results: For both current injection simulations and ANF synaptic inputs, as the value of g_gap is increased, the amplitude of BC action potentials (APs) decreases. Within a range of g_gap values, the cells help each other fire APs, whereas if g_gap is too large, all cells stop firing even though one of them receives high enough input to cause an AP in the absence of gap junctions. Preliminary simulations with ANF synaptic inputs indicate that multiple APs may still occur within one cycle of a low-frequency stimulus, in contrast to the physiological data. Inhibitory inputs from D-stellate and tuberculoventral cells within the network may therefore also be required to generate synchrony enhancement.
Conclusions: Gap junctions have profound effects on the excitability and firing behavior of modeled BCs. The spread of excitability may be a part of the mechanism that enhances the synchronization behavior seen in ANFs, but the gap junction conductance and strength of inhibitory inputs needed for such enhancement is still under investigation.

SA53. Repopulating Microglia Reinstate Developmental Functions in Auditory Brainstem Circuit Refinement

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Category: Brainstem: Structure and Function
Background: Neural circuit formation is achieved through multipartite signaling between neurons and glia. The brain's primary immune cells, microglia, are involved in the development and maturation of connections in the auditory brainstem. During the first postnatal week, microglia populate the brainstem, appearing in the ventral cochlear nucleus by postnatal day (P) 0 and in the medial nucleus of the trapezoid body (MNTB) by P6. At first, microglia display an ameboid morphology, characterized by large cell bodies and short branches. Just after hearing onset, microglia increase in number and display more complex branching and smaller somata. Eliminating microglia through treatment with a colony stimulating factor-1 receptor inhibitor, BLZ945, in the first ten postnatal days results in impaired calyceal pruning and diminished levels of glial fibrillary acidic protein (GFAP), a marker for mature astrocytes, in the MNTB. BLZ945 treatment also results in elevated auditory brainstem response (ABR) thresholds, decreased amplitudes, and increased ABR inter-peak latencies. Following the cessation of treatment, microglia return in a lateromedial progression, as we observed during normal development. The repopulation of microglia is associated with improvement in all of the defects caused by BLZ945 treatment. Once microglia fully repopulate the brainstem, at four weeks of age, monoinnervation is restored in MNTB, but GFAP levels remain diminished. By seven weeks of age, following an extended period of microglial return, GFAP levels are comparable to controls and ABR amplitudes and inter-peak latencies largely recover. Interestingly, these functions do not recover with continuous microglial elimination. These findings point to a role for microglia in circuit sculpting in the auditory brainstem and suggest that repopulating microglia take on characteristics of normally developing microglia.
Methods: To test this hypothesis, we compared morphology and phagocytosis in developing and repopulating microglia. We used CX3CR1+/EGFP mice in which microglia express enhanced green fluorescent protein. We characterized microglia at different ages in control and BLZ945-treated mice. Microglial size, complexity, and lysosomal marker CD68 were measured through 3D reconstruction using Imaris software.
Results: We found that compared to age-matched control mice, BLZ945-treated mice show decreased microglial surface area, volume, number of branches, and branch length prior to complete microglial repopulation. Once microglia reoccupy the brainstem, these microglial characteristics are comparable to controls. Next, we tested whether microglia engulf synaptic protein material during a period of circuit recovery. Using high resolution confocal imaging and surface reconstruction, we found that microglial CD68 contains excitatory and inhibitory pre-synaptic protein markers.
Conclusions: Together, these data suggest that repopulating microglia acquire the form and function of developing microglia, allowing them to carry out synaptic pruning at late postnatal ages.

SA54. Synaptic Drive Onto Inhibitory and Excitatory Principal Neurons of the Mouse Lateral Superior Olive

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Category: Brainstem: Structure and Function
Background: The lateral superior olive (LSO) nucleus in the brainstem is critical for horizontal sound localization. LSO principal neurons (PNs) compare excitatory inputs driven by the ipsilateral ear with inhibitory inputs driven by the contralateral ear. There are distinct glutamatergic excitatory and glycinergic inhibitory LSO PNs types that do not overlap (Mellott, et al, 2021; Ito, et al, 2011), however, interactions between transmitter type differences in intrinsic properties and their synaptic inputs have not been explored.
Methods: We examined inhibitory and excitatory LSO PNs in mouse brainstem slices using whole-cell patch-clamp and two-photon imaging. To target specific cell types, we used knock-in reporter mice that co-express the fluorescent protein tdTomato with vesicular glutamate transporter 2 (vGlut2). PNs were selected based on size, shape, and electrophysiology. Glutamatergic PNs had prominent somatic fluorescence. Other PNs exhibited a distinct lack of fluorescence and were deemed inhibitory. We assessed intrinsic membrane properties in current-clamp mode and recovered morphology for most of these cells. We also analyzed spontaneous and minimal evoked excitatory post synaptic currents (EPSCs) in separate voltage-clamp experiments.
Results: Inhibitory LSO PNs had larger input resistances (61% difference, $p<0.0001$) and correspondingly lower rheobase (40% difference, $p<0.0001$). This difference in activation threshold between LSO PN transmitter types may provide an additional means to organize information in upstream processing centers,

however, synaptic drive could accentuate or offset these differences. Preliminary data for minimal evoked EPSCs suggests inhibitory LSO PNs have smaller amplitudes (I:-87.9±14.3pA, n=3; E:-355.6±182.2pA, n=5, p=0.27) and faster kinetics (10-90%rise, I:0.34±0.03ms, E:0.67±0.17ms, p=0.18; decay τ, I:0.75±0.03ms, E:1.55±0.17ms, p=0.38). Paired pulse ratios were not different at 10 ms interval (I:0.995±0.05ms, n=2, E:0.95±0.42ms, n=3, p=0.94). Spontaneous EPSC amplitudes (I:-23.4±2.5pA, n=7, E:-19.7±2.2pA, n=5, p=0.31) and kinetics (10-90%rise, I:0.59±0.04ms, E: 0.58±0.04ms, p=0.85; decay τ, I:0.44±0.03ms, E:0.46±0.04ms, p=0.78) were not different. However, excitatory LSO PNs had higher spontaneous EPSC frequency suggesting they may have more excitatory synapses (I:9.5±2.1; E:27.6±7.9, p=0.025). Consistent with this hypothesis, excitatory LSO PNs also had higher dendritic branching complexity than inhibitory ones with larger total dendritic length (I:426.90 ±28.09µm, n=29, E:603.70 ±49.35µm, n=41, p=0.007), more primary dendrites (I:3.52 ±0.20, E:4.41 ±0.26, p=0.013), and more dendritic branch points (I:2.41 ±0.27, E:3.83 ±0.48, p=0.025). Results are reported as mean ±SEM and compared using t-test at alpha-level of 0.05.

Conclusions: These preliminary synaptic data suggest that inhibitory LSO PNs have weaker synaptic drive potentially offsetting transmitter type differences in activation threshold. Findings also suggest excitatory LSO PNs may be better tuned for integrative sound localization functions with a larger number of similarly sized synapses, but this may depend more on the number of independent fibers. Further experiments will increase sample size, examine stimulus pulse trains, test for differences in inhibitory synaptic inputs, and acquire miniature synaptic events with TTX application.

SA55. Electrical Signaling in Cochlear Efferents Driven by an Intrinsic Neuronal Oscillator

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Category: Brainstem: Structure and Function

Background: Efferent neurons are believed to play essential roles in maintaining auditory function. However, lateral olivocochlear (LOC) neurons are poorly understood. Here we present an electrophysiological analysis of identified LOC neurons in juvenile and young adult mice. We found that LOC neurons display a slow rate of spike burst firing driven by an intrinsic Ca2+-dependent oscillator. LOC neurons receive excitatory inputs from T-stellate cells in the cochlear nucleus (CN) and pyramidal cells in the auditory cortex. Their inhibitory inputs originate from MNTB. Both inputs function to regulate the patterned activity of LOC neurons.

Methods: Two groups of mice were used, P9-11 and P17-42 mice. We crossed ChAT-IRES-Cre transgenic mice with a tdTomato reporter line, in order to visualize cholinergic LOC neurons within the lateral superior olive. GCamp6f- (for calcium imaging) or channelrhodopsin-expressing (for optogenetic and whole-cell patch-clamp experiments) adeno-associated virus (AAV) was injected into the brain. LOC neurons filled with 1% biocytin were reconstructed with Neurolucida.

Results: LOC neurons were disc-shaped neurons having extensive dendritic expansion along the rostrocaudal axis but rather restricted on the mediolateral axis. Calcium imaging of LOC neurons revealed extremely slow (~0.1 Hz) waves of Ca2+ activity in ~90% of LOC neurons. Both cell-attached and whole-cell current-clamp recordings unveiled bursts of Na+ spikes lasting for seconds interleaved by lengthy silent periods – a patterned activity resembling the Ca2+ signal periodicity. Bursts persisted with the blockade of AMPA receptors and voltage-dependent sodium channels but was abolished by L-type Ca2+ channel antagonist, indicating an intrinsic oscillator dependent on L-type Ca2+ channels. Such activity was not observed at P9-11.

When an anterograde AAV was injected to the CN, light flashes evoked excitatory postsynaptic currents (EPSCs) from the ipsilateral LOC neurons. These EPSCs were blocked by GYKI, and their I-V relations showed little inward rectification. Both observations indicate GluA2-containing postsynaptic AMPA receptors. EPSCs of similar properties were also observed by injecting a retrograde AAV into the inferior colliculus that expressed channelrhodopsin in T-stellate cells, indicating that T-stellate cells play a major role in the CN projection to the LOC. Surprisingly, injection of the same anterograde AAV into the auditory cortex also resulted in EPSCs in the LOC neurons. Moreover, electrically stimulating MNTB evoked robust inhibitory postsynaptic currents (IPSCs) in LOC neurons. Finally, injecting a simulated inhibitory conductance into LOC neurons curtailed the bursts and hence increased the burst frequency. Vice versa, injection of excitatory conductance interrupted the silent periods and reset the burst pattern.

Conclusions: Taken together, we suggest that LOC neurons are driven by a potent intrinsic oscillator, and synaptic inputs act as "light switches" to control the features of these oscillations. Such patterned activity could be important in driving release of diverse transmitters from LOC nerve terminals in the cochlea.

SA56. Human Spinocerebellar Ataxia Type 13 (SCA13) Causes Prolonged Action Potentials in LSO Neurons

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Category: Brainstem: Structure and Function
Background: SCA13 is a neurodegenerative disease characterized by pleiotropic deficits, including motor coordination and sound localization. SCA13 is caused by a single point mutation in the KCNC3 gene which encodes for the Kv3.3 voltage-gated potassium channel subunit, which are highly expressed in cerebellar Purkinje neurons and the auditory brainstem. This late-onset SCA13 serves as a distinct single-factor-origin model to study common cellular mechanisms of hearing loss and neurodegeneration. Kv3.3 containing channels enable rapid repolarization, fast action potentials (APs) and high firing rates. Studies from SCA13 patients carrying the R420H mutation suggest cerebellar Purkinje neurons and neurons of the sound localization pathway to be most impacted by the mutation1,2. We have generated an R420H mouse model of SCA13 to address two questions: 1) Are sound localization deficits caused by neurodegeneration in the auditory brainstem? 2) Are the passive and active properties of neurons changed in the in the sound localization pathway, e.g. lateral superior olive (LSO)?
Methods: The ataxia phenotype of SCA13 correlates to significant cerebellar atrophy in the second to third decade of life in humans. However, significant auditory deficits were often reported before the onset of ataxia. Therefore, neuronal deficits or degeneration might be detectable at an earlier age in auditory neurons. Accordingly, we performed histological and electrophysiological experiments at 1, 3 and 6 months of age, comparing R420H mice to wild type littermates. Immunohistochemical markers of neurodegeneration such as cell size and p62, were monitored prior to apoptosis; as such markers could indicate the interplay of the unfolded protein response, autophagy and ubiquitinated proteins. Finally, Nissl staining was used to count neuron numbers and assess cell loss. In electrophysiological experiments whole-cell patch-clamp recordings are performed from acute brainstem slices of R420H mutants and littermate controls at the same three ages.
Results: Histological data from 3-month old R420H mutants suggest LSO neurons do not show the extent of degeneration compared to the known cerebellar atrophy. However, LSO neuronal soma diameter was reduced from 22 ±4µm in wild type to 16 ±2µm in R420H mutant mice (mean ±SD; n=10 neurons/genotype; age 3 months). The electrophysiological data revealed a prolongation in AP half width from 0.18 ±0.01ms in the wild type to 0.69 ±0.33ms in the R420H mutant (mean ±SD; n=3 neurons/genotype; age 3 months) measured in response to stimulating excitatory synaptic inputs.
Conclusions: The R420H mutation causes a more than 3-fold increase in LSO neurons' AP duration, which may reduce firing rates in vivo and thus effect processing of interaural level differences. Additionally, reduced activity could also relate to the reduced cell diameter observed in R420H LSO neurons as an early sign of neurodegeneration.

SA57. An Integrated Physiological, Morphological and Transcriptomic Atlas of the Mouse Cochlear Nucleus

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Category: Brainstem: Structure and Function
Background: The cochlear nucleus (CN) is the termination site of the auditory nerve and initiates all central auditory processing. A variety of key cell types and their physiological and anatomical properties have been studied over the past 100 years. However, in order to explore how the CN develops and is altered in disease, an unbiased, complete description of cell types and their gene expression is needed.
Methods: Here, a massively parallel single nucleus RNA-sequencing (snRNA-seq) was performed using micro-dissected CN tissues to generate a comprehensive taxonomy of transcriptomic cell types. To register molecular cell types defined by transcriptomic profiling to known CN cell types, we used Patch-seq, an approach combining patch-clamp recording, morphology recovery, and single-cell RNA-sequencing on CN

neurons. Spatial expression patterns of gene markers were further validated with dual-color fluorescent in situ hybridization (FISH) in wild-type and transgenic mouse lines.
Results: From a population of ~31,000 snRNA-sequenced CN neurons, we identified 13 molecular types, including 6 major excitatory and 7 major inhibitory types across the dorsal and ventral CN divisions. In parallel, patch-seq recording across distinct CN subregions gave access to the transcriptome of major classically-defined cell types, which were assessed by their electrophysiological and morphometric parameters. By mapping the transcriptomic data from the patch-seq'd cells to the snRNA transcriptomic clusters, we were able to assign cell type labels to the major molecular cell types in a bijective (one-to-one and onto) fashion. We validated this multi-modal correspondence and identified dozens of novel markers using methods that match molecular expression to electrophysiology, morphology, and locations. Previously described type-specific gene markers were largely consistent with the major clusters. Roughly 65% of isolated nuclei were from auditory granule cells, while over 10% were from unipolar brush cells, confirming these as major cell classes in auditory function. Of special interest were cell classes defined by well-known physiological/anatomical criteria that were distributed across multiple nearby clusters, indicating that the traditionally defined cell types were composed of multiple subtypes. For example, bushy cells were distributed into two molecularly distinct groups with different electrophysiological and morphological properties and were differentially distributed across the ventral CN. Similarly, cells classified as T-stellate cells appeared in two distinct groups based on gene expression, firing properties, and cell-body locations.
Conclusions: This study provides a complete molecular profiling of the cell classes of the mouse CN, including a correlation of gene expression to electrophysiological and anatomical features at the single-neuron level. We reveal a hitherto unrecognized diversity in cell sub-types, and elucidate prominent gene markers for the array of cell types. Mouse cre-lines based on these markers will enable more precise identification of CN neuronal subtypes, including in vivo cell type functions in sound processing and how they are altered following hearing loss.

SA58. Specialized Potassium Channel Properties Help Define the Tonotopic Gradient in the Avian Cochlear Nucleus Magnocellularis

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Category: Brainstem: Structure and Function
Background: Synchronized ionic currents play an essential role in supporting fast and precise signal processing within the auditory brainstem. The avian cochlear nucleus magnocellularis (NM), an analogous structure to the mammalian anteroventral cochlear nucleus (AVCN), has a specialized tonotopic gradient. Neurons in the rostromedial region receive medium- to high-frequency sound inputs via large endbulb of Held synapses. Conversely, NM neurons in the most caudolateral region (denoted NMc) receive low-frequency inputs via small synaptic boutons. These NMc neurons have very distinct structural and functional differences compared to "traditional" higher-frequency NM neurons (Hong et al., 2018). Aside from synaptic differences, the expression of different types of intrinsic potassium channels and their resulting currents are also unique compared to higher frequency NM regions, but more specific details into the functions of these differences remain unknown.
Methods: Using whole-cell patch clamp electrophysiology on ex vivo chicken embryo brainstem tissue (embryonic [E] ages E19-E21) , I recorded tail currents in response to a range of voltage clamp commands to further investigate intrinsic potassium channel properties across the tonotopic gradient. Voltage commands were very short in duration to mimic more naturalistic synaptic inputs and to avoid experimentally confounding buildup of ions near and around the neuron (Rathouz and Trussell, 1998). These experiments utilized PTX, APV, CNQX, and TTX to block both synaptic and voltage-gated sodium currents, respectively.
Results: We determined that NMc neurons exhibit a potassium reversal potential that deviates from that of higher-frequency NM neurons, reflecting a change in driving force and total potassium current magnitude in response to varying voltage commands. In addition, the calculations for rates of activation and deactivation affirm the unique potassium channel properties that are distinct between the populations of NM and NMc neurons.
Conclusions: These data provide evidence for the specialization of potassium channel currents that support the respective firing properties of NM and NMc neurons across the tonotopic gradient. Future work will

investigate how altering the developmental trajectory of NM may impact these potassium channel properties, which would influence neuronal excitability.

SA59. Species-Specific Morphometry of Structural Compartments of MNTB Principal Neurons

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Category: Brainstem: Structure and Function

Background: Neurons in the medial nucleus of the trapezoid body (MNTB) are glycinergic, globular cells dominated by somatic excitation, which precisely relay information to elicit a rapid feedforward inhibition. Since the MNTB can be addressed in all mammals investigated so far, this circuit appears evolutionary highly conserved. Moreover, several features including cell size are supposed to vary along the tonotopic axis of the MNTB. The dendritic compartment of these neurons is thought to support action potential generation by sodium conductance and to serve as a current sink to accelerate EPSPs. Most of this detailed knowledge originates from mice and rats, while this structure exists in every mammal investigated so far. A detailed reconstruction of the dendritic, somatic and initial axonal compartment of MNTB neurons, and their species-dependency is lacking.

Methods: We have analysed the structure of single cell labelled MNTB neurons from five different species and used immunofluorescence to determine somatic arrangements in a total of eight species. The sampled species include mouse lemurs, tupaia, guinea pig, two bats, mouse, gerbil and Etruscan shrew.

Results: We find that average soma size is correlated with brain size and that it increases with tonotopic frequency in tupaia, gerbil, mouse and Etruscan shrew, while it tends to decrease in both bat species and guinea pig. The average number of primary dendrites is lowest in bats and highest in gerbil. In all species, dendritic arbours extend beyond the MNTB boarder defined by VGluT-positive calyx of Held synapses. Sholl analysis reveals dendritic distances from the soma of more than 200 µm even in Etruscan shrews. Only in gerbils the complexity of the dendritic arbour, its orientation and shape are correlated with the tonotopy. The initial dendritic segment before the first branch point is longest in bats, and generally no correlation with brain size was observed. Across species, the diameter of the initial dendritic and axonal segment is correlated with soma volume, suggesting an adjusted current sink coupling. Dendritic and unexpectedly axonal compartments seem to receive synaptic inputs, judged by overlapping fluorescence of VGluT1 and GlyT2 labelling with the cellular marker.

Conclusions: Taken together, dendrites of MNTB neurons are large, highly complex and receive different synaptic inputs. The detailed analysis shows species-dependent differences in MNTB arrangement and their neuron’s morphology. These differences cannot all be explained by differences in brain size, indicating species-dependent adaptations in this highly conserved brainstem structure.

SA60. Consequences of Perinatal Nicotine Exposure on Functional Development of a Central Auditory Synapse

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Category: Brainstem: Structure and Function

Background: Prenatal exposure to cigarette smoke increases the risk of sudden infant death syndrome as well as auditory processing deficits in the brain that persist into adulthood. In prenatal nicotine exposure models, disrupted glutamatergic signaling in the auditory cortex and impaired auditory temporal processing have been reported. Recent in vivo work demonstrates the importance of nicotinic acetylcholine receptors in signal-in-noise detection in the medial nucleus of the trapezoid body (MNTB) of the auditory brainstem. It is currently unclear if nicotinic signaling is required for functional calyx formation within the MNTB, and how perinatal nicotine exposure (PNE) may impact functional MNTB development.

Methods: Using whole-cell patch clamp recordings of MNTB neurons in mouse auditory brainstem, we studied nicotinic receptor currents and synaptic transmission of the calyx synapses in PNE mice and control (vehicle-treated mice). In addition, auditory function was interrogated in vivo with distortion product otoacoustic emissions, auditory brainstem response, and auditory startle tests.

Results: In drug naïve animals, ACh-induced nicotinic receptor currents in postsynaptic MNTB neurons show an age-dependent decrease during postnatal development (from P8 to P16). In addition, ACh puff increased the frequency of mini EPSCs in an age-dependent manner, indicating presynaptic glutamate release is affected by nicotinic receptor expression and activity during this developmental period.

Furthermore, PNE mice have increased nicotinic receptor-mediated currents in MNTB neurons. However, there are no significant differences in ABR or DPOAE thresholds across frequencies between PNE and control animals (n=7-11 mice/group; ABR: 2-way ANOVA, p=0.41; DPOAE: 2-way ANOVA, p=0.37).
Conclusions: Our data suggest nicotinic acetylcholine receptors are present the calyx of Held-MNTB synapse during early auditory brainstem development. PNE during early postnatal development alters nicotinic acetylcholine receptor properties at the calyx synapse. In vivo auditory tests display no deleterious effects on outer hair cell function or auditory brainstem transmission in PNE mice, but more complex processing could be impacted.

SA61. Assessment of Hints Protocol Training in Medicine Students: A Quasi-Experimental Study
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Category: Clinical Otolaryngology and Pathology
Background: HINTS (head impulse, nystagmus, and test of skew) protocol is widely used for emergency physicians, otolaryngologists, neurologists, and internal medicine doctors to differentiate central from peripheral vertigo. The training on this protocol usually occurs during residency programs, however, there is a lack of information about how the training in future general practitioners is, who are usually the first responders to the attention of patients with vertigo. There is scarce evidence about how medical students comprehend, applicate and understand the HINTS protocol. We conducted a quasi-experimental study to assess how is the understanding, applicability, and comprehension of HINTS in trainees after a theory-practice session.
Methods: A quasi-experimental study was conducted in June, 2022. Two experimented healthcare vestibular professionals conducted this research. Pre-test and post-test evaluation was performed before and after a full academic session of HINTS including videos given by one experimented Otoneurologist. A practical session was conducted by one in-site Otoneurologist, who checked the performance of HINTS in each student. One post-test was done one month after the implementation of HINTS. Statistical analysis included ANOVA test and descriptive statistics using mean, absolute and relative frequencies were calculated.
Results: A total of 21 students were included, mean age was 23.7 (SD 5.8). 61% of students were females. In the pre-test, students were slightly familiar with HINTS (57%), somewhat comprehended the protocol (40%), understand this (40%) and just 38% of students were familiar with HINTS. Post-tests results indicated an increased comprehension of protocol (Δ40%), understanding (Δ60%), clarity (Δ38%), and applicability (Δ48%) compared to results pre-test. Head impulse (Δ 39%), nystagmus evaluation (Δ 10%) and test of skew (Δ 39%) shown an increased understanding and comprehension after the academic session. A slight decline was observed on the comprehension (Δ 10%), understanding (Δ 10%) and clarity (Δ 10%) 1-month after, as well as a slight decline for the practical and knowledge skills on nystagmus evaluation (Δ 10%) and test of skew (Δ 10%). Students indicated they can do by themselves the HINTS without assistance after 1-month follow-up (52%), head impulse (33%), nystagmus evaluation (52%) and test of skew (52%) (p=0.001). 80% of students indicated they can differentiate a central vertigo from peripheral 1-month after. In 4 questions performed for external validation, using worldwide literature, students were able to respond the questions about central (90%) and peripheral (90%) vertigo using HINTS.
Conclusions: Increased comprehension, understanding, clarity and applicability of HINTS was observed in medical students after an academic and practice session. Decline over time of HINTS understating and comprehension was slight, which could be associated with diminished hands-on sessions and practice in patients. Further practice and follow-up are needed to check out new variables involved.

SA62. Potential Prognostic Factors and Their Influence in the Decision-Making Between CI and ABI in Inner Ear Malformations: A Histopathological Study
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Category: Clinical Otolaryngology and Pathology

Background: Although much has been discovered on bony and cellular abnormalities of inner ear malformations (IEMs), the results of cochlear implantation (CI) in some cases are unpredictable. It is challenging to identify cases that would not benefit from CI based on imaging tests. The objective of this study is to – based on otopathological analysis – identify prognostic factors that could influence in the decision-making process towards CI or auditory brainstem implants (ABIs).
Methods: From two otopathology archival collections we selected specimens with IEMs. Temporal bones were processed, sectioned at 20um, and every 10th section stained (H and E). IEMs were classified as per Sennaroglu et al. We calculated the percentual loss of hair cells in 5 adjacent sections (midmodiolar and 2 adjacent stained sections), as well as the population of spiral ganglion neurons.
Results: Our study included a total of 20 specimens (2, hypoplasia (CH) type II; 14, CH-III; 2, incomplete partition (IP) type II); and 2, normally developed cochleae with absent cochlear nerves). In CH-II cases, we observed mild loss of inner and outer cochlear hair cells, and a 25% loss of spiral ganglion neurons as compared with normative data. CH-III cases presented with high anatomical and cellular variability. We found mild to moderate loss of cochlear hair cells in most cases and an average loss of 62% of spiral ganglion neurons. We found a direct correlation between the diameter of the bony cochlear nerve canal with the number of spiral ganglion neurons. In all CH-III cases the diameter of the internal auditory canal was normal, but in three specimens we found hypoplastic cochlear nerves that ran adjacent to the anterior wall of the canal. The two IP-II specimens had severe loss of cochlear hair cells (90%) and 41% loss of spiral ganglion neurons, which could be representative of the age of the patient (90 years old). In the 2 cases with cochlear nerve aplasia, the cochlear and vestibular bony anatomy and organ of Corti structures were normal. However, the cochlear nerve, spiral ganglion neurons, and efferent nerve fibers were all absent.
Conclusions: Our findings indicate that CH-II, CH-III, and IP-II cases seem to have favorable anatomy towards CI. The bony aperture for the cochlear nerve seems to be a good indicator of the presence of neural structures in the cochlea, as well as nerve viability. In some cases, the cochlear nerve was present but running adjacent to the anterior wall of the internal auditory canal, and therefore may not be visible using current imaging techniques and therefore can be deemed “aplastic”. Our findings indicate that – although most cases of CH and IP are favorable for CI, imaging tests must be combined with comprehensive audiological examination to determine the optimal hearing rehabilitation strategy.

SA63. USH1C Vision and Balance Natural History Studies and Approach to Sharing Clinical Data

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Category: Clinical Otolaryngology and Pathology
Background: Usher syndrome (USH) is a rare genetic disorder characterized by the multi-sensory loss of hearing, balance, and vision. Four clinical types (USH1-4) and 10 genes (subtypes) are associated with USH based on the severity of the hearing loss, presence of imbalance, and age of onset of the vision loss. Approximately 10% of USH1 is caused by mutations in the USH1C gene, but nearly all cases among the Acadian populations in Canada and Louisiana are caused by the c.216G>A mutation in the USH1C gene (USH1C). The loss of hearing, balance, and vision are present at different ages in USH patients, however, the natural clinical course – when these losses begin and how quickly they progress – is not known.
Methods: We are conducting several natural history studies (NHSs) with USH1C patients at all stages of disease progression to improve our understanding of the natural progression and identify potential clinical trial participants and robust outcome measures that can be used to guide future clinical trials.
Results: Currently, 109 participants are enrolled in a retrospective NHS of USH in Louisiana and throughout the world. Of these, 75 have genetic confirmation of USH1C disease and are currently being

invited to participate in two prospective NHSs on vestibular and visual loss in USH1C. Demographic, eye and ear histories, genetic, patient-reported surveys, and longitudinal hearing, balance, and vision clinical data are being collected from pediatric, young-adult, and adult USH1C patients (n=12/50). Additionally, we have developed several databases designed to store, analyze, and share USH1C genotype-phenotype data.
Conclusions: Natural history and outcome measures data for USH1C patients are important to guide treatment trials. The approach to develop these studies in USH1 patients highlights challenges specific to this population.

SA64. Deep Immunophenotyping of Patients With Hearing Instability Disorders

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Background: Hearing instability (HI) disorders consist of a group of idiopathic hearing disorders characterized by either fluctuation or sudden changes in hearing thresholds or speech understanding, tinnitus, a sensation of fullness in the ear, and, in some cases, vertigo. HI disorders include, but are not limited to, Meniere's disease, autoimmune inner ear disease, sudden sensorineural hearing loss, and enlarged vestibular aqueduct syndrome. Some patients with HI disorders demonstrate evidence of endolymphatic hydrops (EH) on imaging of the inner ear using contrast-enhanced delayed FLAIR MRI. The poorly understood pathogenesis of these disorders contributes to diagnostic delays and the lack of effective treatment options. While components of the immune system, including both immune cells and cytokine signaling pathways have been implicated in some HI disorders, their role in the development of these disorders is unclear. It has been suggested that phenotyping of patients with HI disorders may enable the identification of subgroups more amenable to certain treatments and facilitate the identification of more effective therapies. The objective of this study was to characterize the cellular immune profile of a cohort of patients with HI disorders at their baseline evaluations as part of a deep phenotyping protocol at the NIH Clinical Center (NIH CC). The ultimate goals of this clinical protocol are to identify prognostic and diagnostic biomarkers and to facilitate the identification of novel therapies. We hypothesize that HI patients with EH will exhibit distinct cellular and proteomic profiles from those without EH.
Methods: Patients with evidence of HI disorders were recruited under the longitudinal deep phenotyping clinical protocol at the NIH CC and grouped based on the presence of MRI-proven EH. PBMCs were isolated analyzed using FSFC with a 40-fluorescent marker panel. As part of this clinical protocol, deep phenotyping of the immune system consisting of cellular immunophenotyping by full spectrum flow cytometry (FSFC) and single cell RNA-Seq (scRNA-Seq) of peripheral blood mononuclear cells (PBMCs) as well as cytokine and proteomic profiling are investigated.
Results: The baseline analysis of immune cells from patients in the two groups associated monocytes and natural killer cells (NK-cells) to EH. The involvement of these immune cells and preliminary analysis of scRNA-Seq data suggests that innate immune response in the inner ear may contribute to the development of EH in these HI patients.
Conclusions: Preliminary investigations suggest that HI disorder patients with EH may have distinct immune profile characteristics. Ongoing patient recruitment and longitudinal assessment of patients with HI disorders with multiple deep phenotyping measures including FSFC, scRNA-Seq, and proteomic profiling may build support for the involvement of the immune system in these poorly understood disorders. Deep phenotyping may facilitate the identification of potential therapeutic targets in the future.

SA65. Quantitative Assessment of Inner Ear Fluid Spaces and Endolymphatic Hydrops on Delayed Contrast-Enhanced Flair MRI in Patients With Hearing Instability

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Background: Hearing instability (HI) disorders, including Meniere's disease, autoimmune inner ear disease, and sudden sensorineural hearing loss, are characterized histopathologically by endolymphatic hydrops (EH), an expansion of the endolymphatic space. Recently, fluid sensitive magnetic resonance imaging (MRI) techniques combined with gadolinium-based contrast agents (GBCAs) have been used to differentiate different fluid spaces in the labyrinth as such agents slowly accumulate in the perilymph but not in the endolymph due to the blood-labyrinth barrier. While several studies have quantified these fluid spaces and EH in the inner ear based on such MRI techniques, no studies to date have attempted to quantify or grade EH in a longitudinal fashion. We sought to present a systematic approach to quantify the endolymphatic space in the human inner ear over time.
Methods: Delayed contrast-enhanced 3D Fluid Attenuated Inversion Recovery (FLAIR) MRI was performed 4-8 hours after intravenously administering gadoteridol (0.2 mmol/kg) in HI patients at 3-6 month intervals, under a deep phenotyping protocol at the NIDCD. A 3D Short Tau Inversion Recovery (STIR) sequence was used to visualize perilymph and endolymph spaces while a FLAIR sequence was used to identify the perilymph. A custom MR image processing and analysis pipeline was developed to quantify different inner ear compartments. A semi-automated workflow, which included 3D reconstruction of the inner ear volume and interactive vestibulocochlear nerve removal, was applied to measure perilymph and endolymph volumes among the cochlea and vestibule compartments. Quantification of the endolymph-containing space was performed by image subtraction of the delayed FLAIR signal from the STIR signal. 3D inner ear masks were manually separated into cochlea and vestibule to quantify individual fluid compartments.
Results: We present the details of our image analysis pipeline with example cases to illustrate the key processing steps. Endolymph and perilymph volumes in the cochlea and vestibule are quantified over time in selected patients via the semi-automated workflow described above. This resulted in easily distinguishable hydrops, particularly in the vestibule, with endolymph space more difficult to illustrate in the cochlea and semicircular canals given the irregular geometry. We identify opportunities for further automated image processing and analysis by machine learning.
Conclusions: The presented image quantification strategy expands upon existing clinical grading schemes for EH by performing semi-automated quantitation of the perilymph and endolymph spaces from the combination of FLAIR and STIR MRI sequences. Assessment of patients with HI utilizing delayed contrast-enhanced FLAIR MRI may allow for the correlation with other biomarkers to identify diagnostic and prognostic biomarkers of EH and HI.

SA66. Chemosensory Function Recovery in COVID-19 Patients: A Cross-Sectional Study

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Category: Clinical Otolaryngology and Pathology
Background: Current literature on COVID-19-related smell loss uses self-report and survey data to analyze chemosensory function recovery post-COVID-19, but surveys and self-reports are not adequate substitutes for objective measures of smell and taste capabilities. Assessing the smell and taste capabilities of these patients requires a standardized, quantitative scale. This study aims to assess smell and taste function standardized against those who never contracted COVID-19 (the COVID-naïve).
Methods: This prospective, observational, cross-sectional study convenience sampled subjects at the University of Miami Otolaryngology Clinic in Miami, FL between September 2021 and August 2022. Those previously COVID-19 positive composed the experimental group, those who reported being COVID-naïve composed the control group. Mean total score for the UPSIT Smell Test, and the Burghart Taste Strip test were the primary outcome measures.
Results: 70 adult subjects (35 former COVID-positive, 35 COVID-naïve) were enrolled in the prospective arm, with 21 females and 14 males in each group. 87% of all subjects were white and were almost distributed evenly between Hispanic and non-Hispanic. Mean UPSIT total score for the experimental group was 30.6 (95% CI 28.9 – 32.3), mean UPSIT total score for the control group was 31.2 (95% CI 29.7 – 32.8). Mean Burghart total score for the experimental group was 11.3 (95% CI 10.6 – 12.0), mean Burghart total score for the control group was 10.7 (95% CI 9.7 – 11. 8). These showed a significant overlap of the 95% CI of the mean total score between the control group and the experimental group, suggesting no significant difference between the two groups. Linear regression analysis of VAS responses (graded on a scale from 0 being no problem at all, to 100 being total sensory loss) found no statistically significant

relationship between either VAS taste and Burghart total score (p=0.85, 95% CI: -3.15 – 3.82; Pearson's r=-0.02, p=0.89) or VAS Smell and UPSIT total score (p=0.47, 95% CI: -2.38 –1.12; Pearson's r=-0.12, p=0.32).
Conclusions: This cross-sectional study found no significant difference between the objective measures of smell and taste capabilities between COVID-naïve subjects and subjects who have recovered from COVID-19 infection. While these results are promising for the prognosis of COVID-related smell and taste disturbance, it is merely a jumping-off point. More research is needed in this area to draw more generalizable conclusions or develop treatments regarding COVID-related smell and taste disturbance.

SA67. Hearing Outcomes of Ganciclovir and Quercetin Combination Therapy in a Murine Cytomegalovirus Model

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Category: Clinical Otolaryngology and Pathology
Background: Ganciclovir (GCV) has been well-documented as an effective antiviral treatment for the prevention of cytomegalovirus (CMV) induced sensorineural hearing loss. However, dose-dependent adverse effects (particularly neutropenia) require strict, burdensome monitoring, limiting GCV use in some patients. Quercetin is a flavonoid with antioxidant properties that has demonstrated antiviral therapy against CMV. Although relatively more benign than GCV, quercetin has limited therapeutic value due to its poor solubility. Solubilizing quercetin with poloxamer P188 and combining with GCV therapy has demonstrated amplified CMV inhibition in vitro. Our experiment sought to determine the efficacy of combining quercetin-P188 (QP188) with a subtherapeutic dose of GCV to treat CMV-induced sensorineural hearing loss in a well-established murine CMV model.
Methods: BALB/c mice were infected with 200 plaque-forming units of murine CMV via intracerebral injection into the left, mid-parietal region on postnatal day 3 (p3). Quercetin was solubilized in saline using P188 (Kolliphor® P188 BIO).3 Treatment groups received either 1 mg/kg GCV, 220 mg/kg of QP188, a combination therapy of GCV+QP188, or 220 mg/kg of a blank P188 solution (delivery vehicle control) BID at 12-hour intervals via intraperitoneal injection. All treatment groups were treated for 14 days starting at p3. Uninfected controls were treated with the combined regimen and P188 delivery vehicle. Auditory thresholds were assessed using distortion product otoacoustic emission (DPOAE) and auditory brainstem response (ABR) testing at 4, 6, and 8 weeks of age. Temporal bones from separate CMV-infected groups were harvested at p10, and viral load was determined by quantitative polymerase chain reaction. Differences in ABR/DPOAE thresholds and viral load were analyzed by the nonparametric Kruskal-Wallis test.
Results: CMV-infected mice receiving combination therapy GCV+QP188 demonstrated significantly lower ABR ($p<0.001$) and DPOAE thresholds ($p<0.001$) compared to mice treated with GCV monotherapy, QP188 monotherapy, and P188 delivery vehicle at 4, 6, and 8 weeks of age. GCV only and QP188 only treated mice did not demonstrate significantly lower hearing thresholds than infected mice treated with P188 only. GCV+QP188 combination therapy did not affect hearing thresholds compared to control, uninfected mice. GCV+QP188 combination therapy resulted in significantly lower viral titers than both GCV and QP188 monotherapy (p=0.02974).
Conclusions: Our results suggest that GCV and QP188 have a synergistic therapeutic effect on hearing outcomes in a CMV infected murine model. This may be explained by a reduction in viral titers in mouse temporal bones, consistent with prior in vitro studies. Combining GCV and QP188 did not cause adverse hearing outcomes in uninfected animals.

SA68. Characterization of the Cochlea in the COVID-19 Anosmia Hamster Model

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Category: Clinical Otolaryngology and Pathology
Background: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is responsible for the pandemic, coronavirus disease 2019 (COVID-19). This systemic disease affects multiple organs and has

caused an expanding list of clinical manifestations including audio-vestibular dysfunction. Our goal was to provide the first characterization of cochlear changes induced by SARS-CoV-2 in a hamster model of COVID-19.
Methods: We conducted an immunohistochemical analysis of the temporal bone in SARS-CoV-2 infected hamsters, used in a previous study on anosmia and corresponding olfactory histological damage (Sci Rep. 2022 Jan 12;12(1):628). Syrian golden hamsters were inoculated intranasally with SARS-CoV-2 alpha strain in phosphate-buffer saline (PBS) or PBS as a mock control. The temporal bones were harvested at days post-infection (dpi)- [2 dpi (n=3), 3 dpi (n=4), 5 dpi (n=3), 8 dpi (n=4), 12 dpi (n=4), 21 dpi (n=3), 35 dpi (n=4), 42 dpi (n=4), and 2 dpi mock (n=3)]. The temporal bones were processed into paraffin blocks, thin sectioned and processed for H and E staining and labeling with the SARS-CoV-2 nucleocapsid antibody.
Results: In the hamsters infected with SARS-CoV-2, minor structural damage including bulging and tearing was observed in the middle ear mucosa (3 dpi, 8-42 dpi), stria vascularis (8 dpi), and Reissner's membrane (3-5 dpi). Hemorrhage was noted in the middle ear mucosa (3 dpi, 12-42 dpi) and scala tympani (21 dpi). The SARS-CoV-2 antigen was detected in the spiral ganglion (12-35 dpi), stria vascularis (8-35 dpi), and middle ear mucosa (3 dpi, 8-42 dpi), whereas no positive staining was observed in the mock infected hamsters. SARS-CoV-2 antigen was first detected in the middle ear mucosa, then observed in the cochlea, where the damage peaked at 21 dpi.
Conclusions: A time lag was observed between olfactory dysfunction and inner ear changes. While the damage in olfactory epithelium and corresponding anosmia peaked around 2-5 dpi, the inner ear changes were prominent at 8-35 dpi. From our results, we suggest three possible mechanisms of viral transmission to the temporal bone. First, a direct invasion of the virus to the middle ear through the Eustachian tube. Second, via the bloodstream, supported by the positive SARS-CoV-2 antigen detection in the stria vascularis. Third, through the endolymphatic sac and central nervous system, supported by the bulging of Reissner's membrane demonstrating endolymphatic hydrops and existence of the SARS-CoV-2 antigen in the perilymph and perineural area. We speculate the SARS-CoV-2 virus affects hearing through different mechanisms. Early onset hearing loss could result from the progression of SARS-CoV-2 from the nose to the middle ear mucosa, causing middle ear effusion. Later onset hearing loss could develop from the virus reaching the cochlea through the bloodstream and CNS, remaining for longer periods. More research including evaluation of audio-vestibular behavior is needed to confirm these findings.

SA69. The Hispanic Vestibular Patient: A Qualitative Characterization Using Social Media

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Category: Clinical Otolaryngology and Pathology
Background: Patients with vestibular disorders use social media to search for specific information related to diagnosis, treatment, and relief exercises, and to solve other colloquial questions. Previous studies have explored how social media has been utilized in the medical realm in the US. To date, there is no available data on Hispanic vestibular patients. The aim of this study is to evaluate qualitatively the content of communication on Facebook, Instagram, and Youtube in the Spanish Language related to vestibular disorders
Methods: A network analysis was performed using content analysis strategies. A descriptive codification and categorization from a sample of 150 Facebook posts, 100 Youtube videos, 50 Instagram posts, and 50 Instagram reels were done. Two researchers independently checked the posts, videos, and reels. Discrepancies were solved for the senior author.
Results: From an initial set of 150 Facebook posts, we found 36% were related to Shared experiences/Looking for Help, followed by General Information. Besides these categories, others mostly seen were searched for social support and information about treatment. Females (66%) used to use Facebook to search for information regarding vestibular disorders. Comments were the most common interaction on Facebook (n=3520). Comments on posts regarding Meniere's Disease (17%) were mostly found. Information regarding general information, Shared experiences/Looking for Help on Meniere's disease were commonly reported. Other interactions seen on Facebook posts were associated with information related to treatment and shared experiences/looking for help in unspecified vertigo. On Instagram, 50 posts were

reviewed. 36% of posts mostly showed information about services and testing on vertigo and dizziness clinics, followed by general information about vestibular disorders (32%). Most of the posts (42%) reported information about unspecified types of vertigo, followed by vestibular migraine (24%). Of the 50 reels examined, 36% contained general information about vertigo and dizziness, followed by recommendations for vertigo relief (18%). Benign paroxysmal positional vertigo (54%) was the most common disorder found on reels, followed by vestibular migraine (26%). On YouTube, 100 videos were reviewed. In this social media, visualizations were the most common interaction seen (12.263.566) videos containing exercises for vertigo relief were the highest category visualized (32%) followed by information about medications (27%). Videos with information about medications also were the category with more likes (28%) and comments (60%).
Conclusions: Hispanic patients mostly look for information about shared experiences/looking for help in social media as well as exercises for vertigo relief and information about medications for vestibular disorders. Information about Meniere's disease, unspecified types of vertigo, benign paroxysmal positional vertigo and vestibular migraine were usually found in diverse social media formats. Characterizing information-seeking and information sharing of patients will have an increased potential to improve the quality of life and healthcare providers attention to vestibular Hispanic patients.

SA70. The Future of Eustachian Tube Assessment Using Sound: An Optimization Study of Sonotubometry

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Category: Clinical Otolaryngology and Pathology
Background: Eustachian tube (ET) dysfunction is a disease strongly linked to otitis media and other middle ear diseases. Regardless of its importance, its assessment still heavily relies on subjective patient assessment. Sonotubometry, the only objective assessment method which does not interfere with ET function through artificial changes in pressure, has failed so far to find clinical acceptance due to its unreliability. In sonotubometry, a sound is applied to the nostril using a speaker, and a microphone is placed at the outer ear canal, allowing measurement of the impedance of the nose-ear transmission line. This study addresses the persisting issues with sonotubometry by using an engineering design optimization approach. The sound type and sound amplitude were identified as the two key parameters. To the author's knowledge, no such direct comparison and analysis of the method have been performed to date.
Methods: An assessment protocol consisting of 24 measurements was designed and tested on 27 healthy volunteers. For optimal control of the parameters, a custom sonotubometer was built. This new device allowed sound amplitudes up to 125 dB, which is significantly higher than the current standard in research (usually up to 110dB). Three different sound types were tested and compared for their efficacy (white noise, sweeps and an 8kHz-tone). To induce an ET opening, swallowing without water was used as this created the least amount of swallowing noise.
Results: The findings illustrate that a louder sound leads to a notable improvement in the reliability of sonotubometry. ET opening was detected in 66.7% of the volunteers at 120dB, whereas only 48.1% showed ET opening at 100dB. This lies below the average sound amplitude used in most papers. However, more than 50% of their measurements recorded a spike-type pattern which has been associated with swallowing noise rather than an effective ET opening. This is caused by insufficient excitation sound amplitude. In fact, 17 out of 21 past papers studying sonotubometry used an amplitude of □110dB. Furthermore, broadband sounds proved to be more reliable than a single-frequency sound. Comparing the results with the control recordings (where no sound was applied), the importance of sufficient sound amplitude became even more evident as there is a measurable amount of swallowing noise present, which affects the recordings below 110dB disproportionately.
Conclusions: The evidence demonstrates the capability of this optimized sonotubometry method to measure ET opening objectively. The results further suggest that sonotubometry has the potential to become the new clinical standard for ET dysfunction assessment. A reliable and objective assessment of the ET will improve the diagnosis of ET dysfunction and research related to its management. Future research will focus on a case-control study to assess the specificity and sensitivity of this improved method to identify ET dysfunction.

SA71. Identification of Retinoic Acid Target Genes in Organ of Corti Development

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Category: Development: Cellular/Systems

Background: Retinoic acid (RA), a derivative of vitamin A, is an essential component of cell-cell signaling during vertebrate organogenesis. In the developing nervous system, RA has two main roles: patterning of spatial identity and induction of neuronal differentiation. Studies suggest RA may play similar roles in vertebrate inner ear development. Specifically, ectopic RA was found to induce supernumerary hair cells in the organ of Corti in vitro. While this phenotype is reminiscent of observations made after blocking Notch signaling, a causal relation between RA and Notch signaling remains to be determined.

Methods: To address this question, we used a bioinformatics approach to identify RA target genes. Due to its critical role in organ of Corti development, we focused on the RA receptor alpha (RARA), a nuclear receptor that functions as a transcription factor. Next a gene regulatory network analysis was performed using scRNA/ATAC-seq data of the postnatal day 2 organ of Corti. RARA target genes were extracted and validated in vitro using organ of Corti explants in combination with fluorescent in situ hybridization.

Results: The RARA regulon represents a set of supporting cell specific genes, such as Lfng, S100b1, and Fgfr3. We performed gene set enrichment analysis using a single cell RNA sequencing data set of the E14.5 cochlea and determined that the RARA regulon is differentially active in the nascent supporting cells of the organ of Corti. Together, these data suggest that binding of RA to RARA induces supporting cell specific genes in the cochlear floor at E14.5. To test this hypothesis, we exposed E14.5 organ of Corti explants to ectopic RA (500 nM) and after 72h a significant increase in Lfng expression was detected in the cochlear apex.

Conclusions: In this work, we used scRNA/ATAC-seq data to predict target genes of RA signaling in organ of Corti development. In combining bioinformatics and experimental approaches, we identified Lfng as a RA target, which is known to attenuate Jag1-Notch signaling between cells. Interestingly, attenuated Notch signaling, for example as a consequence of hypomorphic Notch loss-of-function alleles, has been reported to induce supernumerary HCs. Together, these findings indicate that ectopic RA potentially modulates Notch signaling in the developing organ of Corti.

SA72. Developmental Origin of Cell Type Diversity in the Cochlear Nuclear Complex

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Category: Development: Cellular/Systems

Background: To date, it is unclear how novel brain structures evolve. A big question is to what extent they were built by the use and modification of pre-existing developmental genetic programs (DGP). The cochlear nuclear complex (CNC) in the auditory brainstem represents an example of an evolutionary novelty. Current data suggest that the DGP for the CNC integrated elements of two pre-existing ones: the DGP to generate neurons of the developing brainstem and the DGP to generate neurons of the cerebellar cortex.
The CNC originates from the developing brainstem and therefore generates neuronal diversity (e.g. inhibitory vs. excitatory) by variation along three axes: the anteroposterior axis (rhombomeres), the dorsoventral axis (microdomains), and the temporal axis (birthdates). Not much is known about how the DGP for the CNC exploits this mechanism. Moreover, the CNC shares some neuron types with the cerebellar cortex. The DGPs for the CNC and cerebellum have not been compared in much detail before. Here, we examined to what degree the CNC exploits the DGPs to generate neurons of the developing brainstem and the cerebellar cortex. Our analysis revealed novel insight into the developmental and evolutionary origin of the high cellular diversity in the mammalian CNC.

Methods: To determine rhombomere and microdomain that contain the progenitor for a CNC neuron type, the contributions of the Egr2 and Lbx1 lineages were examined. For comparison with the cerebellar cortex, the contribution of the Lbx1 lineage to cerebellar cell types was analyzed. For these purposes, genetic lineage tracing was combined with cell type-specific molecular marker analysis.

Results: The analysis of the Egr2 lineages uncovered that the CNC neuron types are derived from four different rhombomeres. For the CNC, we show that Purkinje cell-like cartwheel cells and stellate cells, but not Golgi cells, express Lbx1 as part of their DGP. For the cerebellum, we show that Purkinje cells do not express Lbx1 as part of their DGP, whereas stellate cells and Golgi cells do.

Conclusions: Our study illustrates how neuronal diversity in the CNC is generated by origin in different rhombomeres and different microdomains. Our comparative analysis strongly suggests that the DGP for the cerebellum was both used and modified by that for the CNC.
Take-Home Message:
The evolutionary novel auditory nucleus CNC was most likely generated by the use and modification of the pre-existing DGPs to generate different neurons in the developing brainstem and the cerebellar cortex.

SA73. Co-Release of GABA and ACh from Medial Olivocochlear Efferent Fibers During Development
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Category: Development: Cellular/Systems
Background: During development, inner hair cells (IHCs) in the mammalian cochlea are unresponsive to acoustic stimuli but instead present intrinsic electrical activity, crucial for the normal development of the auditory pathway. During this same period, neurons originating from the medial olivocochlear complex (MOC) transiently innervate the soma of IHCs. This synapse is mediated by acetylcholine (ACh), activating nicotinic receptors assembled by alpha9 and alpha10 subunits and ultimately controlling IHC excitability. Although it is a cholinergic synapse, previous evidence indicated the presence of abundant GABA and presynaptic GABAB receptors (GABAB-R). Moreover, application of GABAB-R agonists generated a reduction in ACh release.
Methods: Transgenic Chat tm2(crc)Lowl/J-Cre (ChAT-cre) and Gad2 tm1(cre/ERT2)Zjh/J-Cre (GAD2-cre) mice of either sex were mated with a floxed channel rhodopsin 2 (ChR2) line, Ai32, to drive specific ChR2 expression. Whole cell patch clamp was performed in P9-11 IHCs while either cholinergic (ChAT-Cre/ChR2) or GABAergic (GAD2-Cre/ChR2) fibers were optogenetically stimulated. Additionally, immunohistochemistry techniques were used to characterize cholinergic and GABAergic expression in B6.Cg-Gt(ROSA)26Sortm14(CAG-tdTomato)Hze/J (tdTomato) crossed with GAD2-Cre. Furthermore, we performed calcium imaging experiments at the same age range on Balb-C mice. For this, IHCs were dialyzed with a fluorescent calcium indicator (Fluo 4) and MOC electrically stimulated during the application of a GABAB-R antagonist (CGP 36216, 200 μM).
Results: Firstly, eIPSCs were optogenetically triggered in ChAT-cre/ChR2 mice, and these responses could be partially blocked with alpha-bungarotoxin (60.8 ± 4.4%, N=5). Optogenetic experiments were also performed in GAD2-cre/ChR2 mice producing eIPSC that could also be blocked by alpha-bungarotoxin (82.3 ± 7.2%, N=4). This result strongly suggests that a cholinergic response could be elicited in IHC in GAD-expressing neurons. Also, immunohistochemistry experiments done at the base of IHCs showed approximately 30% co-localization of GABAergic and cholinergic labelling.
Finally, calcium imaging experiments were performed with stimulation of MOC fibers, allowing us to resolve the activation of single synaptic sites. The application of the GABAB-R antagonist, CGP 36216, produced an increase in the activation probability of individual calcium hotspots in the context of heterogeneous responses in different sites of a single IHC.
Conclusions: In conclusion, here we provide evidence suggesting that GABA and ACh could be co-released from MOC terminals. Whereas ACh acts postsynaptically activating α9α10 receptors, the role of GABA is presynaptic, as a negative feedback signal to locally regulate cholinergic inhibition of IHCs. Calcium imaging experiments suggest that GABA modulation operates differently at each synaptic site.

SA74. The Role of Nfe2 in Development and Oxidative Stress Response in Zebrafish Inner Ear Function
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Category: Development: Cellular/Systems
Background: Noise exposure is one of the most common reasons for auditory dysfunction. Noise mechanically destructs sensitive structures of the inner ear including hair cells, and it can intensify their metabolic activity, which leads to the excess production of reactive oxygen species (ROS). ROS production then leads to cell death pathways in mammalian cochlear and vestibular hair cells. Since the inner ear depends on hair cells to properly function, the death of these sensory receptors leads to hearing loss. Thus, understanding cellular pathways involved in combating oxidative stress is important for enhancing our

understanding of noise-induced hair cell death. To neutralize increased ROS and prevent oxidative stress from inducing severe damage, cells initiate the oxidative stress response (OSR). Disruption to OSR components, such as the nuclear factor erythroid 2-related factor 2 (Nrf2), is known to cause hearing and balance dysfunction, while OSR activation is shown to protect hair cell function. Considering that Nrf2 plays an essential role in the defense mechanism against oxidative stress in the ear, we hypothesized that other transcription factors in the same family will have a similar role. Recent work using larval zebrafish revealed that Nfe2 shares binding sites with Nrf2 and implicated its involvement during development and in the OSR. We subsequently found that in zebrafish nfe2-morphants, the inner ear did not develop normally as the otic vesicles migrated towards each other and the semicircular canal system failed to develop. Additionally, between mutant Nfe2 knock-out (KO) and wild-type (WT) zebrafish larvae, several genes with putative Nfe2 binding sites were regulated. These findings indicate that Nfe2 may have developmental and protective roles in the inner ear, especially during oxidative stress.
Methods: Therefore, to test our hypothesis that Nfe2 is involved in the OSR and necessary for protecting hair cells during oxidative stress, we measured inner ear microphonics in Nfe2 KO and WT larvae both during control conditions and after oxidative stress exposure. Given the role of the inner ear in driving acoustic startle responses, we are also examining whether Nfe2 KO fish display altered escape responses or reduced escape response probabilities compared to WT larvae.
Results: Our preliminary results include data from an RNA-Seq screen indicating potential downstream targets of Nfe2, which supports how Nfe2 may contribute to inner ear development by regulation of genes expressed in the ear. These findings have prompted recent experiments both examining the candidate genes and determining whether their disruption compliments or rescues mutant Nfe2 phenotype especially during oxidative stress.
Conclusions: Due to high genomic and molecular similarities between zebrafish and humans, studying Nfe2 in zebrafish will further both our understanding of its function and the potential development of novel therapeutics for noise-induced hearing loss and other hearing disorders.

SA75. The Presence of Profibrotic Markers TGFb-1 and CTGF Immunofluorescence (IF) in the Implanted Cochlea

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Category: Auditory Prostheses
Background: Cochlear implants (CI) provide highly successful auditory rehabilitation for patients with sensorineural hearing loss (SNHL); however, implantation can lead to insertion trauma and foreign body reaction resulting in intracochlear new tissue formation. This can increase impedance as well as spiral ganglion neuron death. While the exact mechanism is unclear, better understanding of this process can provide an opportunity for early intervention. Transforming growth factor beta-1 (TGFb-1) plays an important role in fibrosis as well as osteogenesis. Inhibition of TGFb attenuates ectopic bone formation in mice. TGFb-1 stimulates the expression of connective tissue growth factor (CTGF) which is involved in various physiologic and pathologic processes including cell proliferation, tumorigenesis, and fibrosis. Treatment with CTGF anti-sense oligonucleotides significantly reduced fibrosis around breast implants in rats. Given the importance of TGFb-1 and CTGF in fibrosis, the present study aims to characterize the expression of these proteins in the human implanted cochlea.
Methods: Human temporal bone (HTB) samples acquired from 5 patients with prior CI (3 diagnosed with SNHL and 2 with otosclerosis) and 5 age-matched controls were serially sectioned after fixation and celloidin embedding. Histopathologic analysis of fibrosis and osteoneogenesis was conducted using H and E. Protein expression was characterized using immunofluorescence and co-localization studies were conducted using laser confocal microscopy.
Results: A fibrous sheath with adjacent osteoneogenesis was found to surround the electrode path. The capsule was more prominent and thicker towards the modiolus. Profibrotic markers, TGFb-1 and CTGF, were upregulated in CI HTB samples independent of underlying pathology (otosclerosis, SNHL) while there was minimal expression in healthy age-matched controls. TGFb-1 was expressed diffusely within the fibrous capsule while CTGF was primarily expressed in the thickened portion towards the modiolus. Intracellular expression of TGFb-1 was also seen within the fibrosis. Finally, there was strong expression of both TGFb-1 and CTGF at the junction between fibrosis and new bone formation.

Conclusions: Fibrosis and osteoneogenesis following CI placement can lead to implant malfunction and loss of residual hearing. Understanding the pathogenesis of this process is critical to developing effective intervention. To our knowledge, this is the first study to characterize the expression pattern of TGFb-1 and CTGF in HTB following CI implantation. Expression of both profibrotic markers in the fibrotic capsule and at the fibrosis-osteogenesis junctions suggest their importance in new tissue formation following CI and candidacy for targeted therapy. Interestingly, dexamethasone inhibits the expression of TGFb-1 in vitro and may partially explain the efficacy of dexamethasone-eluting electrodes in reducing post-implant damage. Lastly, asymmetric fibrosis and expression of TGFb-1 and CTGF in conjunction with increasing bone maturation towards the modiolus also suggest electrical conduction may play a role and warrants further investigation.

SA76. PRDM16 Expressed in the Kölliker's Organ Regulates Underlying Mesenchyme to Develop the Spiral Limbus

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Category: Development: Cellular/Systems

Background: PR domain containing 16 (PRDM16) is a key transcriptional regulator in the development of multiple tissues including craniofacial, adipose, and neural tissues. Our lab has recently identified PRDM16 expression within Kölliker's organ (KO) cells. Gene trap model of Prdm16 deletion showed defects in Kölliker's organ, spiral limbus (SL) and tectorial membrane during development. Because Prdm16 null mice die at birth, we have generated an inner ear specific Prdm16 conditional knockout (cKO) mouse model that enabled characterizing postnatal cochlear development.

Methods: Prdm16 deletion efficiency in conditional mutant mice (Prdm16lox/lox; Fgf20Cre/+) was validated by immunostaining of cochlear sections at P0. Whole mount and inner ear sections were used to characterize cochlear phenotype in Prdm16 cKO mice by immunostaining and H and E staining at birth as well as postnatal time points (P7, P14 and P21). Proliferation of SL mesenchymal cells at different time points was analyzed using the 5-ethynyl-2′-deoxyuridine (EdU) cell proliferation assay injected 2 hours before sacrificing mice. Immunostaining for mesenchyme extracellular matrix (ECM) proteins was used to identify extracellular matrix deposition defects.

Results: Analysis of Prdm16 cKO at P0 showed hypoplastic KO, shortened cochlear duct, increased density of hair cells (HCs) and supporting cells in the apical turn as well as multiple isolated ectopic HCs within the KO domain. This phenotype persists throughout the first week (P7) of postnatal cochlear maturation. Analysis of P14 and P21 cochlear sections revealed hypoplastic spiral limbus, lack of inner sulcus and detached and deformed tectorial membrane, as well as high density hair cells in the middle and apical turns. No ectopic HCs were noticed at these time points. Analysis of SL mesenchymal cell proliferation showed increased percentage of mesenchymal cells incorporating the EdU maker in the apical turn in Prdm16cKO compared to control at E18.5. At P5, we observed persistent proliferation of SL mesenchymal cells in Prdm16 cKO in the middle and apical turns. Immunostaining for SL ECM proteins at P0 showed loss of collagen II staining and reduced Tgfbi staining (marker of SL mesenchyme) in the middle and apical sections in Prdm16cKO indicating a defect in ECM protein deposition.

Conclusions: Our work showed Prdm16 necessity for normal development and maturation of tectorial membrane, spiral limbus and inner sulcus. Also, we show that loss of SL occurs secondary to persistent proliferation, lack of differentiation and ECM deposition defect in Prdm16 cKO SL mesenchymal cells.

SA77. Kinase Signaling Regulates Hair Cell Planar Polarity and Reinforces the Line of Polarity Reversal in the Mouse Inner Ear

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Category: Development: Cellular/Systems

Background: Planar polarity is the polarized organization of cells and cellular structures within the plane of an epithelium and is evident in the organization of hair cell stereociliary bundles. Core planar cell polarity (PCP) signaling coordinates the orientation of hair cells along a common axis, however it remains unclear how PCP signaling is interpreted by individual cells during their differentiation. We are addressing this

using the utricle and saccule of the mouse because they contain hair cells divided between two groups, each with oppositely oriented stereociliary bundles, that meet at a boundary called the Line of Polarity Reversal (LPR). Although formation of the LPR requires the transcription factor Emx2, the effectors acting downstream of Emx2 that determine how hair cells respond to PCP signaling and establish the LPR are not known.
Methods: Using a bulk RNAseq approach we identified Stk32a, a serine-threonine kinase gene expressed on one side of the LPR that is transcriptionally repressed by EMX2. STK32a was evaluated through a combination of gene deletion and AAV-mediated overexpression assays in mouse vestibular hair cells. The impact of these manipulations on planar polarity, hair cell development and GPR156 distribution was evaluated by immunofluorescent labeling.
Results: Stk32a expression in vestibular hair cells is negatively regulated by EMX2 and the two genes are expressed on opposite sides of the LPR with Stk32a expression expanding in Emx2 mutants and disappearing when Emx2 is ectopically expressed. Following Stk32a gene deletion in mice, the intrinsic polarity of the bundle is no longer aligned with the core PCP proteins. As Stk32a is only expressed in hair cells on one side of the LPR, only bundle orientation in these regions is impacted in knockout mice. In contrast, Stk32a over-expression on the opposite side of the LPR is sufficient to reorient Emx2-expressing hair cells. GFP-tagged STK32a is enriched at the apical cell surface and stereociliary bundle consistent with a role in regulating this structure. Finally, STK32a regulates the subcellular distribution of GPR156 because this receptor is lost from the surface of hair cells ectopically expressing STK32a where GPR156 would normally function to regulate bundle orientation.
Conclusions: We propose a model in which STK32a functions opposite of EMX2 to establish and maintain the position of the LPR. In hair cells not expressing EMX2, STK32a aligns the bundle with the PCP axis so that those cells point in a single direction. This function is blocked by EMX2-mediated repression of Stk32a. In hair cells expressing EMX2, GPR156 reverses bundle orientations resulting in formation of the LPR. In this model the position of the LPR is determined by EMX2 since it actively represses the STK32a-mediated polarity pathway and STK32a reinforces LPR positioning by inhibiting GPR156 function at the cell surface.

SA78. Mitochondrial Morphology Regulates Development of Tonotopy in the Chick Cochlea
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Category: Development: Cellular/Systems
Background: In the vertebrate inner ear, the positions of sensory hair cells (HCs) along the basal-to-apical long axis of the cochlea determine the frequencies to which they are tuned (tonotopy). Despite growing understanding of how HC fate is specified versus the surrounding astrocyte-like supporting cells (SCs), the specific factors driving phenotypic refinement within cells along the tonotopic axis remain unclear. Mitochondria exist in an equilibrium between an interconnected, network-like state, as well as in discontinuous, fragmented populations. This fusion/fission state is emerging as an important regulator of excitable cell fate and maturation. Therefore, we aimed to characterize mitochondrial morphology and function in HCs and SCs along the tonotopic axis of the developing chick cochlea and hypothesized that mitochondrial dynamics play an important role in cochlear development and tonotopic patterning.
Methods: We used a combination of live and super-resolution imaging to examine mitochondrial dynamics in HCs and SCs of the avian basilar papilla between E7 and E14. Tetramethyl rhodamine methyl ester (TMRM), Mitoview and SiR-actin dyes were loaded into live tissue explants allowing quantification and statistical analysis of mitochondrial morphology and energetics during the specified developmental time course. Our quantitative approach was verified using pharmacological inhibition of the mitochondrial fission machinery using mitochondrial division inhibitor-1 (mdivi-1) and M1. Additionally, mitochondrial morphology was measured at E8 and E14 using transmission electron microscopy (TEM) and serial blockface scanning electron microscopy (SBF-SEM). Mitochondrial dynamics were modulated experimentally by culturing cochleas dissected at E8 in vitro for 4 days with pharmacological inhibitors of fission and mitochondrial biogenesis.
Results: Super-resolution imaging of whole-mount cochleae firstly revealed distinct groups of mitochondria within each HC, which we designate as as subnuclear, supranuclear and supracuticular populations. By observing mitochondria at timepoints during development we identified a window (E8-E10) in which mitochondrial morphology and biogenesis rapidly diverge in HCs at different tonotopic positions, and these

findings were verified using electron microscopy. During this window, differences in mitochondrial metabolism between HCs and SCs were also established. Disturbing mitochondrial biogenesis and fusion/fission balance using pharmacological inhibitors disrupted tonotopic organization of the cochlea, as evident by changes in cell size and sterocilial organization.
Conclusions: Our data suggest that mitochondrial remodelling is linked to the functional refinement of HCs and SCs along the tonotopic axis of the developing cochlea.

SA79. Interplay Between the Different Mechanisms Regulating Hair Cell Orientation

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Category: Development: Cellular/Systems
Background: Auditory and balance functions rely on hair cells (HCs) being properly oriented to detect directional vibrations. We recently showed that polarized enrichment of the G protein-coupled receptor GPR156 acts via inhibitory G protein alpha (Gai) to trigger a reversal of HC orientation downstream of the transcription factor EMX2. In mouse mutants where the EMX2>GPR156>Gai cascade is inactivated, one HC population in macular organs is inverted by 180° because it fails to undergo normal reversal during development, abrogating mirror-image HC organization. Interestingly, auditory HCs are similarly affected, with outer HC1 (OHC1) and OHC2 generally inverted by 180° in mutants.
Core PCP proteins regulate HC orientation via intercellular communication at HC-supporting cell junctions. It was proposed that the early off-center migration of the basal body in nascent HCs is oriented by asymmetric core PCP cues, and that GPR156>Gai regionally reverses the interpretation of these cues to define the orientation of the mature HC. In other systems, core PCP mutants generally show randomly oriented structures. In contrast, auditory IHC and OHC3 are respectively inverted in Fzd3;6 and Vangl2 mutants, reminiscent of, and complementary to, OHC1-2 inversion in Gpr156 mutants. Finally, auditory HC misorientation is corrected in time in core PCP, but not Gpr156 mutants. To try to make sense of these puzzling similarities and differences, we started to investigate whether and how these two classes of regulators interact at apical junctions to define HC orientation.
Methods: We analyzed auditory HCs of constitutive Gpr156 mutants and conditional Vangl1;2 and Fzd3;6 double mutants at birth using confocal microscopy. We used the semi-dominant Vangl2Looptail strain to explant E17.5 cochleae, and pertussis toxin to block EMX2>GPR156>Gai signaling in culture.
Results: The polarized distribution of VANGL2 and FZD6 is largely normal in Gpr156 mutants despite inverted OHC1-2. In contrast, the GPR156 crescent generally follows aberrant HC orientation in Vangl1;2 and Fzd3;6 double mutants, thus remaining in register with the HC cytoskeleton. VANGL1;2 but not FZD3;6 are required for normal enrichment levels of GPR156. We used the tonotopic gradient of HC maturation to follow in pseudo-time how HC orientation is corrected in core PCP mutants. Interestingly, measurements reveal that the GPR156 crescent corrects its orientation ahead of the basal body in Fzd3;6 mutants, evoking a possible role for GPR156>Gai in the correction process. To test this idea, we bred the Vangl2Looptail and Gpr156 strains, but were unable to obtain double mutants. Instead, we explanted Vangl2Looptail cochleae and established that correction can occur in culture. We are currently using pertussis toxin to block GPR156>Gai and ask whether correction is impaired in Vangl2Looptail mutants.
Conclusions: This work in progress generally addresses how EMX2>GPR156>Gai reversal relates to core PCP function, and could clarify how auditory HCs correct their orientation in time.

SA80. Single Nucleus Transcriptome and Epigenome of Human Inner Ear Sensory and Non-Sensory Cells

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Category: Development: Human Subjects
Background: The human utricle is a vestibular sensory organ responsible for balance. In recent years, single-cell omic sequencing technologies have been revolutionizing our understanding of molecular biology and diseases. Although some single-cell RNA-seq studies were performed in mouse utricle uncovering the cellular heterogeneity and molecular networks, no single-cell omic data for human utricle is yet available.

Therefore, we used emerging single-nucleus (sn) multiomic sequencing technology to simultaneously reveal individual nuclei transcriptomic and epigenomic landscapes. Our data shed light on utricular cell heterogeneity, cell type enriched and specific genes, chromatin accessibility, and gene regulatory networks.
Methods: We used thermolysin to collect sensory epithelia from two gestational age week 19 fetuses, and nuclei were isolated using a cell membrane lysis buffer. We acquired multiomic datasets performing Chromium Single Cell Multiome ATAC + Gene Expression (10x Genomics). For data analysis, we performed quality control based on RNA- and ATAC-seq metrics and applied dimension reduction and clustering techniques using Seurat, Signac, cisTopic, and other bioinformatic tools.
Results: We integrated snRNA- and snATAC-seq data from both samples and obtained robust projection and cell clustering. Clusters of hair cells, supporting cells, and transitional epithelial cells present specific differentially expressed genes and accessible peaks. We also discovered motifs enriched in accessible peaks (transcription factor-peak link) and calculated the correlation between peak accessibility and gene expression (peak-gene link). Combining these results, we inferred gene regulatory networks.
Conclusions: We generated the first fetal utricle single-nucleus multiomic dataset. Our data uncover cell type-specific gene regulatory networks of the human utricle and will contribute to advancing translational research to treat balance disorders.

SA81. Development of Gene Therapy for Usher Syndrome 1F Using Rational Protein Engineering
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Category: Gene Therapy
Background: Usher syndrome is an autosomal recessive disorder characterized by hearing loss, progressive retinal degeneration, and vestibular dysfunction. Mutations in protocadherin-15 (PCDH15) –an essential component of the inner ear hair-cell tip links – cause Usher syndrome type 1F. PCDH15 also plays an important role in the retina, but its function is less understood. Adeno-associated viral (AAVs) vectors have been found to be effective in delivering gene therapies to the inner ear. However, the coding sequence of PCDH15 is too large to fit into a single AAV capsid. The extracellular domain of PCDH15 comprises 11 similar but not identical extracellular cadherin (EC) repeats. We engineered novel "mini-PCDH15" constructs that retain the most important domain but lack 3-5 of EC repeats and that consequently fit in AAV. To test this therapy, we used a Pcdh15-/- mouse model, which exhibits profound deafness and balance deficit (circling behavior and inability to swim). Since a similar mouse model has been reported to have a visual deficit, we also examined the vision phenotype in our Pcdh15-/- mice. Here, we demonstrate the efficacy of mini-PCDH15s in treating the vestibular symptoms of USH1F and further evaluate if our mouse model displays a visual phenotype.
Methods: Newborn Pcdh15-/- mice were injected with AAVs encoding mini-PCDH15 at P0 through the round window, and then their vestibular phenotype was evaluated at 1 and 9 months of age using an open field, swim, and rotarod tests. The direct neural response was measured in vestibular stimulus-evoked potential measurements. Furthermore, to measure the electrical activity of the retina in Pcdh15-/- and Pcdh15+/- littermates, we carried out dark-adapted and light-adapted electroretinogram recordings (ERGs). In addition, we evaluated the anatomy of the vestibular organs and of the retina using standard immunohistochemistry techniques.
Results: AAV-mediated mini-PCDH15 delivery to the inner ears of Pcdh15-/- mice rescued their balance phenotype as assessed by behavioral testing. When compared to their uninjected Pcdh15-/- littermates, injected mice were able to swim, performed well on a rotarod test and showed reduced number of rotations in open field test similar to those measured in wild-type mice. Upon evaluation of the retinal phenotype, we have confirmed the visual deficit in our Pcdh15-/- mice, in agreement with previous reports. Namely, Pcdh15-/- mice show a substantial decline in ERG amplitudes and mislocalization of key phototransduction proteins in photoreceptors.
Conclusions: A single injection of mini-PCDH15 rescues the balance function in Pcdh15-/- mice, while the presence of the visual deficit in our Pcdh15-/- mice enables further studies with AAV-mediated delivery of mini-PCDH15 to the mouse retina as part of its preclinical evaluation. Furthermore, our findings promote the use of rational, iterative, structure-based mini-gene approaches to develop gene therapies for other large proteins.

SA82. Preclinical Development of an Adeno Associated Vector-Based Gene Therapy (SENS-501) for the Autosomal Recessive Non-Syndromic Deafness 9 (DFNB9)

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Category: Gene Therapy

Background: OTOF is a gene expressed in the inner hair cells (IHC) of the cochlea and encodes for Otoferlin. Otoferlin is a calcium sensor protein critical for the transmission of the signal from IHC to the spiral ganglion neurons (SGNs). The autosomal recessive deafness 9 (DFNB9) is caused by pathogenic biallelic loss of function variations in OTOF leading to the failure of synaptic transmission, resulting in congenital severe-to-profound auditory neuropathy. Cochlear implantation is the only option proposed to young patients thus far. Although this medical device improves the quality of life and language acquisition, hearing quality is limited, and a treatment for DFNB9 is necessary to address this unmet medical need.

Methods: We have developed SENS-501, a dual hybrid Adeno-Associated Virus (AAV) vector for DNFB9. Indeed, the size of the OTOF coding sequence largely exceeding AAV packaging capacity, OTOF coding sequence has been split into two AAV vectors.

SENS-501 was delivered into congenitally deaf DFNB9 mutant mouse ears through the round window (RW) at different doses. In parallel, SENS-501 was administered to Non-Human Primates (NHP) using the surgery and device that will be used in human. Early tolerability and biodistribution of SENS-501 studies were conducted in both species.

Results: The therapeutic candidate was validated through demonstration of otoferlin expression and integrity upon reconstitution of the full-length sequence in vitro and in vivo both in mice and NHP. In both species, IHC-restricted Otoferlin expression and good preliminary tolerability were demonstrated. Post-natal intracochlear injection of SENS-501 into the DFNB9 mutant mouse inner ear lead to improvement of hearing thresholds as early as 3 weeks post-injection, and long-term auditory-evoked brainstem responses, in a dose dependent manner, with efficacy demonstrated for at least six months. Dose-response experiments, early biodistribution studies in mice and NHP after intracochlear injection were performed.

Conclusions: SENS-501 appears safe and well tolerated. The selected AAV vector components allow to efficiently target IHC at levels compatible with therapeutic intervention in human and provide long-term efficacy data in DFNB9 mutant mouse model, which constitute a major step toward future clinical trials in DFNB9 patients. Dose range finding studies and early biodistribution studies, as well as the good preliminary safety profile of SENS-501, helped to design the ongoing GLP toxicity and biodistribution stun studies.

SA83. Comparative Study of Different Delivery Routes for Auditory Gene Therapy in Mice

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Category: Gene Therapy

Background: Gene therapy is a promising technique in the treatment of genetic hearing loss. It has already shown that it can provide complete restoration of hearing thresholds in an Otof KO mouse model (Akil et al, 2019). Its efficacy depends on many factors such as the stage of injection, the type of deafness, and the injection technique.

Methods: The aim of this study was to analyse different intra-labyrintic delivery routes in order to compare their transduction efficiency and their consequence on the hearing of wild-type mice at a mature stage (P15-20) with the AAV2-CBA-GFP vector. Administration through the round window membrane (RWM) with or without fenestration of the posterior semi-circular canal (PSCC), though the PSCC with or without fenestration of the RWM, and diffusion without injection through the RWM with or without fenestration of

the PSCC were studied. Then, we compared the outcome of the dual Otof gene therapy (Akil et al, 2019) using two different routes in the Otof KO mouse model.
Results: We showed that the PSCC delivery pathway in mice allows an equally efficient transduction of the cochlea and a better transduction of the vestibular organs compared to the RWM delivery. Moreover, the delivery via PSCC allowed a complete conservation of the hearing of wild-type mice, contrary to the other routes with opening of the RWM. Finally, PSCC also allowed more frequent hearing restoration in Otof KO mice compared with RWM delivery (n=8/12, 67% versus n=3/6, 50%) with better hearing thresholds ($p<0.0001$, 2-way ANOVA test).
Conclusions: The delivery via PSCC seems to better way of administration of gene therapy in genetic hearing loss mice model.

SA84. Application of a Novel Exon-Skipping Strategy on a Common USH2A Mutation in a Human Induced Pluripotent Stem Cell-Derived Inner Ear Organoid System

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Category: Gene Therapy
Background: USH2 is the most common form of Usher syndrome and mutations in USH2A gene, encoding the Usherin protein, are the main cause for USH2. In the inner ear, USH2A is expressed in hair cells where it forms links between the lower ends of stereocilia. Human USH2A gene consists of 71 coding exons. Twenty-three of exons encode for either Laminin EGF-like or Fibronectin type 3 repetitive domains. The most frequent mutation in USH2A is a single nucleotide deletion (2299delG; p.E767fs) in exon 13. Recently, an exon-skipping approach designed to remove exon 13 was tested in retinal organoids differentiated from an USH2A patient and a mutant zebrafish model that carries the c.2299delG mutation in the Ush2a gene. Herein, we explore effects of a CRISPR/Cas9-based exon-skipping strategy on hearing loss (HL) using the inner ear organoid system derived from patient-derived induced pluripotent stem cells (hiPSC) carrying c.2299delG mutation in the USH2A gene.
Methods: hiPSC was derived from a patient carrying compound heterozygous mutations, c.2299delG and c.14791+2 T>C, in the USH2A gene. The disruption of exon 13 in patient hiPSC was achieved by CRISPR/Cas9 genome editing technology. Additionally, hiPSC from the healthy sibling carrying the c.14791+2 T>C mutation serves as a healthy control.
Inner ear organoids were induced from three hiPSC lines: patient, patient with the exon 13 skipping edit, and the healthy control. Briefly, cells were aggregated on the induction day -2. Various small molecules and recombinant proteins were supplemented between induction day 0 and 15. Maturation medium was changed every two days for the long-term culture. Samples were collected for immunohistochemistry and single cell RNA-sequencing analyses.
Results: Multiple paired sgRNAs were first tested in WERI-Rb1 cells to determine the deletion efficiency in the exon 13 of USH2A. sgRNA with the highest editing efficiency was used to produce in-frame exon skipping transcripts in the patient hiPSCs.
Differences in the hair bundle structure and the localization of Usherin were examined using immunohistochemistry. Single cell RNA-sequencing analyses revealed the differential gene expression in hair cells in inner ear organoids derived from patient, patient with the exon 13 skipping edit, and healthy control hiPSCs.
Conclusions: This study demonstrated the utility of a novel exon skipping approach to the Usher syndrome in HL. A common mutation, c.2299delG, in the Ush2A gene was targeted and hiPSCs were derived from a patient bearing this mutation. An hiPSC-based model system was used to directly analyze the effect of this exon skipping approach on human cells. Moreover, it insights into the potential application of this novel approach in treating HL resulted from Usher syndrome.

SA85. Bedside to Bench: A Patient-Centered Pipeline for Gene Therapy for TMPRSS3 Hearing Loss

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Category: Gene Therapy
Background: Hearing loss demonstrates extreme genetic heterogeneity, with 122 causative genes and over 8,000 variants identified to date. This means that any molecular therapy requires a molecular diagnosis and precision-tailored and patient-specific approaches will be required for effective therapy.
TMPRSS3 (transmembrane serine protease 3) associated hearing loss, the fifth most common genetic cause of hearing loss and the most common genetic cause among recipients of cochlear implants, causes nonsyndromic, autosomal recessive sensorineural hearing loss. Variants in TMPRSS3 are associated with two discrete hearing phenotypes based on location and severity of variants; DFNB8 presenting mild, progressive, high-frequency hearing loss; and DFNB10 a severe profound, prelingual high-frequency hearing loss. Importantly, adult patients with TMPRSS3 mutations, compared to other genetic forms of hearing loss, have (1) more variable speech recognition scores, and (2) a physiologic reduction in spiral ganglion neuron function in vivo. However, the function of the TMPRSS3 protein is not known, but a prior mouse model showed rapid and total loss of cochlear hair cells just prior to the onset of hearing (P10-P15). Using a new patient-specific pipeline, we sought to model an individual patient's hearing loss in lab, to characterize the molecular mechanisms underpinning TMPRSS3-associated hearing loss and develop precision therapy tailored to this patient's hearing loss.
Methods: A patient presented to Otolaryngology clinic at Boston Children's Hospital with progressive bilateral, severe, high-frequency hearing loss. Word recognition scores decreased from 80% in 2017 to 20% in 2020. Genetic panel testing revealed a compound heterozygous variants in TMPRSS3:p.His70ThrfsTer/p.Ala426Thr.
We utilized CRISPR/Cas9 to generate mouse models of this patient's variants. This includes a compound heterozygous mouse model of the well-characterized, severe Y260X mutation on one allele and the patient derived p.A426T (m.p.A447T) on the other allele. Additionally, homozygous p.A426T and p.Gly443fs/p.Gly443ArgfsTer4 models were generated. Characterization of these lines to include hair cell counts, auditory brainstem responses, vestibular evoked potentials, and in situ hybridization assessment of TMPRSS3 expression is ongoing.
Results: Preliminary VsEP data shows no significant differences in A462T homozygous animals from wild type, whereas Y260X homozygous and G443fs homozygous animals show severe, progressive vestibular impairment. Similarly, ABRs and DPOAEs show profound hearing loss in Y260X and G443fs homozygous animals and no significant elevation in Y260X heterozygous and A426T homozygous animals at 16 weeks. In these models, we will assess if there are two separate mechanisms responsible for divergent phenotypes, to ultimately develop gene therapy in hopes of returning this treatment to the clinic.
Conclusions: We present a model of patient-centric precision medicine; taking a TMPRSS3 variant from "bedside" genetic diagnosis, to the "bench" to decipher the molecular mechanism of hearing loss, ultimately developing and testing novel therapies to return to patients. Models like this will be required for development of personalized therapies for hearing loss.

SA86. Adeno-Associated Virus (AAV)-Mediated Cochlear Glial Cell Targeting
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Category: Gene Therapy
Background: Sensorineural hearing loss (SNHL) accounts for more than 466 million people worldwide as of 2021. Those with SNHL subtypes, including acoustic neuropathy (AN) and cochlear nerve deficiency (CND), benefit little from CI due to disruption in signal transduction. In mammals, the auditory nerve does not spontaneously regenerate, thus, regenerative restoration of spiral ganglion neurons thus holds enormous potential. One potential source for in situ epigenetic reprogramming are the cochlear glial cells. Few studies have attempted transfecting glial cell tissues in the inner ear with adeno-associated viral vectors (AAVs), thus the viral tropism profile of AAV serotypes in the cochlear glial tissue in the osseous spiral lamina (OSL) and Rosenthal's canal (RC) remain unknown. In this project, we sought to identify AAV serotypes with selective tropism towards glial cells in the inner ear.

Methods: We selected the following 5 AAV serotypes: AAV-1, 6, AAV-DJ, AAV-PHP.eB and AAV.rh10, each harboring CMV promoter-driven eGFP. The selected AAVs were injected into neonatal mice between P2-5 via the posterior semicircular canal approach. Sham injections were also conducted by injecting fast green FCF dye with phosphorus buffered saline (PBS). The neonatal mice were euthanized one-week post-injection to harvest the cochlea for wholemount and cryosection preparations for immunofluorescence and confocal microscopy. The samples were stained with anti-Sox2 and Sox10 glial cell markers in the inner ear. Auditory brain responses (ABRs) in selected mice were recorded one-month post-injection to assess for injection-related hearing changes.
Results: Groups of 3-5 CBA/CaJ neonatal mice were microinjected with each of the 5 AAV serotypes; two sham injections with fast green FCF dye were conducted, and contralateral ear served as internal control. AAV-PHP.eB and AAV-DJ demonstrated robust GFP expression co-localizing with Sox2 and Sox10 positive cells in the OSL and RC regions. Both AAV-1 and AAV-6 demonstrated moderate GFP expression in OSL and is seen with limited expression in Sox10+ cells. AAV-6 also showed moderate GFP expression in the interdental cells, inner sulcus cells and Hensen's cells. AAV.rh10 demonstrated sparse GFP expression within the OSL and RC, with no expression in other parts of the cochlea and no co-localization with Sox10+ cells. The ABRs demonstrated no significant differences between, vector injected, sham injection, and contralateral control ears.
Conclusions: Our preliminary results demonstrated that AAV-PHP.eB and AAV-DJ have robust tropism towards glial cell population in the inner ear and co-localize with Sox2+ and Sox10+ cells in OSL and RC in neonatal mice. AAV-1 and -6 co-localizes weakly with Sox10+ cells, while AAV.rh10 demonstrate little to no expression in Sox10+ cells. Further investigation is necessary to optimize targeted gene delivery to glial cells with glial cell specific promoters and evaluation of age-related changes of viral gene expression.

SA87. Serum and Plasma miRNA Expression Levels in Sudden Sensorineural Hearing Loss Patients
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Category: Genetics A: Genomics and Gene Regulation
Background: Sudden sensorineural hearing loss (SSNHL) is an acquired idiopathic hearing loss that develops under 72 hours. Serum levels of small, non-coding RNAs, microRNAs (miRNAs) miR-195-5p/-132-3p/-30a-3p/-128-3p/-140-3p/-186-5p/-375-3p/-590-5p are differentially expressed in SSNHL patients within 28 days of hearing loss onset compared to normal hearing individuals. miRNAs are transmitted in both serum and plasma. Exosomes are transmitted in plasma and exosomal miRNAs are believed to be more readily available to cells. Serum transmits extracellular miRNAs. It is unknown if changes in serum or plasma miRNA differ in their ability to act as markers of disease related changes in cellular miRNA. Therefore, we sought to determine if there are difference between the serum and plasma expression levels of miRNAs previously identified to be differentially expressed in SSNHL patients' serum.
Methods: We collected serum and plasma of consenting adult SSNHL patients at presentation or during subsequent clinic follow-up. We extracted total RNA including miRNAs from 200µl of patient's serum and plasma using miRNeasy Mini Kit (Qiagen, Toronto, ON, Canada) then reverse transcribed (RT) the RNA with a TaqMan cDNA synthesis kit utilizing a preamplification step. We analysed the product by quantitative real-time PCR, normalizing target miRNA expression levels based on hsa-miR-191-5p. We calculated the miRNA expression level using the delta Ct method. We compared the serum and plasma expression levels of the miRNAs of interest with a Bonferroni corrected Welch's t-test (SPSS version 26).
Results: Paired serum and plasma samples from 17 (9 male) SSNHL patients' (mean age 51.9 years, Std. deviation 13.9 years) were analyzed. There was an inter-group difference in the mean expression levels of miR-590-5p, -4.49 and 3.37 in serum and plasma samples respectively (Bonferroni corrected $p<0.01$). However, the expression levels of miR-128-3p/-132-3p/-375-3p/-30a-3p/ -140-3p/-186-5p/-195-5p were similar in both groups.
Conclusions: We identified that miRs-128-3p/-132-3p/-375-3p/-30a-3p/ -140-3p/-186-5p/-195-5p expression levels in plasma and serum were similar, and that miR-590-5p expression level in plasma was higher than in serum in SSNHL patients.

SA88. Alpha1beta1 Integrin as a Potential Druggable Target for Alport Glomerular and Inner Ear Pathologies

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Category: Genetics A: Genomics and Gene Regulation

Background: Chronic kidney disease with early end stage renal failure is the most catastrophic aspect of Alport syndrome. Hearing loss is less studied but poses considerable emotional stress on young patients with the disease. We have been probing the mechanism of hearing loss in an autosomal recessive Alport syndrome (ARAS) mouse model which is rooted in dysfunction of the stria vascularis. We found common features in the pathological mechanisms underlying initiation and progression of the disease in both the kidney glomerulus and cochlear stria vascularis. In Alport glomeruli, laminin α2 and collagen α1(III) activate receptors on cells that promote inflammation and metabolic dysfunction, driving the progression of the disease. Blocking ETAR-mediated CDC42 activation prevents accumulation of these two ECM molecules in both organs and leads to cytoskeletal changes affecting cell adhesion and motility. Blocking CDC42 activation with small molecules that block endothelin A receptor (ETAR) improves function in both the kidney and the ear.

Methods: Given that Alport syndrome affects both glomerular and strial function, we did a comparative analysis between the two tissue compartments. We have identified ETAR receptor signal transduction through CDC42 as a principal initiator of glomerular disease. CDC42 is also regulated by integrin α1β1. As an initial step towards defining the similarities/differences of α1 integrin deletion on Alport glomerular and strial pathology RNA-seq was used to compare pathologic features of the glomerular and strial compartments in wild type, Alport, and integrin α1-null Alport mice. The stria vascularis was isolated from three 9-week-old wild type, Alport, integrin α1-null and integrin α1-null Alport (DKO) mice.

Results: We show that α1 integrin-null ARAS (DKO) mice treated with ramipril (ACE inhibitor, the standard of care for Alport patients) live > 3X longer that untreated ARAS mice, which is unprecedented. Glomerular pericytes from DKO mice are refractory to CDC42 activation and do not accumulate appreciable laminin α2 and collagen α1(III) in the GBM. The stria vascularis from DKO mice expresses all the receptors for these ECM molecules. Importantly, the RNA-seq data shows normalization of chemokine and cytokine expression in DKO in contrast to their upregulation in ARAS mice. In addition, DKO mice show normalization of expression of many genes previously associated with deafness.

Conclusions: We propose that α1β1 integrin may be superior to ETAR as a druggable target for the treatment of both glomerular and strial dysfunction associated with Alport syndrome.

SA89. Transcriptional Regulation of MYO7A Isoforms

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Category: Genetics A: Genomics and Gene Regulation

Background: Unconventional myosin VIIA (MYO7A) is essential for hair cell development, and our previous study provided evidence that MYO7A is critically involved in tensioning the tip link. Isoforms of MYO7A are differentially expressed in the inner and outer hair cells (IHCs and OHCs). Interestingly, these isoforms have distinct transcription start sites, suggesting different regulatory mechanisms for their expression. In this study, we investigated cis and trans-regulatory factors that regulate cell-type specificity and tonotopy of Myo7a expression.

Methods: ATAC-seq databases were consulted to predict novel cis-regulatory units of the mouse Myo7a gene. The function of regulatory unit candidates was evaluated by luciferase assay in RPE cells. Enhancer-specific deletion mouse lines were generated. Myo7a expression levels were measured by qPCR and immunofluorescence microscopy. UniBind was used for identifying TF candidates. Hair cell-specific Six2 KO mice were analyzed.

Results: By analyzing ChIP-seq and ATAC-seq databases, we identified multiple novel Myo7a enhancer candidates in the mouse genome. We screened candidates using luciferase assays and selected the most robust candidate to generate an enhancer-specific deletion mouse. The deletion of this enhancer reduced MYO7A in both IHCs and OHCs in a tonotopic manner in P5, with a ~80% reduction of MYO7A levels at basal turns and ~50% reduction at apical turns. MYO7A expression diminished progressively during

maturation, resulting in nearly undetectable MYO7A signals at all regions in adult mice, which resulted in profound hearing loss at 8-weeks.
The center of this enhancer features a binding motif for the transcription factor SIX2. This binding site is highly conserved in mammals and shows robust interaction with SIX2 in mouse and human kidney tissue. Immunofluorescence imaging showed that SIX2 is differentially expressed in the cochlea, with predominant expression in OHCs and a lower expression in IHCs. In OHCs, Six2 immunofluorescence increases from apex to base in a tonotopic manner. This expression pattern correlates well with the newly identified isoform of MYO7A (MYO7A-N). To investigate the function of SIX2, we deleted SIX2 in hair cells specifically. Our imaging analysis shows a stronger reduction of MYO7A in OHCs than IHCs, indicating its role in regulating the expression of MYO7A in OHCs. More importantly, the hair cell-specific deletion of SIX2 resulted in hair bundle disorganization and hair cell loss. This suggests that in addition to the hypothesized role in regulating MYO7A, SIX2 has a fundamental role in hair cell maintenance. In future studies, we will identify SIX2 target genes in the inner ear by applying single-cell RNA-seq, and investigate its global chromatin interaction using ChIP-seq.
Conclusions: Our previous study revealed an unexpected complexity of Myo7a isoforms in hair cells. Here we identified potential cis and trans-regulatory factors of Myo7a expression, indicating a role of transcriptional regulation of MYO7A isoforms in tuning hair cell function

SA90. A Maf Transcription Factor Code for Establishing Type I SGN Synaptic Heterogeneity
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Category: Genetics A: Genomics and Gene Regulation
Background: Heterogeneity at the inner hair cell (IHC)-spiral ganglion neuron (SGN) synapse is critical for transducing complex sound information. Each Type I SGN creates one to two synapses onto IHCs. Immunohistochemical analyses have found variations in the volume of glutamate receptor puncta among SGN postsynaptic terminals. Single-cell RNA sequencing has shown that Type I SGNs can be divided into three molecularly distinct subtypes. However, the molecular drivers of SGN postsynaptic heterogeneity remain elusive. Maf family transcription factors stand out as excellent candidates due to their known roles in synapse development in other regions of the nervous system. Here, we show that these transcription factors create a combinatorial code across SGN subtypes to establish the synaptic heterogeneity that is critical for normal hearing function.
Methods: To define the contributions of Mafb and cMaf to synaptic heterogeneity, we assessed single and double conditional knock-out strains for changes in synaptic morphology, gene expression, and auditory responses. Conditional knockouts were made using bhlhb5Cre and floxed cMaf and Mafb alleles; controls were wildtype or heterozygous. Synaptic puncta number and volume were quantified from wholemount cochleae stained for GluR2 and CTBP2. Auditory brainstem response (ABR) thresholds and and Wave-I amplitudes were measured. All experiments were conducted on at least 4-5 littermates (P6 and adult) from each genotype. cMaf and Mafb protein expression was quantified by staining SGNs along with a subtype marker. 10X single-cell RNA sequencing (scRNAseq) was used to detect changes to synaptic genes or subtype identity in knockout strains.
Results: cMaf/Mafb double-knockouts have severe synaptic and functional hearing deficits. Double-knockouts have more variable GluR2 puncta volumes and are less likely to be paired to an opposing ribbon compared to littermate controls. ABR wave-I amplitudes are severely reduced in double knockouts and thresholds are increased. Analysis of scRNAseq datasets and protein staining revealed that cMaf and Mafb are expressed in complementary patterns across SGN subtypes, suggesting they might have opposite effects on SGN synaptic heterogeneity. Indeed, whereas Mafb mutant SGN terminals contain significantly smaller GluR2 puncta, GluR2 puncta volume is increased in cMaf mutants. ABR wave-I amplitudes are reduced in Mafb and cMaf single-knockouts compared to control suggesting that each of these transcription factors independently play a role in synaptic differentiation. Importantly, neither of the single-knockout ABR or synaptic phenotypes are as severe as the double knockout, further supporting their complementary functions. Consistent with this idea, single-cell RNA sequencing revealed that cMaf and Mafb likely target both distinct and overlapping synaptic and synaptic plasticity genes.
Conclusions: The complementary expression patterns, single-knockout synaptic phenotypes, and more severe synaptic and functional deficits in the double-knockouts suggest that cMaf and Mafb establish a combinatorial transcriptional code to establish synaptic heterogeneity across type I SGN subtypes.

SA91. miRNA Expression Levels in Sudden Sensorineural Hearing Loss Patients: Acute Serum Samples Compared With Samples Drawn 3-12 Months After Hearing Loss Onset

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Category: Genetics A: Genomics and Gene Regulation

Background: Sudden sensorineural hearing loss (SSNHL) is a type of acquired idiopathic hearing loss that develops under 72 hours. Serum levels of small, non-coding RNAs, microRNAs (miRNAs) miR-195-5p/-132-3p/-30a-3p/-128-3p/-140-3p/-186-5p/-375-3p/-590-5p are differentially expressed in SSNHL patients within 28 days of hearing loss onset compared to normal hearing individuals. This study determines if these changes persist by comparing the miRNA expression profile in the serum of SSNHL patients within 1 month of hearing loss onset with that of patients 3-12 months after hearing loss onset.

Methods: We collected serum of consenting adult SSNHL patients at presentation or during subsequent clinic follow-up. We matched patient samples drawn 3-12 months after the onset of hearing loss (delayed group) by age and sex to samples drawn from patients presenting within 28 days of hearing loss onset (immediate group). Total RNA including miRNAs was extracted from 200µl of patient's serum using miRNeasy Mini Kit (Qiagen, Toronto, ON, Canada). After total RNA extraction, Reverse transcription (RT) was undertaken with a TaqMan cDNA synthesis kit utilizing a preamplification step followed by TaqMan™ MicroRNA quantitative real-time PCR. We normalized the miRNA expression levels based on reference miRNA, hsa-miR-191-5p. Then we calculated the miRNA expression level using the delta Ct method. We compared the expression levels of the miRNAs of interest between the immediate and delayed group using Welch's t-test SPSS version 26. In order to confirm the diagnosis of SSNHL, categorize the degree of hearing loss at presentation, and determine patient's hearing recovery status we performed pure tone audiometry. We calculated the averaged air conduction pure-tone audiometric thresholds in affected ears at 4 low (0.5,1,2 and 3 or 4kHz) or 3 high (3 or 4,6 and 8kHz) frequencies. The same frequencies selected at the initial audiogram were averaged in all follow-up audiograms for each patient. We classified the patients hearing outcome status as either hearing recovered or not recovered. Patients with PTA averaged hearing gain on follow-up of 10 dB or greater were categorized as hearing recovered. We undertook inter-group comparisons of hearing outcome status, initial and final averaged PTA thresholds in the affected ear with a Chi-squared test and independent samples Student's t-tests.

Results: 7 delayed group serum samples from 7 male SSNHL patients' (mean age 56.8 years, Std. deviation 17.3) and 5 immediate group (4 male) SSNHL patients (mean age 57.53 years, Std. deviation 17.63) were analyzed. There was an inter-group difference in the expression level of miR-195-5p and miR132-3p ($p<0.005$). However, the expression levels of miR-30a-3p/-128-3p/-140-3p/-186-5p/-375-3p/-590-5p were similar in both groups. There was no significant inter-group difference in hearing recovery status, initial and final averaged PTA thresholds in the affected ears.

Conclusions: There is evidence of a change in the serum miRNA expression profile of SSNHL patients over time.

SA92. Early Postnatal RFX1/3 Depletion Leads to Outer Hair Cell Loss and Severely Impaired Auditory Function

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Category: Genetics B: General

Background: Our laboratory has previously identified the RFX transcription factors (TFs) as essential for hearing and outer hair cell (OHC) survival. Specifically, a loss of Rfx1 and Rfx3 together (Rfx1/3), at embryonic day (E) 16, using the Rfx1/3;Gfi1-Cre mouse model, leads to an abrupt loss of OHCs shortly after the onset of hearing and profound hearing loss as early as postnatal day (P) 16. To better understand the role of RFX1/3 in the development and maintenance of HCs during the postnatal period, we evaluated the auditory function and histology of a newly generated Rfx1/3 conditional knockout mouse model.

Methods: Rfx1/3 floxed mice were crossed with Myo15-Cre mice (onset of cre-recombinase expression in cochlear HCs at ~P0) to conditionally deplete Rfx1/3 from hair cells (HCs) (Rfx1/3;Myo15-Cre mice). Auditory thresholds and OHC function were measured using auditory brain response (ABR) tests and distortion product otoacoustic emissions (DPOAEs), respectively, at P30 and P45. HC loss was evaluated with whole mount cochlear immunohistochemistry at early postnatal time points, P30, and P45, using the actin stain Phalloidin and nuclear stain DAPI.
Results: Rfx1/3;Myo15-Cre mutant mice demonstrate significantly increased hearing thresholds as well as impaired OHC function in comparison to their littermate controls. Additionally, Rfx1/3;Myo15-Cre mutant mice exhibit OHC loss at P30 and P45.
Conclusions: Our data reveal that expression of Rfx1/3 is not only necessary during embryonic for OHC development and/or maintenance and auditory function, but also postnatally. Follow up studies are required to identify the beginning of OHC loss and Rfx1/3 regulatory pathway.

SA93. Does WFS1 Have a Role in the Mouse Cochlea?

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Category: Genetics B: General
Background: Wolframin ER Transmembrane Glycoprotein (WFS1) is localized to the endoplasmic reticulum (ER). Its functional role is largely unknown. Studies using animal models and cultured cells suggest that WSF1 promotes ER homeostasis, via modulating calcium signalling or the unfolded protein response. In humans, more than 50 point mutations have been identified in the WFS1 gene. They are associated with mild-to-moderate syndromic or non-syndromic hearing loss. WFS1 mutations underlie the autosomal recessive Wolfram syndrome, a rare degenerative syndrome associated with diabetes mellitus, optic atrophy, and sensorineural hearing loss. Rodent studies have shown that WFS1 is expressed in the brain and pancreas. WFS1 immunoreactivity has also been shown in the organ of Corti and spiral ganglion neurons of adult mice and marmosets. We are interested in the regulation of ER homeostasis in the cochlea and in the consequences of ER stress, defined as the accumulation of misfolded proteins, in hair cells. As WFS1 has been linked to ER stress in other models, we were interested to find its possible role in ER stress regulation in hair cells.
Methods: We studied the expression and functional significance of WFS1 in a KO mouse model where exon 8 is deleted, resulting in a loss-of-function phenotype. In this KO model, the deleted exon is replaced by the LacZ reporter gene, enabling us to study WFS1 expression. This was important, as we did not find any reliable WFS1 antibodies for immunohistochemical detection.
WFS1 KO and wildtype mice were under the 129S/Svevtac/C57BL/6J hybrid background. We measured ABRs and quantified the numbers of hair cells and ribbon synapses of inner hair cells between P8 and 6 months of age. Contralateral ears were processed for X-Gal staining to detect LacZ expression.
Results: We did not find LacZ expression in the cochleas of heterozygote or homozygote individuals of the WFS1 KO line. In these experiments, we had brain hippocampal and pancreas tissue as positive controls. Consistent with the lack of WFS1 expression in the cochlea, ABR thresholds at the 4 to 40 kHz frequency range were comparable in the KO and wildtype mice. The KO mice lacked a diabetic phenotype, but had reduced body weight. The genetic background used did not cause age-related hearing loss, assessed by ABRs at 6 months of age. Consistent with the lack of WFS1 expression in the cochlea, KO mice did not show hair cell loss and their ribbon synapse numbers were comparable to wildtype mice.
Conclusions: Our results suggest that WFS1 is not expressed and does not have a role in the normal mouse cochlea. Therefore, considering the human data showing a link between WFS1 mutations and hearing loss, there is not a correspondence between the WFS1 mouse model and the human disease phenotypes.

SA94. Copy Number Variants in Genetic Hearing Loss

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Category: Genetics B: General
Background: Structural variants comprise different changes in genomic structure, such as copy number variants (CNVs), inversions, insertions, and rearrangements. CNVs have been identified as a significant

cause of hearing loss, making their detection an important part of comprehensive genetic testing. Challenges related to genomic regions of high homology, mapping, and variant classification may preclude detection and correct classification of CNVs. Here, we present the largest study to date exploring the impact of CNVs in syndromic and non-syndromic hearing loss (NSHL).
Methods: We used targeted genomic enrichment and massively parallel sequencing to screen all known deafness-associated genes in a large ethnically diverse cohort with hearing loss. All genes were assessed for single nucleotide variants, indels, and CNVs with a customized bioinformatics pipeline. Variants were discussed in the context of clinical and familial history data.
Results: Of 6172 probands tested for a genetic cause of hearing loss, we identified approximately 1000 CNVs in over 80 different known hearing loss-associated genes, with 13.4% of probands carrying at least one CNV. The most common type of CNVs in our cohort are deletions, ranging from multi-gene deletions (~42%), whole gene deletions (~9%), to partial gene deletions (~17%). Duplications and gene-to-pseudogene conversions make up the remainder of the CNVs (~32%). We also identified one inversion event in PAX3.
A genetic cause of hearing loss was identified for 43% (2626) of our probands, with 18% involving at least one causative CNV. CNVs were the sole causative variants in nearly 10% of autosomal recessive hearing loss diagnoses and in ~2% of autosomal dominant hearing loss (ADHL) diagnoses. Of the CNVs involved in ADHL, two-thirds were causative for syndromic HL, and one-third was causative for NSHL.
Overall, CNVs in STRC were the most common finding (67%), followed by CNVs in OTOA (8%), USH2A (2%), and the DFNB1 region (2%). For ADHL, CNVs were most common in TBX1 (22%), followed by EYA1, MITF, and POU3F4 (12% each).
Conclusions: Targeted gene panels that include regions of homology are an efficient strategy to detect CNVs. Our findings show that the contribution of CNVs to hearing loss is both inheritance and phenotype dependent. Our data emphasize the importance of a comprehensive CNV screening in all genetic testing to ensure accurate patient diagnoses, leading to more effective care and treatment options.
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SA95. The Spen/Starliner Zebrafish Mutant Has Central Processing Deficits in Hearing and Balance

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Category: Genetics B: General
Background: Previous studies have shown that genetic forms of human hearing loss mainly affect the peripheral auditory system. In contrast, the genetic basis of central defects in the auditory/vestibular system remains largely unexplored despite the existence of central forms of hearing loss and imbalance in humans. Using a forward genetic approach, we identified a novel zebrafish mutant, starliner, which has recessive auditory/vestibular defects within the central nervous system, offering an opportunity to explore central dysfunction.
Methods: The starliner mutant was isolated from an F3 mutagenesis screen. The mutation in starliner was identified using bulk RNAseq combined with RNAMapper analysis. Standard immunohistochemistry, in situ hybridization, and EdU labeling methods were used to characterize the phenotype of spen mutants. Behavioral tests, including the AEBR, VIEM, VSR, and OMR, were conducted and quantified as previously described (Maeda et al., 2017; Gao and Nicolson, 2020; Roeser and Baier, 2003).
Results: We identified a nonsense mutation in the split ends (spen) gene, which encodes a transcription factor that is enriched in the brain. In humans, de novo mutations in SPEN are associated with childhood intellectual disabilities and sensorineural hearing loss. Analysis and validation of RNAseq data revealed that transcripts for smc1a, which encodes a DNA cohesin ring protein, are strongly downregulated in spen mutants. Mutations in both SPEN and SMC1A are implicated in Cornelia De Lang Syndrome, which also involves intellectual disabilities and hearing loss. SMC1a is critical for DNA replication and our EdU labeling experiments demonstrate that cell proliferation in the brain is delayed in spen mutants between 4 and 7 days postfertilization. In terms of behavioral deficits, the AEBR was greatly diminished, and the VSR was attenuated in mutants, yet the VIEM and touch startle reflex were normal. Visualization of tone-induced responses in the CNS via phospho-ERK labeling revealed that most regions showed less activity in spen mutants with the exception of a midline area of the hindbrain, which exhibited a striking increase in activity.

Conclusions: The above results suggest that spen plays a role in the development of the central auditory/vestibular system. The transient yet complete block of cell division within the proliferation zones of the developing brain in spen mutants suggests that this short time window during larval development is critical for development and/or function of central pathways of the auditory/vestibular system. We hypothesize that this delay leads to aberrant signaling in the hindbrain in spen mutants in response to sound. Such a delay in neural proliferation could also explain the delayed development and intellectual disabilities seen in human patients.

SA96. Generation of F1 Gad670GFP and CBA Backcross Improves ABR Thresholds

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Category: Genetics B: General
Background: Gad67-GFP knock-in mice express GFP in the inferior colliculus (IC) but have elevated hearing thresholds as they age, as demonstrated via auditory brainstem response (ABR). The Gad67-GFP knock-in mouse strain used in this study is bred on a WT Swiss Webster background. Swiss Webster mice develop delayed onset hearing loss roughly around 4 months of age and exhibit elevated ABR thresholds (Drayton et al., 2006). CBA/CaJ mice are considered as the gold standard of age-related hearing loss in a murine model due to slow hearing loss as they age, much like what is seen in most humans (Frisina et al., 2011). Based on what is known about age-related hearing loss in control mouse models, we hypothesized that a backcross of Gad67-GFP with CBA would improve hearing thresholds (as determined by ABR) while maintaining enhanced GFP expression in the IC under control of the endogenous Gad67 promoter.
Methods: Adult glutamic acid decarboxylase-67 (Gad67)-GFP knock-in mice were bred with CBA mice to obtain eight filial generations of mice. For this study, the F1 generation was used as the primary basis of comparison. Auditory brainstem response testing was conducted under anesthesia (2-3mg/kg acepromazine, 100mg/kg ketamine, 6mg/kg xylazine) on Gad67-GFP knock in mice (N=10), CBA/CaJ (N=10), WT Swiss Webster (N=11), and the F1 generation (N=7) at 6 months and 12 months of age. Sound stimuli were presented via subdermal electrodes placed at the vertex of the skull and behind each ear. Hearing thresholds were determined via Wave I generation in decreasing intensity as measured in decibels (dB) across flat noise, 4kHz, 8kHz, 16kHz, 32kHz, and 40kHz stimuli. After 12-month ABR data was compiled, mice were euthanized via transcardial perfusion to collect the brain and cochlea for histology.
Results: Preliminary data strongly suggest that the F1 backcross retains robust GFP expression in GABAergic neurons and additionally exhibits improved hearing across all frequencies in comparison to three control groups at 6 months and at 12 months of age. Further histological analysis is ongoing.
Conclusions: Backcrossing the Gad67-GFP mouse model (generated on a SW background) with the CBA mouse model significantly improves hearing thresholds in an aging model in one generation while maintaining robust expression of GFP in the IC. Further analysis is needed to determine potential causes of age-related hearing loss in control mice in comparison to the F1 generation.

SA97. Investigating the Hearing Phenotype and the Efficiency of Flpo Recombinase Within the Cochlea in R26FlpoER Mice

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Category: Genetics B: General
Background: B6N.129S6(Cg)-Gt(ROSA)26Sortm3(CAG-flpo/ERT2)Alj/J mice, commonly known as R26FlpoER, express a tamoxifen-inducible optimised FLPe recombinase variant known as Flpo (Lao et al., Cell Reports 2012). In both cells and animals, Flpo recombination efficiency is higher than the conventional FLPe in deleting DNA between nearby FRT sites in the genome (Kranz et al., Genesis 2010; Raymond and Soriano, PLoS One 2007, Genesis 2010). Although R26FlpoER mice are a helpful tool for manipulating murine genetics, neither the hearing of these mice nor the efficiency of Flpo recombinase in the inner ear have ever been studied.
Methods: Auditory Brainstem Responses were recorded from ketamine/xylazine anaesthetised mice at 4, 8, and 14 weeks old in response to click stimuli and tone pips ranging from 3-42kHz.
The efficiency of Flpo recombinase was investigated by crossing the R26FlpoER mice with RC::FLTG mice, also known as B6.Cg-Gt(ROSA)26Sortm1.3(CAG-tdTomato,-EGFP)Pjen/J (Plummer et al., Development 2015). A STOP codon flanked by FRT sites is located upstream of the tdTomato gene in

RC::FLTG mice, inhibiting its expression. Only upon tamoxifen injection, Flpo is activated and can recognise the FRT sites which flank the STOP codon upstream of tdTomato and a robust tdTomato fluorescence is expected in Flpo-expressing cells/tissues. The efficacy of Flpo recombinase has been evaluated 2 weeks after a single tamoxifen injection (0.2ml/mg IP) at postnatal day 17.
Results: At all tested ages, Flpo heterozygotes and homozygotes had similar ABR thresholds to their wild-type littermate controls. This study demonstrated that up to 14 weeks, R26FlpoER mice do not exhibit any hearing loss making them suitable for experimental studies up to that age. Preliminary findings suggest tamoxifen injection at postnatal day 17 produces widespread activation of Flp recombinase assessed using a tdTomato reporter. Additional ages of tamoxifen injection, including neonatal, juvenile, and adult mice, are underway.
Conclusions: These mice have the potential to be widely employed for genetic manipulation in the field of hearing research, thus it is critical to determine whether or not Flpo recombinase activity varies with age within the inner ear before proceeding with any further research with them. Our results suggest the R26FlpoER mice are suitable for this use.

SA98. Identification of Variants Contributing to Impaired Hearing in Older Adults

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Category: Genetics B: General
Background: Adult-onset hearing loss is a common, heterogeneous condition with a strong genetic component. Although over 150 genes have been identified as contributing to human hearing loss, many more remain to be discovered, and most of the underlying genetic variation remains unexplained. Family studies have been invaluable in discovering rare variants with moderate to large effects, but the contribution of common variants is harder to ascertain.
Methods: Our primary cohort consisted of 532 older adult volunteers, for whom we had both audiometry and whole exome sequencing data. The exome sequence data were processed and filtered stringently, resulting in a list of 29,807 high quality variants, a selection of which were confirmed by Sanger sequencing, with an accuracy rate of 95.8%. We designed an algorithm to test each variant, comparing the average thresholds for pure tones at individual frequencies of participants separated into groups by genotype and sex. Any variants which appeared to be linked to markedly different thresholds in carriers were subjected to a permutation test to ascertain how likely the observed differences were to have arisen by chance in our cohort.
Results: We identified 40 individual variants which appeared to contribute to differences in audiometric thresholds, and we have found variants linked to better hearing (eg TCEANC2) in carriers as well as those linked to poorer hearing (eg CAPN9). In some cases, we observed a difference in phenotype between male and female carriers. We attempted to replicate our findings in TwinsUK, where a subset of participants also have both exome sequencing and audiometry data. From these analyses we have chosen genes for further investigation in silico and in vivo, to confirm their link to hearing loss in older adults.
Conclusions: We have developed an algorithm which identified multiple individual candidate variants associated with changes in pure-tone thresholds in carriers. Most variants are not in previously known deafness genes, suggesting novel candidates to add to the list of genes underlying hearing loss. Interestingly, a subset of variants are associated with better thresholds in older adult carriers.

SA99. Two New Mouse Alleles of Ocm and Slc26a5

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Category: Genetics B: General
Background: Oncomodulin (Ocm) and prestin (Slc26a5) are two key proteins for the correct functioning of the outer hair cells (OHCs) and are required for normal hearing in mice. Oncomodulin is a calcium-binding protein that functions as a cytosolic Ca2+ buffer. Prestin is a voltage-sensitive motor molecule that changes

conformation in response to changes in membrane potential. It is thought to be responsible for amplifying cochlear responses to low-level sounds. Targeted deletion of Ocm in mice causes progressive cochlear dysfunction and late-onset OHC loss (Tong et al.,2016), and prestin knockout mice have shorter OHCs and hair cell degeneration (Liberman et al.,2002).
Methods: Here, we present two new mouse alleles: Ocm<tm1e(EUCOMM)Wtsi> and Slc26a5<tm1(EGFP/Cre/ERT2)Wtsi>. Both lines have a large cassette inserted into intron 2-3 (in Ocm<tm1e>) or intron 8-9 (in Slc26a5<tm1Cre>) that interferes with transcription, resulting in expression knockdown. We assessed the hearing sensitivity in Ocm<tm1e> and Slc26a5<tm1Cre> mice using Auditory Brainstem Response (ABR) measurements and characterised the associated structural and molecular features by scanning electron microscopy (SEM), immunohistochemistry, and RT-qPCR.
Results: Slc26a5<tm1Cre> homozygous mice exhibit severe hearing loss from four weeks old and Slc26a5<tm1Cre> heterozygous mice have raised thresholds only at the high frequencies. ABR thresholds were normal in Ocm<tm1e> mice at 4 weeks old. However, by 8 weeks old, Ocm<tm1e> homozygous mice showed high-frequency hearing loss, which progressed over time, affecting also the lower frequencies by 14 weeks old. At all ages studied, mice heterozygous for the Ocm<tm1e> allele looked identical to wildtype mice. SEM revealed that Ocm<tm1e> homozygous mice have a significant degeneration of OHC stereocilia bundles at 4 weeks old, even when ABR thresholds are normal. The OHC bundle degeneration worsens by 8 weeks old, and by 10 weeks old, Ocm<tm1e> homozygous mice show severe OHC degeneration, which is progressive towards the high-frequency locations, as determined by prestin staining of the OHCs.
It has been reported that Ocm mutants show abnormal prestin expression in a different mouse line (Tong et al.,2016), so we investigated whether Ocm and Slc26a5 interact or regulate each other. We used RT-qPCR to investigate how knocking down each of the genes affected the expression of the other in the inner ear of 5-days-old and 10-weeks-old mice. The mRNA levels of Slc26a5 were unaffected in Ocm<tm1e> homozygotes, and Ocm levels were unaffected in Slc26a5<tm1Cre> homozygotes. Our findings indicate that it is unlikely that oncomodulin interacts with or regulates prestin, or vice versa. In support of this, we found that the OHC length was unaffected in Ocm<tm1e> mutant mice, indicating that prestin is not affected by oncomodulin deficiency.
Conclusions: In conclusion, we have characterised two new mouse alleles of Ocm and Slc26a5, which provide new insights into the roles of oncomodulin and prestin in the mouse inner ear.

SA100. Exome Sequencing is an Effective Diagnostic Tool for Pediatric Hearing Loss, including Unilateral and Asymmetric Forms

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Category: Genetics B: General
Background: Clinical algorithms often incorporate genetic testing for pediatric patients with bilateral symmetric sensorineural hearing loss (SNHL), but may exclude children with asymmetric or unilateral SNHL, given previously reported low diagnostic rates of genetic testing for these patients. However, the studies underlying these clinical algorithms largely do not target pediatric patients, include parental testing, use exome sequencing (ES), or have a large sample size. Our goal was to determine the diagnostic yield of ES for pediatric patient with asymmetric and unilateral compared to bilateral SNHL in a large cohort of pediatric patients.
Methods: ES was performed from buccal-derived DNA for pediatric patients with confirmed Weird SNHL without a known genetic or environmental etiology. Biological relatives were also tested (typically trios). ES mapping and variant calling, including copy number variants, was performed with the DRAGEN Bio-IT Platform (Illumina). Primary variant analysis focused on 366 known and candidate hearing loss genes.
Results: ES was performed for 254 pediatric hearing loss patients and their biological relatives that were available for testing (648 participants, including 153 trios). This cohort was clinically heterogenous in terms of SNHL phenotypes, including probands of varied laterality of SNHL, configuration of audiogram, age of onset of SNHL, and presence of other clinical features. A genetic cause of SNHL was identified for 31.9% of probands (n=81) with causative variants in 42 genes. The overall genetic diagnostic rate was 40.7% for

bilateral, 27.0% for asymmetric, and 16.7% for unilateral hearing loss, with syndromic diagnoses made in 20.3%, 30.0%, and 58.3% of cases in each group, respectively. In several cases of a syndromic SNHL diagnosis, a genetic syndrome was not suspected, and no other clinical features were appreciated prior to study enrollment or post-diagnosis.
Conclusions: We identified a genetic cause of SNHL in a substantial percentage of pediatric patients with asymmetric and unilateral SNHL. Syndromic SNHL was more common in these cases compared to bilateral SNHL. Increased access to genetic testing for patients with all SNHL phenotypes will facilitate tailored intervention, early referral to appropriate specialists, and improved prognostic and recurrence information for families.

SA101. Deafness Gene Identification: Variant Prioritization in a Large Cohort of Hearing-Impaired Individuals Without a Genetic Diagnosis

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Category: Genetics B: General
Background: Over 200 genes have been associated with hereditary hearing loss (HL), yet many cases remain unexplained. One reason for this is that pathogenic variants are in still “unknown” deafness genes. Identification of these genes is important for patients and their families to provide prognostic information, for genetic counselling and for eligibility for future therapies. With a variant prioritization approach, we aim to identify new (candidate) genes for HL.
Methods: In a collaboration of Dutch university medical centers, we collected exome-wide sequencing (ES) data of a large cohort of subjects with HL with a (likely) genetic cause, for which no cause was identified in routine genetic testing (ES-based gene-panel analysis). We defined two groups: individuals with a likely autosomal dominant (AD) inheritance (group 1, n = 121) and with a likely autosomal recessive (AR) or unknown pattern of inheritance and isolated cases (group 2, AR n = 35, unknown/isolated cases n = 305). We prioritized variants based on allele frequency (<0.1% in group 1; <1% in group 2), (predicted) effect on the encoded protein (e.g. truncating), gene component in which the variant is located (e.g. the splice site region), and predicted pathogenicity of missense variants and predicted effects on splicing by in silico prediction tools. We followed up variants in known or candidate deafness genes based on literature, preferential inner ear expression and/or mouse studies.
Results: Currently, a number of candidate genes with variants in multiple families are being addressed with segregation and functional analyses. Also, structural variants are being assessed in a subset of group 2 samples with a truncating monoallelic variant in a known gene for ARHL. So far, we have solved two cases with variants in known deafness genes (TRRAP, DFNA75 (group 1); ABHD12, syndromic HL and cataract (group 2)).
Conclusions: Our study shows that with the current knowledge, the diagnostic yield of exome-wide after HL gene panel analysis is limited. Also, it is essential to include family members for a follow-up of candidate variants. We need to address structural variants which are likely to significantly contribute to HL. Further elucidation of genetic causes of HL will also require larger cohorts of subjects and a focus on genome sequencing. The latter will allow the assessment of intronic and regulatory variants.

SA102. A Study on the Mechanism of a Novel Candidate Gene CEP250 Cause Hearing Loss

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Category: Genetics B: General
Background: CEP250 encodes the C-Nap1 protein that belongs to the CEP protein family. Which includes more than 30 proteins that form active centrosome components and play important roles in centrosome aggregation and cell cycle progression. C-Nap1 is expressed in photoreceptor cilia and interacts with other ciliary proteins, including Rootletin and NEK2. It is also known that CEP250 is involved in cilia formation

in a study on retinal cilia formation in ARPE19 cells. It is known that nonsense mutations in CEP250 cause atypical Usher syndrome, characterized by early onset sensorineural hearing loss and relatively mild retinitis pigmentosa.
Heterozygous nonsense variants in CEP250 were identified in five Japanese family members with syndromic features of cone-rod dystrophy and sensorineural hearing loss. Their ophthalmologic phenotype was mild, which was indicative of atypical Usher syndrome.
A novel homozygous nonsense variant in CEP250 was identified in a family with non-syndromic sensorineural hearing loss. This study was conducted to determine whether CEP250 variations can cause non-syndromic hearing loss and elucidate potential pathomechanism of the variant in CEP250.
Methods: Whole exome sequencing and variant calling, filtering and evaluation of variants.
pRK5-Myc-CEP250 recombinant vector construction and mutagenesis.
Cell culture and transfection.
Immunoblotting.
Immunocytochemistry.
Inner ear immunoblotting.
Inner ear immunohistochemistry.
Results: We identified a novel variant in CEP250 in members of the YUHL251 family who did not have GJB2 and SLC26A4 variants.
Immunoblotting of the CEP250 (c.3511C>T) variant produced a band of 150 kDa, which was smaller than that of WT, which was 250 KDa. This result indicates that truncating CEP250 protein could be escaped from ER quality control and may harm cellular functioning, which in turn may affect the cochlea in a way that causes hearing loss.
CEP250 WT was localized to centrosomes, but CEP250 p.Gln1171Ter was not and instead was dispersedly expressed in the cytosol.
Cep250 protein was expressed in inner hair cells, outer hair cells, and spiral ganglions.
Conclusions: The novel nonsense CEP250 (c.3511C>T) variant was found in patients with moderate NSHL caused by autosomal recessive inheritance. Early truncating protein of CEP250 (p.Gln1171Ter) had a deficit in trafficking toward centrosomes and ciliary elongation. The fact that Cep250 was expressed in the cochlear haircells and spiral ganglion indicates that defect in the centrosomal localization of CEP250 and impaired ciliary elongation caused by the nonsense CEP250 variant may have resulted in the hearing deterioration. Based on these results, CEP250 can be considered a candidate gene for causing NSHL.

SA103. Effect of K+ Relief of Block in gK,L on Non-Quantal Transmission at the Vestibular Hair Cell-Calyx Synapse

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Category: Hair Cells: Anatomy and Physiology
Background: Type I hair cells, which detect head motion, sit within and transmit to cup-shaped terminals (calyces) of afferent neurons. These neurons guide motor reflexes that maintain gaze, balance, and our sense of orientation. In addition to glutamate release (quantal transmission), ions flow through the basolateral hair cell (pre-synaptic) membrane into the synaptic cleft and through the inner calyx (post-synaptic) membrane (nonquantal transmission-NQT). The synaptic cleft (SC) cannot be accessed by reference electrodes without disrupting its structure and function. As a result, gradients in ion concentrations [Ion] and electric potential (ϕ) within the cleft cannot be measured and pre-/post-synaptic membrane voltages cannot be directly obtained. We have developed a computational, biophysical, model of the synapse to overcome this limitation.
Methods: To simulate transmission between hair cell and afferent neuron, our model uses Hodgkin-Huxley-style ion currents based on whole-cell recordings, continuity equations to describe changes in electric potential within hair cell, cleft, afferent calyx and fiber, and electro-diffusion equations for cleft K+ and Na+. Step or sinusoidal hair bundle deflection or voltage step protocols are used as input.
Results: We previously presented the sequence of events underlying NQT and the role of [K+]_SC and ϕ_SC in driving post-synaptic currents during hair cell stimulation (bioRxiv 2021.11.18.469197, in review). We now present simulations that explore K+ relief of block (KRB) in gK,L (Contini et al. 2020). This was accomplished by extrapolating the concentration dependence of voltage block parameters from two (4 and 20 mM) datapoints. gK,L is depolarization-activated. When hair cell intracellular potential (ϕ_H) is

depolarized, [K+]_SC and ϕ_SC are greatest at the base and fall to the perilymph value at the apex. As a result, the hair cell trans-membrane voltage (V_H = ϕ_H - ϕ_SC) is less negative and E_K more negative towards the cleft apex. These properties result in greater gK,L open probability and K+ driving force (V_H - E_K) towards the apex of the cleft. KRB works in the opposite direction: gK,L open probabilities are enhanced towards the base, where [K+]_SC is largest.
Conclusions: Upon replacing the steady-state activation-voltage curve for gK,L with the KRB ([K+]_SC and voltage-dependent) curve, I_K,L is slightly reduced and resting ϕ_H shifts positively. This may be of relevance to quantal transmission. The KRB activation curve is broader, with higher open probability negative to rest and lower open probability positive to rest. For sinusoidal hair bundle displacements, use of the KRB curve had modest effects on the amplitude and phase of the simulated calyx postsynaptic potential. More experimental data at different concentrations are needed to refine the KRB model.
Supported by NIH-NIDCD R01 DC012347

SA104. Postsynaptic Afferent Neurons Contain Specialized Mitochondrial Architecture at the Hair Cell to Afferent Neuron Synapse in the Zebrafish Lateral Line

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Category: Hair Cells: Anatomy and Physiology
Background: Mitochondria are pleomorphic organelles that adopt specific morphologies to impact cellular function. For example, elongated mitochondria demonstrate improved ATP production and calcium buffering. Ample literature suggests mitochondria play a role in synaptic transmission at both pre and postsynaptic sites. Presynaptically, mitochondria provide necessary ATP for vesicle release and recycling, while postsynatpically, mitochondria buffer calcium and fuel structural changes during plasticity. Mitochondrial accumulations with distinct morphologies have been characterized in auditory synapses, including the presynaptic calyx of Held (Rowland et al., 2000) and the postsynaptic bushy cells of the anteroventral cochlear nucleus (Cant and Morest, 1979). However, little is known about mitochondria at the hair cell to afferent neuron synapse. This first synapse of the auditory system supports high rates of synaptic transmission while converting a mechanical, graded signal into a binary signal. Therefore, the synapse requires precise temporal regulation at the molecular level at both pre- and postsynaptic sites. It is also highly sensitive to insult. Noise or ototoxin overexposure results in synaptopathy and ribbon loss presynaptically, and excitotoxicity postsynaptically. These changes occur prior to cell death, potentially leading to hidden hearing loss. Presynaptically, we have shown that hair cells have distinct, networked mitochondria surrounding the synaptic ribbons in a manner dependent on synaptic transmission. The mitochondrial morphology of the postsynaptic terminals, however, remains unexplored. We therefore asked whether postsynaptic afferent terminal mitochondria at this synapse also form stereotyped morphologies that optimize information transfer and preserve synapse health.
Methods: We use the zebrafish lateral line as a model for hair cells and afferent neurons. This allows ease of genetic manipulations paired with direct structure-function comparisons. We reconstructed hair cell and afferent neuron mitochondria using serial block-face scanning electron microscopy.
Results: We have previously shown that hair cell mitochondria assume stereotyped architectures dependent on synaptic transmission. This includes large, networked mitochondria near the hair cell synaptic ribbons. Here, we further show that afferent neurons also develop complex mitochondrial architectures in an activity-dependent manner. Wildtype terminals contained elongated mitochondria juxtaposed to hair cell ribbons, and could even extend into terminals adjacent to other hair cells. These mitochondrial structures in the afferent neuron terminals were eliminated upon removing hair cell mechanotransduction or synaptic transmission.
Conclusions: We have shown that mitochondria assume particular morphologies at the hair cell to afferent neuron synapse at both presynaptic hair cell and postsynaptic neuron sites. This points to the potential role of mitochondrial morphology in shaping the molecular environment for optimal transmission at this synapse, and provides insights into mechanisms that could preclude hidden hearing loss.

SA105. Super-Resolution Microscopy of Cochlear Tissue Using Expansion Microscopy

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Category: Hair Cells: Anatomy and Physiology

Background: We introduce our method of applying expansion microscopy (ExM) to image cellular ultrastructure and protein expression in the cochlear tissue, using a novel light microscopical super-resolution technique. In current inner ear research, imaging often achieves either high throughput (widefield light microscopy) or high resolution (electron microscopy) with a significant sacrifice of the other, limiting utility in studies involving cell- and tissue-level research questions. Using the presented method, resolving hair cell stereocilia and synaptic structures becomes possible in super-resolution using a relatively easy-to-implement protocol for conventional light microscopy and fixed tissue samples.
Methods: Cochlear tissue was fixed using paraformaldehyde (PFA) and was manually trimmed to produce whole-mounts of the organ of Corti. Whole-mounts were embedded in an acrylamide-based hydrogel, which is able to swell isotropically in water. Whole-mounts with fluorescently labeled antibodies (Alexa) tagged to proteins of interest swelled isotropically together with the hydrogel, effectively de-crowding the biological components and thus enabling higher resolving power. Samples were imaged using widefield or structured illumination light microscopy (SIM using Zeiss Apotome), with a 40X NA 1.0 objective. Multi-channel Z-stacks (3D images) were acquired all along a whole-mount with the focus on the hair cells of the organ of Corti. The entire hair cell population of a mouse cochlea was imaged in a whole-mount preparation. Entire hair cells were imaged in Z-stacks.
Results: In PFA-fixed mouse cochlear tissue, we achieved ~50 nanometer resolving power when detecting stereocilia using fluorescent primary-secondary antibodies without prohibitively expensive imaging systems or highly specialized fluorescent tags. We were able to accurately localize specific stereociliary proteins to their predicted locations along a single stereocilium of outer hair cells in the high-frequency region of the adult mouse cochlea. We were able to quantify a subtle stereociliary pathology not detectable by conventional confocal microscopy. Further, cochlear ribbon synapses and afferent neurites could be resolved in a similar high resolution.
Conclusions: The ExM method enables accessible super-resolution study of the inner ear soft tissue, e.g. hair cell stereocilia or synapses, in fixed samples. This allows new discoveries in pathology or physiology of cochlear cells that were previously hard to reach because of low resolving power or slow/expensive sample preparation. The advantages give the presented method extensive utility between the spatial scales of light and electron microscopy.

SA106. Glutamatergic Quantal Transmission in Peripheral Vestibular Function

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Category: Hair Cells: Anatomy and Physiology
Background: Type-3 vesicular glutamate transporter in the cochlea (Vglut3) mediates quantal synaptic transmission at inner hair cell afferent synapses. Quantal synaptic transmission in vestibular endorgans is understood to be glutamatergic as well (Sadeghi et al., 2014), and Vglut3 expression has been reported in the vestibular hair cells (VHCs; Wang et al., 2007; Schraven et al., 2012). Although genetic deletion of Vglut3 leads to deafness in mice due to the absence of cochlear afferent transmission (Seal et al., 2008; Ruel et al., 2008), the dependence of vestibular function on Vglut3-mediated quantal transmission is unknown.
Methods: Here, we investigated the vestibular phenotype of Vglut3-/- mice at the cellular, systems, and behavioral levels.
Results: In utricles and cristae of WT, but not Vglut3-/- mice, we observed strong Vglut3 immunoreactivity in type-II VHCs and weaker immunoreactivity in type-I VHCs. Rarely, very weak and sparse Vglut2 immunoreactivity was detected in VHCs of WT mice without any apparent upregulation in Vglut3-/- mice, while Vglut1 immunoreactivity was absent in both. In utricles of ~3-week-old Vglut3-/- mice, with whole-cell patch-clamp recordings of postsynaptic currents in calyces receiving input from VHCs, we observed near complete absence of quantal synaptic transmission (97% reduction in evoked EPSC frequency when compared with the WT, tested at two conditions), suggesting a strong dependence of VHC quantal transmission on Vglut3. In vivo recordings of spontaneous activity in the vestibular nerve revealed similar action potential rates and spike-timing regularity in WT and Vglut3-/- mice (WT: 65 spikes/s, CV* = 0.32;

KO: 40 spikes/s, CV* = 0.27). In contrast, recordings from the auditory nerve showed no spontaneous spiking in Vglut3-/- mice, suggesting divergent underlying mechanisms. In behavioral studies, although Vglut3-/- mice were unresponsive to acoustic startle, they exhibited no major visible sensorimotor or balance deficits in RotaRod, balance beam, or CatWalk tests.
Conclusions: The lack of overt balance deficits, the persistence of spontaneous activity in the absence of Vglut3 despite lack of compensation by Vglut1/2, and the paucity of quantal transmission support the view that non-quantal transmission is the predominant mode of neurotransmission between VHCs and vestibular afferent neurons (Goldberg, 1996; Holt et al., 2007; Eatock, 2018; Contini et al., 2022; Mukhopadhyay and Pangrsic, 2022). We propose that non-quantal transmission alone underlies the apparently normal vestibular behavioral function in Vglut3-/- mice.

SA107. Effects of Increased Cav1.3 Ca2+ Currents in Inner Hair Cells of Cav1.3-DCRDHA/HA Mice on Hearing and Synapses, and Consequences of an Acoustic Trauma

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Category: Hair Cells: Anatomy and Physiology
Background: Cav1.3 channels with a disrupted C-terminal modulatory domain (Cav1.3DCRDHA/HA) show altered gating behavior in mouse inner hair cells (IHCs), where Cav1.3 currents contribute to >90% of the total Ca2+ current. IHC Ca2+ currents of mice carrying this disruption (HA mice) were 30% larger compared with wildtype (WT) (Scharinger et al., 2015). We hypothesized that the increased Ca2+ influx into IHCs of HA mice would cause Ca2+ excitotoxicity in IHCs/type I afferents, and that a noise trauma would increase the damage.
Methods: Mice aged 7-8 weeks were subjected to white noise (8 – 16 kHz, 106 dB SPL) for 2 h. ABR recordings were performed 2 days before trauma (day -2), directly after the trauma (day 0), and on day 28. Thereafter mice were sacrificed for immunohistochemistry. Ribbons and postsynaptic scaffold proteins of AMPA receptors were labeled with anti-CtBP2 and anti-Homer1, respectively.
Results: ABR hearing thresholds of HA mice were unaltered compared with WT mice at 7 – 12 weeks indicating that subtype Ia (threshold-determining) spiral ganglion neurons innervating part of IHC ribbons were not affected. Ribbon numbers of HA mice were not different compared with WT mice in the apical, medial and midbasal cochlear region but ribbon sizes were reduced by ~30%. In the basal region, the average ribbon number/IHC of HA mice was reduced to ~50% of the WT value suggesting loss of ribbons due to Ca2+ excitotoxicity. The number of postsynapses was similar between WT and HA but a reduction by 20 % was found in the basal region. The median number of unpaired postsynapses/IHC amounted to about 1 for all cochlear regions and both genotypes except the basal region of HA mice, where it was as large as 8.
Four weeks after trauma, ribbon numbers were reduced in the mid-to-high frequency regions compared with unexposed control groups of both genotypes. However, trauma did not further reduce the low number of ribbons in HA IHCs in the basal region. Noise trauma led to orphan postsynapses per IHC in the range of 1 - 2 (medial); 4 - 6 (midbasal), and 3 - 5 (basal) in either genotype underlining once more that in our experimental settings postsynapses are more stable to noise trauma than ribbons.
Conclusions: Altered gating of Cav1.3 channels in HA mice did not affect synapses of threshold-determining (Ia) fibers. In the basal (>32 kHz) region of HA mice, degeneration of ribbons was observed even without acoustic trauma, yet postsynapses were hardly affected. The noise trauma did not further reduce ribbons in basal HA IHCs suggesting that in our experimental settings ribbons susceptible to a Ca2+ overdose had already degenerated by Ca2+ excitotoxicity before the trauma was applied.
Supported by DFG SFB 894 (A8 to JE).

SA108. Myosin 15 Isoform 3 Traffics the Elongation Complex in Postnatal Hair Cells and is Required for Hearing

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Category: Hair Cells: Anatomy and Physiology
Background: Actin-based stereocilia of hair cells assemble into rows of precisely graded heights. Maintenance of hair bundle “staircase” architecture is essential for the senses of hearing and balance.

Mutations in MYO15A, encoding the molecular motor myosin 15, disrupt the hair bundle and cause hereditary hearing loss, DFNB3. MYO15A influences hair bundle architecture via multiple protein isoforms. MYO15A-1 maintains stability of shorter row stereocilia that harbor mechanotransducer channels. MYO15A-2 controls stereocilia elongation during development by trafficking proteins of the elongation complex (EC), WHRN, EPS8, GNAI3, and GPSM2, and, perhaps, by directly stimulating actin polymerization. We recently identified a new isoform (MYO15A-3) expressed in the cochlea. Here, we examine whether MYO15A-3 is necessary for hair cell function and hearing.
Methods: We generated an isoform specific Myo15a-Δ3 null allele in mouse using CRISPR-Cas9 editing. To assess hearing function, we measured auditory brainstem response (ABR) thresholds at 8, 16, and 32 kHz and distortion product otoacoustic emission (DPOAE) amplitudes at 8, 16, and 32 kHz in Myo15a-Δ3 mice and littermates. Hair bundles were examined using scanning electron microscopy. Myo15a-3 expression was quantified using qPCR and BaseScope in situ hybridization. Cellular binding assays of MYO15A and EC proteins were performed by measuring EGFP-MYO15A-3 and mCherry-WHRN / anti-EPS8 in HeLa cells. Endogenous EC proteins were detected using immunofluorescence and imaged with spinning-disk confocal microscopy.
Results: Myo15a(Δ3/Δ3) mice had normal ABR thresholds at P17 compared with littermates. By P40, Myo15a(Δ3/Δ3) mice had ABR thresholds > 90 dB SPL and did not exhibit DPOAE responses at all frequencies measured indicating deafness. Consistent with this, stereocilia had normal architecture at P10 in Myo15a(Δ3/Δ3) cochleae but exhibited height abnormalities by P30. Myo15a-3 expression increased postnatally by P60 and was concentrated in inner and outer hair cells. We hypothesized that MYO15A-3 might traffic the EC in postnatal hair cells and found that EGFP-MYO15A-3 delivered both EPS8 and mCherry-WHRN along filopodia of HeLa cells. As a negative control, EGFP-MYO10 did not accumulate EPS8 or mCherry-WHRN within filopodia. To test if MYO15A-3 similarly trafficked the EC in vivo, we examined the localization of EPS8 and WHRN in hair cells using immunofluorescence. EPS8 and WHRN were absent from stereocilia of Myo15a(Δ3/Δ3) hair cells at P30, whilst we detected robust labeling in littermate controls. By contrast, EPS8 and WHRN were correctly targeted in Myo15a(Δ3/Δ3) stereocilia at P4 when MYO15A-3 is not yet fully expressed, arguing that other MYO15A isoforms (e.g., MYO15A-2) are sufficient for normal EC trafficking in younger animals.
Conclusions: MYO15A-3 is not required for stereocilia development but is necessary for maintaining stereocilia architecture. Our data argue that, during postnatal development, a hair cell switches MYO15A isoforms from MYO15A-2 to MYO15A-3 for trafficking EC proteins to the tips of stereocilia.

SA109. Split-GFP Tagging and Live Imaging of Hair Cell Proteins

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Category: Hair Cells: Anatomy and Physiology
Background: The goal of this project is to develop a tool that makes imaging of protein movements in living hair cells more accessible. The gold standard technique for tracking protein movement is live-cell imaging of tagged proteins. Traditionally, this is achieved by expression of exogenous DNA constructs fused to fluorescent proteins, delivered to cells by gene gun, electroporation or viruses. Transgenic mice also have been employed. These approaches, however, are potentially confounded by overexpression artifacts and low transduction/transfection efficiency. Knock-in (KI) of genetically-encoded fluorescent tags into the endogenous gene loci has become more tractable through modern genome editing methods, but KI of large DNA segments, such as the full-length GFP coding sequence, remains difficult and has the potential to affect gene expression.
Methods: To address these challenges, we developed a mouse tool kit that streamlines fluorescent tagging of proteins for localization and live-cell imaging. To this end, we adopted the Split-GFP strategy. In this two-component system, the endogenous gene of interest is genetically tagged with a small part of GFP ("GFP11"). Co-expressing the remaining (non-fluorescent) portion of GFP ("GFP1-10") reconstitutes GFP fluorescence, allowing imaging by fluorescence microscopy. Due to the small size of the GFP11 fragment (48 bps), CRISPR-mediated KI into the endogenous loci is efficient. We confirmed that the Split-GFP approach faithfully recapitulates the endogenous localization of various hair cell proteins such as LMO7 and XIRP2, by delivering AAV-packaged GFP1-10 into hair cells of Lmo7-GFP11 KI and XIRP2-GFP11 mice, respectively. To circumvent AAV-mediated delivery, we generated a transgenic mouse line that expresses the GFP1-10 fragment globally or in a Cre-dependent manner (GFP1-10 TG mouse).

Results: We knocked in the GFP11 sequence into the N-termini of Lmo7 and Xirp2 and confirmed that the GFP11 sequence does not affect gene expression levels. When crossed with the GFP1-10 TG mouse, reconstituted (recon)GFP-XIRP2 and reconGFP-LMO7 recapitulated the endogenous expression and localization pattern as determined previously by antibody staining. We are now in the process of preforming live cell experiments to study the movement of these proteins in the living hair cell.
Conclusions: The study of hair cells presents many challenges. In particular live-cell imaging and tracking of proteins, important pillars of cell biology studies, have been difficult, mainly due to the lack of a in vitro system and the limited means to transfect hair cells. Considering that the essence of hair cell function is micro- and nanoscale movement, investigations of the dynamic properties of proteins is crucial for a complete understanding of hair cell function. The split-GFP approach provides an effective way to fluorescently tag endogenous hair cell genes.

SA110. CETN2 Promotes Ca2+ - Dependent Assembly of Myosin 15 Biomolecular Condensates

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Category: Hair Cells: Anatomy and Physiology
Background: Hair cell stereocilia transduce sound and are packed with a para-crystalline array of actin filaments that determine their size and shape. Myosin 15 (MYO15A) traffics molecules along stereocilia required for the developmental growth of the actin core. The ATPase "motor" domain of MYO15A generates force and is critical for this trafficking activity in hair cells. A necessary part of the force generation mechanism is the binding of accessory light chain proteins to the motor domain's neck region, which stiffens the neck domain, allowing it to transmit force. In other myosin molecules, light chains modulate ATPase activity, providing a key mechanism to regulate motor force output. MYO15A contains three putative light chain binding sites (LCBS) that are related to IQ domains; however, the endogenous light chains are not known. Here, we identify the centrosome – associated protein, centrin-2 (CETN2), as specifically binding to MYO15A and investigate how this regulates motor activity.
Methods: To identify light chains binding to MYO15A endogenously, we performed immunoprecipitations using immortalized pituitary cells (AtT20) engineered to express an EGFP-tagged MYO15A-3IQ-FLAG protein under a CMV promoter. LC-MS/MS analysis of FLAG-immunoprecipitated MYO15A protein identified CETN2 as a candidate light chain. To study the effects of CETN2 binding to MYO15A, we used the baculovirus system to express the ATPase and neck domain of MYO15A in Sf9 insect cells. Proteins were purified by liquid chromatography and were studied using low-speed sedimentation assays and fluorescence microscopy.
Results: The MYO15A motor domain was expressed either as the full neck domain (MYO15A-3IQ) or with the third LCBS domain truncated (MYO15A-2IQ) and co-expressed with CETN2. CENT2 co-purified with MYO15A-3IQ, and not with truncated MYO15A-2IQ, confirming that CETN2 binds specifically to the third IQ domain. CETN2 is an EF-hand containing protein that was previously shown to polymerize when bound to Ca2+, and we hypothesized that CETN2 might similarly polymerize when bound to MYO15A, forming Ca2+ induced MYO15A/CETN2 polymers. To test this, we incubated MYO15A-3IQ (w CETN2), or MYO15A-2IQ (w/o CETN2) with varying [Ca2+] and performed low-speed centrifugation assays. MYO15A-3IQ + CENT2 sedimented with ≥ 500 μM Ca2+, where MYO15A-2IQ remained in the supernatant at all concentrations tested. Sedimented protein was visualized using confocal fluorescence microscopy and revealed that MYO15A-3IQ + CETN2 formed extensive biomolecular condensates in the presence of Ca2+. Critically, MYO15A-2IQ remained a monomer under identical buffer conditions.
Conclusions: Our experiments show that CETN2 specifically binds to the neck region of MYO15A and is an accessory light chain. Additionally, CETN2 drives calcium-dependent multimerization of MYO15A into high molecular weight structures. We are currently investigating the physical properties of these condensates and how they might contribute to MYO15A's activity in stereocilia.
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SA111. Non-Quantal Transmission at the Type I Hair Cell to Calyx Synapses is Critical for Encoding Head Movements

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Category: Hair Cells: Anatomy and Physiology

Background: With the evolutionary migration from water to land and generation of new forms of head movement, amniote vertebrates (reptiles, birds and mammals) acquired a novel type I vestibular hair cell (HC-I) and a calyx afferent terminal that ensheathes its basolateral walls. This structure provides a unique non-quantal synaptic transmission between HC-I and the calyx (Contini et al. 2012, Songers and Eatock 2013, Sadeghi et al. 2014, Contini et al. 2022). Here, we demonstrate in mouse, that HC-II synapses on the outer surfaces of calyces can provide substantial quantal input to the calyx and that the non-quantal transmission from HC-I alone is sufficient for generating a normal vestibulo-ocular reflex (VOR).
Methods: We used patch clamp recording from calyx terminals in 3-4 week old mice and optogenetic stimulation of hair cells in different mouse lines: Gfil-Cre;Ai32 mice that expressed channelrhodopsin 2 in all hair cells, VgluT3 (vesicular glutamate transporter-3) KO mice, and VgluT3-Cre14;Ai32 mice that sparsely expressed ChR2 in a subset of both HC-I and HC-II.
Results: In Gfil-Cre;Ai32 mice, every recorded HC could be excited by blue light pulses (n = 32) and resulted in an increase in quantal excitatory postsynaptic currents (qEPSCs) with rates from 0.04 ± 0.01 events/s to 3.35 ± 0.79 events/s in calyx terminals (n = 14, p = 0.001, paired t-test). All tested calyx-afferents also showed a non-quantal transmission defined as a steady inward current (n=27). Using loose patch recordings, we found that optogenetic stimulation increased calyx firing rates and was not affected by glutamate receptor blockers.
We then used VgluT3-Cre14;Ai32 mice, which had sparse expression of reporter, providing the opportunity to selectively test the inputs from a single HC-I or a single HC-II to a nearby calyx. ChR2 activation of HC-I induced non-quantal synaptic currents (-19.5 ± 2.5 pA; n=15), with no significant change in qEPSCs. In contrast, activation of a nearby HC-II resulted in an increase in qEPSC rate (4.77 ± 1.30 events/s vs. 0.13 ± 0.08 events/s, n = 6, p = 0.015).
We then used VgluT3 KO mice that are deaf with no startle reflex in response to loud sounds. Furthermore, we found no quantal release of glutamate during voltage clamp recordings from the calyx in response to 40 mM potassium stimulation. Yet, these mice exhibited VOR responses that were comparable to WT mice over a wide range of rotational stimuli.
Conclusions: Calyx terminals receive quantal inputs from HC-II synapses onto their outer surface (and probably from HC-I to their inner surface). The specialized HC-I to calyx synapse enables a unique form of non-quantal transmission in mice, which seems effective in driving afferent activity to produce normal VOR responses.

SA112. Can Prestin Be Used as a Serological Biomarker for Cochlear Damage?

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Category: Hair Cells: Anatomy and Physiology
Background: Mammalian hearing requires mechanical amplification of sound by outer hair cells (OHCs) to produce high sensitivity and sharp frequency selectivity. OHCs are also one of the most vulnerable components in the cochlea and are extremely sensitive to and often damaged from different insults. Therefore, OHC proteins are considered an effective choice for uncovering biomarkers to detect cochlear damage. The lateral membrane of OHCs is largely occupied by the motor protein called prestin, which gives rise to the molecular basis for OHC electromotility (Zheng et al., 2000). By changing its conformation between short and long states, prestin subserves OHC motility when switching between depolarized and hyperpolarized conditions. Several reports suggested that prestin could be used as a serological biomarker for idiopathic sudden sensorineural hearing loss and cochlear damages caused by noise exposure and ototoxic drugs. In fact, recently, prestin was detected in the bloodstream of humans, rats, guinea pigs and mice using a sandwich enzyme-linked immunosorbent assay (ELISA). However, the reported data are inconsistent and lacking a proper negative control. In addition, prestin is also expressed in heart, which raises a question whether the prestin detected in the bloodstream is indeed derived from OHCs. In this study, we measured prestin quantities in the bloodstream using ELISA. HPβCD-treated wildtype (WT) mice were used as a positive control as HPβCD can rapidly kills OHCs and release prestin into extracellular space. Prestin-knockout (KO) mice (Liberman et al., 2002) were used as a negative control.
Methods: WT and prestin-KO mice were injected with 0.9% NaCl or 8000 mg/kg HPβCD dissolved in 0.9% NaCl subcutaneously. 20 hours after the injection, the plasma and cochleae from WT and prestin-KO mice were collected. Prestin concentrations in the bloodstream were measured using mouse prestin ELISA

kit (MyBioSource.com). The expression of prestin in cochleae was verified by immunofluorescence using anti-prestin antibodies targeting both the N-terminal and C-terminal of prestin.
Results: Prestin was expressed in WT-OHCs but not in prestin-KO-OHCs. Single high-dose administration of HPβCD resulted in > 60% OHC death in WT mice. Despite a large amount of OHCs loss in less than 20 hrs, prestin concentration in the bloodstream from both WT and prestin-KO mice were below detectable limitation regardless of whether mice were treated with HPβCD or NaCl. However, the optical densities of samples, which correlates to prestin quantities, were significantly influenced by the severities of hemolysis in the samples.
Conclusions: Prestin concentrations in the samples are significantly affected by the quality of collected plasma. Whether prestin from OHCs is a good and reliable serological biomarker requires further investigation (Work supported by the Knowles Leadership Fund to JZ and NIH R01DC019434-01 to XT).

SA113. Reducing Taperin Expression Restores Hearing in Grxcr2 Mutant Mice

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Category: Hair Cells: Anatomy and Physiology
Background: Human sensorineural hearing loss, the most common form of deafness, is often caused by defects in stereocilia. Taperin, mutations of which cause the autosomal recessive nonsyndromic hearing loss type 79 (DFNB79), localizes at the base of stereocilia. Taperin is able to regulate the actin cytoskeleton, as evidenced by the formation of rod-like actin filaments in COS7 cells and extraordinary stereocilia growth in hair cells when it is overexpressed. GRXCR2, another deafness-related protein, interacts with taperin and regulates its localization at the base of stereocilia. Hair cells lacking GRXCR2 have disorganized stereocilia due to the mislocalization of taperin. Reducing taperin expression in Grxcr2 null mutant mice rescues the morphological defects of stereocilia and partially restores hearing in Grxcr2 null mice.To further validate that GRXCR2 is essential for the
morphogenesis of stereocilia via regulating taperin localization, we generated two novel taperin mutant mouse lines with progressive hearing loss. Then, we crossed Grxcr2 null mice with one of these taperin mutant mice.
Methods: Whole mount immunostaining, scanning electron microscopy, auditory brainstem response measurement.
Results: GRXCR2 interacts with taperin, and taperin was concentrated at the basal taper region of the stereocilia at different developmental stages. Morphology of stereocilia was analyzed
using whole mount immunostaining and scanning electron microscopy. Auditory functions were evaluated by measuring the auditory brainstem responses (ABRs). Our results demonstrate that reducing taperin expression does indeed correct stereocilia morphological defects and partially restore hearing in Grxcr2 null mutant mice.
Conclusions: Recessive mutations in GRXCR2 cause deafness in both humans and mice. In Grxcr2 null hair cells, the sensory receptors for sound in the inner ear, stereocilia are disorganized. Reducing the expression of taperin, a protein that interacts with GRXCR2 at the base of stereocilia, corrects the morphological defects of stereocilia and restores hearing in Grxcr2 null mice. To further validate this finding, this study generated two novel taperin mutant mouse lines that exhibit progressive hearing loss. Then Grxcr2 null mice were crossed with one of these taperin mutant mice. The following morphological analysis revealed that reducing taperin expression indeed corrected stereocilia morphological abnormalities in Grxcr2 null mice. Functional analysis further confirmed that reducing taperin expression partially restored hearing in Grxcr2 null mice.

SA114. Raman Spectroscopic Detection of Cisplatin-Induced Changes in the Inner Ear

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Category: Hearing Loss: Consequences and Adaptation
Background: Cisplatin chemotherapy is a common therapeutic for children suffering from cancer. However, its therapeutic benefits are also associated with ototoxicity for about 60% of patients, manifesting as irreversible hearing loss, tinnitus, or vertigo. Cisplatin has been shown to accumulate preferentially and indefinitely in the stria vascularis, a cochlear region in the inner ear critical to sound detection and can lead to further complications with speech recognition, vocabulary development, communication skills, school

performance, and psychosocial behavior. Early clinical detection of these molecular changes in the inner ear may be advantageous for initiating treatment and preventing potential permanent hearing damage.
Methods: We have developed a murine model for cisplatin-induced hearing loss using the CBA/CaJ strain at 4-6 weeks of age, with a sample size of n = 5 for both the treatment and control group. We auditory brainstem responses (ABRs) to confirm hearing loss. After confirmation, we performed Raman spectroscopy on the two groups.
Results: The ABR results show that there is complete hearing loss on all frequencies (4kHz, 5.7 kHz, 8Hz, 11.3 kHz, 16 kHz, 22.6 kHz, 32 kHz, and 43.5 kHz) in the treatment group of cisplatin and typical hearing thresholds for the control group. The Raman spectroscopic results showed statistically significant chemical signatures, so the two groups are easily differentiable.
Conclusions: We have successfully developed a cisplatin-induced hearing loss model which enables us to further study the full effects of cisplatin chemotherapy. Furthermore, we are able to detect differences in the inner ear of mice that have experienced cisplatin-induced hearing loss using Raman spectroscopy. By developing ways to understand the population at risk, we can greatly advance interventions to improve the care of patients with cisplatin-induced ototoxicity.

SA115. Differential Presbyacusis Phenotype Associations With Cortical Gray Matter Volume
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Category: Hearing Loss: Consequences and Adaptation
Background: Age-related hearing loss or presbyacusis has been consistently associated with lower cortical gray matter volume, particularly in low-level auditory cortex. These findings may reflect sensory and/or metabolic presbyacusis phenotypes, as measured by the audiogram, and other factors. For example, neural presbyacusis, as measured with electrocochleography, has not been examined as a potential contributor to cortical gray matter volume. Here, we examined the extent to which differences in cortical gray matter volume were associated with neural, sensory, and metabolic components of age-related hearing loss. We hypothesized that higher estimates of these presbyacusis phenotypes would each be associated with lower cortical gray matter volume.
Methods: 110 adults (51 – 83 years) with a Mini-Mental Status Examination score of at least 27 were recruited from the Charleston, S.C. community. Neural presbyacusis was defined using auditory nerve compound action potentials that were elicited by a 110 dB pSPL click and processed to measure phase locking (intertrial coherence) across 1100 trials. Sensory and metabolic components of age-related hearing loss (in dB HL) were defined by estimating their contributions to the audiogram based on fitting audiometric shape parameters that have been shown to be characteristic of sensory and metabolic presbyacusis in older adults. Voxel-based morphometry with multiple comparison correction (TFCE $p < 0.05$) was used to identify regions where gray matter volume (8 mm FWHM smoothing kernel) was associated with neural, sensory, or metabolic presbyacusis. Gray matter volume was also averaged within cytoarchitectonic maps for primary auditory cortex (Te 1.0, 1.1, 1.2) for comparisons across presbyacusis phenotypes using linear regression.
Results: The presbyacusis estimates each exhibited significant associations with auditory cortex gray matter volume, where higher estimates of sensory and metabolic components of age-related hearing loss and poorer phase locking occurred with lower volume. These voxel-based associations were spatially specific to auditory cortex for the phase locking measure of neural presbyacusis but were more widespread for the sensory and metabolic estimates. Each presbyacusis estimate was significantly associated with gray matter volume in multiple Te regions of interest, and uniquely associated with gray matter in Te 1.0 and Te 1.1 when included together in the same regression analyses.
Conclusions: The results are consistent with findings from previous studies showing that auditory cortex gray matter volume relates to the magnitude of age-related hearing loss. Both sensory and metabolic components of age-related hearing loss appear to contribute to previous associations between pure-tone thresholds and gray matter measures. In addition, and in contrast to the widespread gray matter associations with sensory and metabolic components, neural presbyacusis appears to exhibit a spatially specific association with lower gray matter volume in auditory cortex.

SA116. Temporal Masking Underlies Reduced Speech Discrimination in Noise at High Sound Intensities
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Category: Hearing Loss: Consequences and Adaptation
Background: Natural sounds, such as speech, are complex time-varying waveforms containing information critical to how we communicate with each other and navigate the external world. Hearing loss results in a breakdown of this information and causes distortions in the neural code. As a result, perception of complex sounds such as speech is compromised. This problem is further complicated by the fact that sound intensity varies in natural settings, both in quiet and in noisy backgrounds. Somewhat paradoxically, despite increased audibility at high sound intensities, perception and discrimination of speech is actually diminished, especially in the presence of background noise, and this effect is further compounded by hearing-impairment. This is known as rollover of speech and its neural basis is poorly understood.
Methods: To investigate this problem, we performed in-vivo electrophysiology in awake and anaesthetized Mongolian gerbils (Meriones Unguiculatus) to investigate how hearing loss affects the neural encoding of speech. We presented 22 Vowel-Consonant-Vowel (VCV) syllables to the gerbil and recorded neural responses from the inferior colliculus (IC). We used a K-nearest neighbor neural classifier to investigate whether IC neurons could discriminate between different consonants in normal hearing (NH) and noise-exposed hearing-loss (HL) animals. We hypothesized that there will be strong effects of temporal masking in the boundaries between consonant and vowels in syllables, and that these effects will be most pronounced at higher intensity levels across conditions. We tested the effects of temporal masking by presenting the consonants isolated from the vowels and reclassifying to observe whether there was an improvement in discrimination.
Results: We found that neural correlates of perceptual rollover were present in the IC and that performance in discrimination decreased when VCVs were presented in +2 dB SNR noise when compared to in quiet. The rollover was a more prominent dropoff in the NH animals whereas the dropoff plateaued in the HL animals. When the consonants were presented in isolation, thereby removing temporal masking interaction effects between syllable elements, the effect of rollover was mitigated, with a greater effect in HL compared to NH animals, despite a decrease in overall discrimination. Thus, there appears to be a critical trade-off between audibility and rollover mediated by temporal masking.
Conclusions: These results demonstrate that the failure of temporal processing, caused by temporal masking in speech syllable elements, results in detrimental speech perception as sound intensity increases for speech in background noise. This advancement in knowledge could help to improve current hearing aid designs by mitigating the temporal masking from incoming sound features in speech. This could also pave the way for better diagnoses and personalized medicine treatments in the future.

SA117. Building a Toolbox With Human Induced Pluripotent Stem Cells for Studying Non-Syndromic Hearing Loss

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Category: Hearing Loss: Consequences and Adaptation
Background: Approximately 80% of non-syndromic hearing loss (NSHL) is inherited. The advancement of sequencing technology has led to the rapid identification of causative genetic variants. The major challenge has become understanding the functional consequences of these genetic variants in normal inner ear biology and HL. One of the major challenges in studying the molecular and cellular underpinnings of HL has been the paucity of readily available patient biopsy tissues. Human induced pluripotent stem cell (hiPSC)-based systems have been utilized and proved to be powerful tools in various fields, e.g., brain, eye, cardiovascular system, etc. In the past decades, several protocols have been established to differentiate stem cells into inner ear cell types. To help move forward our understandings of the molecular mechanisms underlying NSHL and the development of therapeutic strategies, we have committed to establish a hiPSC bank contain lines that represent various NSHL-associated genetic variants. CRISPR genome editing is being used to derive isogenic pairs of lines providing valuable control samples. This bank will serve as an invaluable resource for the inner ear field. Herein, we demonstrated our progress with hiPSC-based system for two NSHL-associated genes, P2RX2 and TMPRSS3.
Methods: hiPSCs were derived from a patient carrying a common TMPRSS3 mutation, c.208delC. The mutation was corrected to generate an isogenic control cell line. A P2RX2 mutation, c.178G>T was introduced using the CRISPR/Cas9 technology. Inner ear organoids were induced from the hiPSCs of P2RX2V60L/V60L and the isogenic control.

Results: Firstly, we showed the successful reprogramming of patient peripheral blood mononuclear cells (PBMC) into hiPSCs. We also demonstrated our capacity of manipulating genomic contents in hiPSCs using the CRISPR/Cas9 technology. Each iPSC line underwent rigorous quality control analysis, including assessment of pluripotency, g-band karyotyping for genomic stability, short tandem repeat analysis, and analysis of the differentiation potential (Trilineage differentiation and staining for primary germ layer specific markers).
Induction of inner ear organoids was shown in both P2RX2V60L/V60L and the isogenic control. Otic vesicles were seen in the induction day 40 and hair cell-baring inner ear organoids were observed by day 60.
Conclusions: With our large NSHL patient population, we are confident that our hiPSC bank will steadily grow. With various differentiation protocols, these hiPSCs will serve as power tools for studying NSHL and testing therapeutic approaches, e.g., genetic therapy.

SA118. Hearing Variability Before and After Noise Trauma in Rodents

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Category: Hearing Loss: Consequences and Adaptation
Background: Most experimental approaches to investigate hearing abilities and noise-induced disorders involve rodents as animal models.
Methods: To reveal species-dependent differences in hearing and especially changes after noise trauma, we measured auditory brainstem responses (ABRs) in mice (C57BL/6), rats (Sprague Dawley) and Mongolian gerbils before, during and several weeks after noise exposure with a stepwise increase of trauma intensity for threshold determination and ABR waveform analysis. Furthermore, we compared in detail prevalent variations in hearing characteristics between four rat strains (Sprague Dawley, Wistar, Long Evans and Lister Hooded) regarding ABR thresholds and their susceptibility to tinnitus based on gap-prepulse inhibition of the acoustic startle response. This way we tried to identify the most suitable rat model that could be used for future optogenetic manipulation for hearing research.
Results: We used noise overstimulation to induce temporary threshold shifts at a suitable hearing frequency and compared short- and long-term dynamics of threshold recovery and ABR waveforms. An increase in noise trauma intensity led to a threshold increase in rats, whereas an upper limit of ABR threshold shift was found in gerbils. Interestingly, the temporary threshold shifts in rats and mice depended on the hearing ability before the noise trauma, with larger threshold shifts occurring in animals with lower pre-trauma ABR thresholds. Regarding the ABR waveforms in the different rat strains, we found that variations were most prominent at later peaks after stimulus onset. Changes in ABR waveforms after trauma, however, were small compared to consistent strain-dependent differences between individual waveform components. Further, the most vulnerable rat strain for temporary and permanent hearing loss was Sprague Dawley, while Long Evans rats yielded the highest tinnitus rate (75%) as.
Conclusions: Our comparative study revealed pronounced species- and strain-specific differences in the development of noise-induced hearing loss and the related processing along the auditory pathway. As an outlook, we present hearing data for the future use of JDO mice (Jackson Diversity Outbreed) in hearing research.

SA119. The Effect of Cochlear Synaptopathy on Adaptation to Noise

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Category: Hearing Loss: Consequences and Adaptation
Background: Adaptation to noise refers to the improvement in word-in-noise recognition as words are delayed a few hundred milliseconds from the noise onset. This adaptation is thought to reflect one or more physiological mechanisms that can adjust the dynamic range of auditory nerve fibers, such as statistical adaptation to the most frequent noise level preceding the words and/or noise activation of olivocochlear efferent reflexes. The loss of cochlear synapses (or synaptopathy) could impair these mechanisms, hence adaptation to noise. The aim of the present study was to investigate the impact of synaptopathy on adaptation to noise. Because synaptopathy predominantly reduces the number of cochlear synapses for

auditory nerve fibers with high thresholds, we expected a larger effect of synaptopathy on adaptation to high level noise.
Methods: For 48 participants with normal-hearing (pure-tone average thresholds at 500-2000 Hz <25 dB HL), we measured (1) speech reception thresholds (SRTs; signal-to-noise ratios at 50% recognition) for disyllabic words delayed 50 or 800 ms in stationary, speech-shaped noise; (2) high-frequency thresholds (HFTs) at 12 kHz; and (3) auditory brainstem responses (ABRs) for clicks presented at 95 and 110 dB ppeSPL. SRTs were measured for fixed noise levels of 55 and 78 dB SPL by adaptively varying the speech level. Adaptation to noise was calculated as the SRT improvement in the 800-ms versus the 50-ms delay condition. Because adaptation is known to be greater for vocoded than for natural words, words were processed through a tone vocoder. The amplitudes of ABR wave I for the two click levels and its rate of growth with increasing level (slope) were used as proxies for cochlear synaptopathy.
Results: Adaptation occurred at the two noise levels (55 dB SPL, mean=0.88 dB, p=0.001; and 78 dB SPL, mean = 1.89 dB, p<0.001). At 78 dB SPL, adaptation was correlated with wave I slope [r(46)=0.089, p=0.039] but not with wave I amplitude [at 95 dB ppeSPL: r(46)=0.024, p=0.30; at 110 dB ppSPL: r(46)=0.013; p=0.43]. At 55 dB SPL, adaptation was not significantly correlated with any ABR measure. Results were similar when the potential confounding effects of HFTs were partialled out.
Conclusions: Cochlear synaptopathy (as assessed by wave I slope) could reduce adaptation to high-level noise. More data are necessary to corroborate these findings. [Work supported by the Spanish Ministry of Science and Innovation (grant PID2019-108985GB-I00), and the European Regional Development Fund.]

SA120. Stress in People With Hearing Loss

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Category: Hearing Loss: Consequences and Adaptation
Background: Psychological and physiological stress have a causal role in depression onset, potentially via alteration of hypothalamic-pituitary-adrenal axis functioning. Hair cortisol assessment, a method that quantifies the concentration of cortisol secreted over time, offers the opportunity to objectively measure chronic stress. Stress could plausibly derive from a sensory deficit such as hearing loss (HL). Yet hair cortisol concentration (HCC) in association with stress, depression or anxiety has not been measured in people with HL.
The objectives of the study were to
(1) prospectively assess HCC in people with and without HL,
(2) investigate associations between HCC and psychological responses, and
(3) pilot paediatric work to assess whether it is feasible to assess HCC in children.
Methods: HCC, depression, anxiety, and stress-relevant variables were assessed in adults (aged 20-89), 160 people donated hair samples over the study period. Immunohistochemistry (ELISA technique) was used to measure HCC. Depression, anxiety, and stress responses were measured using self-report questionnaires from the Patient Health Questionnaire (PHQ-9), Zung's self-rating anxiety scale (SAS), and the Perceived Stress Scale (PSS-10) respectively.
Results: Median HCC was significantly higher in people with HL compared with people who have normal hearing (NH), when measured at the individual timepoints and across the study period (Friedman's ANOVA). Median HCC in NH group was 3.18pg/mg, range [0.02-147]; median HCC in bilateral HL group was 5.04pg/mg, range [0.37-476] and median HCC in unilateral HL group was 5.01 range [0.29-204.5]. A statistically significant difference was recorded between HL and NH groups (Kruskal-Wallis, ANOVA; P=.007). Overall people with HL did not have statistically significantly higher anxiety, stress or depression scores compared with NH participants. Psychological scores were not correlated with HCC for any of the groups at any timepoint. People with BHL exhibited increased HCC when confounding variables were controlled for.
Conclusions: In a pilot paediatric study running in parallel with the adult study we found it was possible to take hair from children and babies with no significant difficulties.
HCC was higher in adults with HL than those with NH. Anxiety, stress, and depression scores were correlated with each other but not statistically significantly increased in those with HL compared with NH participants. Psychological scores were also decoupled from HCC, which could reflect different aspects of stress reactivity. While psychological symptoms were not correlated with HL, the increased HCC in people

with HL suggests that the HPA responded to their HL. This could relate to contextual features related to social evaluative threat or other stress not recorded in this study. Recording HCC in children could be a useful index of distress, particularly important in the pre-verbal population who can't articulate the stress they may feel.

SA121. Assessment of Synaptic Ribbon Mmorphologies Using Super-Resolution Microscopy

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Category: Inner Ear: Anatomy and Physiology

Background: Synaptic ribbons (SRs) are presynaptic structures within inner ear hair cells that are surrounded by neurotransmitter vesicles. While they are best examined with ultrastructural methods, SRs are easily immunolabeled with antibodies to the transcription factor CtBP2 for higher-throughput approaches common in investigations of inner ear synaptopathies. In vestibular epithelia SRs exhibit broad morphologic heterogeneity, particularly in mouse specimens, a proxy for which is represented by immunolabeled puncta size. However, puncta sizes approach the diffraction limit of light microscopy whereby their resolution is subject to considerable optical distortion. Through the present investigation evidence for minimizing this distortion is presented, alongside strategies for efficient segmentation and quantification.

Methods: Vestibular epithelia were harvested from adult mice and chinchillas, fixed in 4% paraformaldehyde (mice; 2-3 hours) or mixed aldehydes (chinchillas: 4% paraformaldehyde, 0.25% glutaraldehyde; 8 hours), and then incubated in 30% sucrose for 3 days. Mouse specimens were cryosectioned (15µm) and placed on glass slides for on-slide immunoprocessing. Chinchilla specimens were embedded in agar-sucrose, incubated in 30% sucrose-PBS for 3 additional days, then cryosectioned (20 – 50µm) and transferred to PBS in wells of a 96-well plate for free-floating immunohistochemistry. The principal antibodies used were anti-CtBP2 and anti-beta-3-tubulin with appropriate secondary antibodies conjugated to AlexaFluor (AF) 488, AF594, or AF647. The sections were imaged using confocal microscopy (Zeiss LSM880 Airyscan), followed by processing either with Airyscan (ZenBlue) or the Huygens deconvolution software (SVI Inc.). Distributions of SR volumes were compared across processing conditions (e.g. fluorophore, post-processing method) using the Kullback-Leibler divergence and bootstrap resampling.

Results: Analyses of manually segmented SRs from an EM stack of the mouse utricle provided a "ground-truth" standard for comparison with volumes obtained from confocal microscopy. The range of EM-imaged volumes for simple ribbons was 0.0002 – 0.02µm3, while the range for clusters was 0.01-0.05µm3. SR volumes determined through Airyscan processing vastly overestimated these ranges, while deconvolution resulted in a volume range closer to that derived through EM segmentation. As expected, volumes of SRs labeled with antibodies conjugated to AF488 were smaller than those conjugated to AF594 for both Airyscan ($p=0.023$) and deconvolution ($p=0.023$) processing. However, deconvolution resulted in smaller SR volumes than those resulting from Airyscan processing (both fluorophores: $p<0.0001$).

Conclusions: The diffraction limits and distortion imposed by light microscopy result in vastly enlarged SRs illustrated by volume comparisons with manual segmentation of EM stacks. The present analyses demonstrated that deconvolution of confocal stacks has an enormous impact on SR volumes, even after data collection with an established super-resolution method. While longer wavelength fluorophores resulted in enlarged SR volumes, the differences were relatively small when contrasted with the impact of deconvolution. These data demonstrated the relative impact of imaging factors contributing to analyses of SR distributions and resulting interpretations of potential alterations.

SA122. Automated Hair Cell Quantification Using the Hair Cell Analysis Toolbox

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Category: Inner Ear: Anatomy and Physiology

Background: Our sense of hearing is mediated by hair cells, precisely arranged, and highly specialized sensory cells of two subtypes: outer hair cells (OHCs) and inner hair cells (IHCs). The one row of IHCs and three rows of OHCs are arranged tonotopically; hair cells at any given location respond to their best

frequency which decreases from base to apex of the cochlea. Loss of hair cells at a specific place affect hearing performance at the corresponding tonotopic frequency. To better understand the underlying cause of hearing loss, a plot of hair cell survival along the cochlear frequency map, known as a cochleogram, can be generated. However, imaging auditory hair cells along the length of the cochlea, or just smaller regions in large datasets for biomedical research, often yields more data than feasible to manually analyze. Currently, there are no widely accepted tools for unsupervised, unbiased, and comprehensive analysis of cells in the cochlea. To facilitate this type of analysis, we present a machine learning-based Hair Cell Analysis Toolbox (HCAT) that automates common image analysis tasks such as counting hair cells, determining their type (OHC vs IHC), calculating their best frequency, and the generation of cochleograms.
Methods: We trained a deep neural-network on confocal micrographs of cochlear hair cells generated in our laboratory and from other laboratories in the larger research community. Our training data covers multiple age ranges, tonotopic locations, experimental conditions, microscopy techniques and species. We also include an automated approach to determining cell frequency from a contiguous piece of cochlear tissue. HCAT is presented as a graphical user interface (GUI) and can analyze both large and small regions of tissue, from single images or batch datasets. HCAT outputs annotated micrographs with detected inner and outer hair cells, accompanying data tables for offline analysis, and automated cochleograms.
Results: Hair cell detection in confocal micrographs of whole mouse cochlear is highly accurate (>98% true positive accuracy) with a misclassification error rate under 0.1%. We found similar accuracy when we further validated our model's detection accuracy on imaging data, from a variety of experimental systems and conditions, sourced from the hearing research community. Finally, to showcase real use applications in a research setting, we used this tool to assess datasets from two previously reported studies and successfully replicated their manually determined findings.
Conclusions: HCAT is highly accurate and fast, facilitating a more comprehensive approach to hair cell quantification. The software is easy to use and can run on an entry-level PC. With the release of this tool, we hope to ease the burden of manual hair-cell analysis for hearing researchers.

SA123. The Sensitivity of Neonatal Rat Spiral Ganglion Neurons to a Muscarinic Acetylcholine Agonist
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Category: Inner Ear: Anatomy and Physiology
Background: Lateral olivocochlear efferent (LOC) neurons directly contact and potentially modulate the peripheral dendrites of spiral ganglion neurons (SGN). The biophysics of LOC modulation is poorly understood but is believed to (at least in part) involve the release of acetylcholine. Our ongoing work asks if the ion channel properties and excitability of SGNs are modulate-able via common efferent-receptor pathways. Here, we focus on the muscarinic acetylcholine receptor pathways. Additionally, as in vitro electrophysiology and recent transcriptomic analyses suggest that SGNs are comprised of biophysically distinct sub-types, likely due to underlying variations in ion channel composition, we also ask if some sub-types of SGNs are more sensitive to efferent driven modulation than others.
Methods: Spiral ganglia from postnatal day 6-13 Long-Evans rats are excised, enzymatically treated, disassociated, and cultured overnight to isolate single somata for patch clamp recordings. Voltage-clamp recordings were made to characterize the whole-cell outward potassium currents, hyperpolarization-activated currents (HCN), and the sensitivity of these currents to the muscarinic receptor agonist, Oxotremorine-M (Oxo-M). Recordings were made in the perforated-patch configuration to preserve intracellular signaling cascades relevant to muscarinic acetylcholine agonism. Results were then mapped onto SGN sub-types (e.g. modiolar/pillar-contacting) by implementing a previously defined predictive model relating the basic electrophysiological properties to the SGN sub-type.
Results: HCN channel properties (e.g., conductance density and half-activation voltage) were broadly distributed across 44 neurons but did not correlate with SGN sub-types. Unlike our recent observations in vestibular ganglion neurons, muscarinic acetylcholine agonism did not alter HCN channel properties. However, so far, in 5 paired recordings Oxo-M significantly increased net conductance in each recording, on average by 10%. Which ion channels contribute to this increase remains to be determined. Consistent with the observed increase in net conductance, SGN became less excitable in response to Oxo-M, as indicated by the larger (20-40 pA in each neuron) currents needed to reach action potential threshold. Preliminary data suggest that putative modiolar-contacting neurons experienced greater increases in net conductance than did

putative pillar-contacting neurons after Oxo-M exposure, although more data is needed for greater resolution.
Conclusions: The action of LOC neurons on the excitability of auditory afferents is not well understood but is generally thought to be neuroprotective. Our findings that one component of efferent modulation decreases the excitability of SGNs supports this protective role. However, it is believed that neuroprotection may not be equal across SGN sub-types as low spontaneous-rate fibers are the most vulnerable to damage from loud noise overexposure. Therefore, there is a need to better understand the differences across sub-groups that cause these variable outcomes to deleterious insults. With our ongoing work, we aim to characterize these differences towards the goal of developing more targeted interventions to prevent noise-induced hearing loss.

SA124. Preoperative Cochlear Coverage Prediction for Cochlear Implant Candidates
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Category: Inner Ear: Anatomy and Physiology
Background: Electro-acoustic hearing has shown improved speech perception scores in cochlear implant patients hinting at importance of preserving residual hearing. Pre-knowledge of expected electrode insertion trajectory could help to optimize cochlear insertions ultimately boosting speech performance scores.
Methods: Pre- and post-operative CT scan pairs of 150 patients implanted with Oticon Medical EVO electrodes were included in this retrospective study. An automatic image analysis software (Nautilus, Oticon Medical, France) was used to analyze the images and obtain segmentation of the ducts, cochlear lateral wall centerline, electrode positions and respective insertion coverages within the cochlea. Based on these outputs, a statistical model was formulated using 100 patient data to predict a-priori the angular coverage, an electrode array is expected to reach as a function of the number of electrodes inserted beyond the round window. The remaining 50 samples were used to assess the accuracy of our model which was also compared to state-of-the-art coverage estimation methods.
Results: The statistical model was able to accurately predict the insertion coverages for all patients with our predictions falling on average within 20º of observed insertion angular coverage (n=50). The statistical model also outperformed state of the art approaches for which the accuracy decreased exponentially with increase in insertion depth. The behavior was contrary to the proposed statistical model for which coverage prediction error plateaued beyond 15 mm insertion depth.
Conclusions: A retrospective analysis of electrode insertions carried out on our clinical dataset suggested that on average the CI electrode only follows an ideal trajectory along the lateral wall after 150º insertion. The proposed statistical model of the electrode insertion trajectory from pre-operative image could be used prospectively to aim at a specific insertion angular coverage potentially leading to better patient outcomes. Our results raise the need to develop implant-specific and surgical technique-specific coverage prediction models to achieve more realistic and safe insertions.

SA125. Competition Between Lateral Line and Inner-Ear Evoked Startle Responses in Larval Zebrafish
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Category: Inner Ear: Anatomy and Physiology
Background: The acoustic escape reflex known as the C-bend startle response (C-start) can be initiated using optogenetics in zebrafish. Optical stimulation of the light-gated ion channel, Channelrhodopsin (ChR2) depolarizes hair cells and triggers C-starts through activation of one of two hindbrain reflex pathways. The faster pathway begins with the Mauthner neuron (M-Cell) and produces short-latency C-starts (SLCs). The slower pathway begins with Midcm 2 and 3 neurons and generates long-latency C-starts (LLCs). When the hair-cell specific promoter (myo6b) drives expression of ChR2, optical stimulation activates both hair cells in the zebrafish inner ear and lateral line sensory systems. Previously, we found that these optically-evoked C-starts display an intensity-dependent probability shift between SLCs and LLCs as well as intensity-dependent SLC latency. However, whether the lateral line system can modulate the acoustic response or evoke C-start responses independently from the inner ear was not explored. Here, we examined the role of lateral line hair cells in both initiating and modulating SLC and LLC C-starts in larval zebrafish.

Methods: We examined C-start probabilities and latencies using ChR2 and the faster optogenetic protein, Chronos. Using the aminoglycoside antibiotic, neomycin, we ablated lateral-line hair cells to study inner-ear evoked C-starts in myo6b:ChR2 larvae. In addition, we cloned inner-ear and lateral-line specific promoters to drive expression of the optogenetic proteins in each system independently. Given that SLCs and LLCs could display overlapping latencies especially at lower stimulus intensities, we utilized the fact that SLCs display large amplitude field potentials to discriminate between the two C-start types by simultaneous measurement of extracellular field potentials and high-speed behavioral videos.
Results: Our data reveal that the lateral line can independently evoke SLCs and LLCs that display intensity-dependent latencies that are each slower than inner-ear SLCs and LLCs. By measuring the difference between the onset of the field potential and the onset of the C-start body bend, we estimated the processing time from excitation of the hindbrain neurons to contraction of the trunk musculature. Analysis of this reflex circuit time showed that the different SLC and LLC pathways are consistent with their different response latencies. These two circuit times were also independent of which sensory system evoked the response. Furthermore, at low stimulus intensity a comparison between lateral line and inner-ear evoked C-starts suggests that there is competition at the level of the hindbrain neurons.
Conclusions: Our findings suggest that the inner ear and lateral line systems can initiate or modulate startle responses in context-dependent ways. Considering the role of corollary discharge in lateral line activity during self-generated motion (e.g., schooling or mating), our study contributes to understanding how multiple sensory systems integrate or compete to initiate reflexes in response to a changing environment.

SA126. Evaluation of PRDM16 Mutant Cochlear Phenotype Using Synchrotron X-Ray Phase Contrast Microcomputed Tomography and ABR

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Category: Inner Ear: Anatomy and Physiology
Background: PR domain containing 16 (PRDM16) is a key transcriptional regulator in the development of craniofacial, adipose, and neural tissues. PRDM16 expression was recently identified in the epithelial cells of the Kölliker's organ (KO), and it is shown to regulate KO proliferation and inhibit KO prosensory gene expression. Since Prdm16 null mice die at birth, we generated an inner ear-specific conditional deletion mouse model which survived and enabled postnatal characterization. Prdm16 cKO cochleae show hypoplastic spiral limbus, lack of inner sulcus, and detached, deformed tectorial membrane via H and E and immunostaining. To circumvent tectorial membrane susceptibility to histological processing artifacts, and to better connect structural and functional phenotypes, we utilized synchrotron X-ray phase contrast microcomputed tomography (μCT) and Auditory Brain Stem Responses (ABR) to phenotype Prdm16 cKO cochleae.
Methods: Prdm16 cKO mice were generated by crossing Prdm16lox/lox and Fgf20Cre mice. Immunostaining showed efficient Prdm16 deletion from KO in the middle and apical cochlear turns, while the basal turn exhibited some expression. P28 cKO and control male and female mice were tested for hearing thresholds using ABR at 4, 8, 16, and 32 kHz; inner ears were then harvested and imaged using synchrotron X-ray phase-contrast and micro-computed tomography at Argonne National Laboratory (resolution of 1.7 μm). These virtual sections were visualized and analyzed using Amira software (ThermoFisher Scientific). The scala media, spiral limbus, and tectorial membrane were manually outlined and segmented. Surface generation was used to generate three-dimensional models, and lastly, Amira's statistical analyses were performed to gather volume and volume per slice measurements.
Results: Reconstruction of μCT slices showed structural changes in the middle and apical regions of the cochlea in Prdm16 cKO compared to controls, including the lack of spiral limbus and inner sulcus. The tectorial membrane lost its modiolar attachment and was either floating in the scala media or attached to Reisner's membrane. Tectorial membrane total volume was 36.5% compared to control with the greatest reduction in the middle and apical turns (n=3/group, Student's t-test p-value<0.05). Spiral limbus total volume was significantly reduced (37% relative to controls) (n=3/group, Student's t-test p-value<0.05). Scala media total volume and volume/slice did not show significant changes. ABR analysis of P28 Prdm16 cKO and littermate controls showed statistically significant elevation in the hearing thresholds at 4KH and 8KH (n=11/group, Student's t-test p-value<0.05) indicating a low to mid-frequency hearing deficit. Such results align with the μCT analysis showing structural defects in the middle and apical turns.

Conclusions: We show that micro-computed tomography can be reliably used to analyze cochlear structure without the need for tissue sectioning. This work expands upon the role of Prdm16 during cochlear development and establishes a structural and functional hearing deficit related to Prdm16 deletion in mature cochlea.

SA127. The Effects of Estrogen on Hair Cells in Plainfin Midshipman (Porichthys notatus) and Zebrafish (Danio rerio)

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Category: Inner Ear: Anatomy and Physiology
Background: Estrogen can alter hearing in vertebrates, including rats, mice, humans, zebra finches, and plainfin midshipman fish (Porichthys notatus). Midshipman are a species of toadfish native to the Pacific coast of North America that communicate vocally during the summer reproductive season. Interestingly, reproductive female midshipman have increased auditory sensitivity relative to non-reproductive females, allowing reproductive females to better encode the higher harmonics of the male's reproductive call. Increased physiological sensitivity is accompanied by increased hair bundle density in the saccule, the main auditory end organ in this species. Exogenous estrogen is sufficient to increase auditory sensitivity in non-reproductive females, and we hypothesize that estrogen will also increase saccular hair bundle density in these animals. We further hypothesize that estrogen will alter hair cell density in other vertebrate models, such as in the zebrafish (Danio rerio) lateral line. This hair-cell based sensory system provides a tractable alternative to midshipman for studying the effects of estrogen on hair cell populations.
Methods: Estradiol treatment in midshipman: Female midshipman were ovariectomized and administered either estradiol or control implants. Following a 23–25-day incubation period, fish were euthanized, and the inner ear was removed. We then quantified the hair bundle density of each inner ear end organ. Additionally, reproductive inner ear end organs were assessed for hair bundle density, cell proliferation, and cell death. Estradiol treatment in zebrafish: Wildtype AB zebrafish larvae were exposed to varying levels of estrogen and/or estrogen receptor antagonist for 72 hours prior to hair cell quantification.
Results: We found that estradiol implantation in non-breeding female midshipman was sufficient to increase saccular hair bundle density, suggesting that estrogen alters the rate of cell turnover in the saccule. Similarly, estrogen exposure alters the number of hair cells in the lateral line of larval zebrafish. These results show that estrogen interacts with hair cells in multiple sensory organs, suggesting that there could be a conserved mechanism of estrogen induced cell turnover in hair-cell based sensory organs.
Conclusions: Our results show that estradiol can increase the saccular hair bundle density in non-breeding female midshipman, consistent with our prior data demonstrating seasonal auditory plasticity in this species. However, the mechanism by which estrogen increases hair bundle density is unknown. In addition, we found that estrogen signaling also modulates hair cell number in the larval zebrafish lateral line, suggesting that zebrafish may be a tractable model to better understand the effects of estrogen on hair cell populations in vertebrates.

SA128. Vibration Analysis Reveals the Fish Saccule Behaves as a Velocimeter Near it's Natural Frequency

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Category: Inner Ear: Anatomy and Physiology
Background: The saccule is often the largest otolithic end organ and most associated with hearing in teleost fishes. It is also posited to serve a vestibular function. Recent studies have demonstrated that in some fishes, the swim bladder can act as a crude "ear drum". Pressure-induced vibrations of the swim bladder are transduced to the saccule, allowing sound pressure detection. Despite the importance of the saccule as a hearing organ, little is known about its dynamic response and resonant frequencies. Previous attempts using either X-ray techniques or "4D" standing wave tube-like setups have not been able to fully capture the dynamic range of the inner ear end organs. Here, we used finite element analysis to predict the natural frequencies and mode shapes of the saccule in the plainfin midshipman fish, Porichthys notatus. Further

analysis using a Matlab transfer function model suggests that the saccule functions as a velocimeter near its natural frequency.
Methods: Adult plainfin midshipman were scanned with a Bruker Skyscan 1172 micro–CT scanner. The sagitta (saccular otolith) was rendered into a 3D volume using 3D Slicer software. This rendering was imported into finite element software (Strand7) and meshed with solid elements. Although the otolithic membrane thickness could not directly be determined from micro–CT scans, we approximated it to be between 20 and 40 microns, based on literature. Material properties of the membrane and sagitta were also taken from literature.
We used the finite element model to determine the natural frequencies and mode shapes in which the sagitta shears the underlying otolithic membrane. Next, a Single-Degree-of-Freedom transfer function model was developed in MATLAB to predict the dynamic range of the sagitta based on these natural frequencies.
Results: Mode shapes illustrate that the sagitta oscillates as a Single-Degree-of-Freedom system, shearing the underlying otolithic membrane containing the auditory hair bundles. Based on the observed natural frequencies and mode shapes, and assuming the saccule behaves as a quasi-critically damped system, our model predicts that the midshipman saccule functions as a low pass filter for measuring acceleration, and as a high pass filter when functioning as a seismometer. However, if the hair cells on the saccular macula are activated as a function of the velocity of the saccular otolith, the saccule functions as a band pass filter, responding maximally at the saccule's natural frequency.
Conclusions: This study represents the first attempt to describe the natural frequencies and dynamic range of the fish inner ear saccule using finite element models. Our results indicate the saccule detects velocity excitation over a narrow bandwidth near the natural frequency of the end organ. At other excitation frequencies, the saccule behaves as an accelerometer or seismometer.

SA129. Test-Retest Reliability of Electrocochleography Measured With Ear Canal and Tympanic Membrane Electrodes

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Category: Inner Ear: Anatomy and Physiology
Background: Human electrocochleography (ECochG) provides a non-invasive measurement of auditory nerve (AN) and cochlear hair cell function. ECochG is useful for establishing normal cochlear function and assessing cochlear dysfunction resulting from hearing loss, loss of AN fibers, Meniere's disease, and other peripheral pathologies. Electrode montages for measuring ECochG in humans typically involve placing an electrode in the ear canal (e.g., "Tiptrode") or on the tympanic membrane (TM electrode). Compared to TM electrodes, ear canal electrodes are more comfortable for patients and often easier to place; however, TM electrodes result in larger ECochG amplitudes and better signal-to-noise ratios (SNRs) compared to ear canal electrodes. Although these factors are important in selecting an electrode when measuring ECochG, an equally important factor is test-retest reliability, especially when experiments include measurements across multiple days. This study compares the test-retest reliability of Tiptrode and TM electrodes for compound action potentials (CAPs) elicited by a high-level click.
Methods: Seventeen young adults with normal hearing participated in this study. We obtained CAP amplitudes elicited by a bilateral, 100 dB peSPL, 80 μs click for a simultaneous, two-channel recording where the active electrodes for the first and second channels were the TM (right eardrum) and Tiptrode (left ear canal) electrodes, respectively. The reference and ground electrodes were located on the high forehead and between the eyes. Two blocks of 1000 sweeps were collected during the initial recording session and again during a follow-up recording session. This design allowed us to assess the within- and across-session test-retest reliability of CAP amplitudes for TM and Tiptrode electrodes. Test-retest reliability was assessed with intraclass correlation coefficients (ICC, two-way random effects, absolute agreement).
Results: Peak-to-peak CAP amplitudes measured with the TM and Tiptrode electrodes averaged 3.3 μV (σ = 0.99 μV) and 0.65 μV (σ = 0.20 μV), respectively. Average noise floors were 0.12 μV for the TM electrode and 0.10 μV for the Tiptrode, resulting in SNRs of 29 dB and 16 dB. Within-session test-retest reliability was excellent for TM (ICC = 0.95) and Tiptrode (ICC = 0.92) electrodes. Similarly, excellent test-retest reliability was observed across sessions and was slightly greater for the TM electrode (TM electrode: ICC = 0.92; Tiptrode: ICC = 0.81). CAP amplitudes were significantly correlated for TM and Tiptrode electrodes ($r = 0.54$, $p = 0.03$).

Conclusions: Within- and between-session test-retest reliability for TM and Tiptrode electrodes are comparable for 100 dB peSPL clicks, suggesting that either electrode is appropriate for experiments using similarly high stimulus levels. These conclusions may not hold for lower-level stimuli, where the higher SNRs for the TM electrode are expected to result in relatively greater reliability compared to the Tiptrode.

SA130. Predicting Supporting-Cell Survival in the Human Cochlea Based on Audiogram and Etiology

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Category: Inner Ear: Anatomy and Physiology

Background: Restoration of cochlear function by transdifferentiation of surviving supporting cells (SCs) after noise damage has been achieved in a mouse model, but when similar approaches were applied in a recent clinical trial, the success was limited. Outcomes might be improved if there were more quantitative and comprehensive data on the extent of SC survival in human cochleas from hearing-impaired subjects with different audiometric patterns or hearing loss etiologies.

Methods: We analyzed human temporal bones from the collection of celloidin-embedded, H and E-stained sections at the Mass Eye and Ear. We chose 175 cases with a descending audiogram (better than 40 dB at low frequencies and worse than 50 dB at high frequencies), 38 cases with a flat audiogram (standard deviation of all thresholds < 10) and 58 cases with profound hearing loss (all thresholds worse than 90 dB), ages ranging from 10 to 102 yrs. Cases with mean air-bone gaps larger than 10 dB were excluded. In each case, SCs were assessed at >100 locations along the spiral using a 5-rank semi-quantitative rating scale based on the fractional survival of inner and outer pillar cells and Deiters cells. We also quantitatively assessed fractional hair cell survival.

Results: Of the three SC types analyzed, inner pillars were the most resistant to degeneration. Even in the profound hearing loss cases, where there was a complete loss of outer and inner hair cells, complete loss of SCs (replaced by an undifferentiated epithelium) was rare and almost never seen in apical to the 2 kHz location. Regardless of audiogram shape, SC survival was better in the apex than the base. Among the cases with either descending or flat audiograms, SC survival in the basal half of the cochlea was highly correlated with mean high-frequency thresholds ($p < .001$). No such correlation was seen in the apex. Among cases with similar high-frequency thresholds, cases with noise-damage history had greater loss of SCs.

Conclusions: There can be good SC survival, even in ears with severe to profound hearing loss. In the context of hair cell regenerative therapies in the animal model where the main source of transdifferentiation is the inner pillar cells, it is notable that inner pillar cells are the most resistant to degeneration. Ongoing work will expand the analysis to other critical structures such as stereocilia, tectorial membrane, stria, and cochlear neuronal populations.

SA131. Non-Contact Biomechanical Imaging of the Organ of Corti in Mice Using Brillouin Microscopy

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Category: Inner Ear: Cochlear Mechanics

Background: The mammalian cochlea's ability to discern frequencies and intensities of sounds depends on the shapes and mechanical properties of various cellular and noncellular structures in the organ of Corti. Due to the lack of measurement tools, the stiffness of the individual components in the organ of Corti remains largely unknown, hindering our understanding of cochlear mechanics. The present study tested the feasibility of using the Brillouin scattering microscopy, a non-contact optical method, to measure the stiffness of various structures in the organ of Corti of mice.

Methods: Upon euthanizing the mouse, the bulla was harvested. The cochlea was extracted and immersed in saline in a dish and frozen. Two hours before imaging, the cochlea was thawed and immersed in fresh saline. The wall in the middle turn of the cochlea was opened, and the scala vestibuli was exposed. The exposed area was imaged using a confocal Brillouin microscope. Two-dimensional Brillouin images, with a lateral resolution of 1×1 μm per pixel, were taken in the plane perpendicular to the basilar membrane in radial direction of the cochlea. The high-frequency longitudinal modulus of various structures in the organ of Corti was derived using the equation M^'=Ω_B^2·λ^2·ρ/4n^2, where Ω_B is the measured Brillouin frequency shift, λ the wavelength of the laser source (660 nm), ρ the density of the measured material, and n the refractive index of the measured material. Although the values of ρ and n vary among samples, previous

data show the ratio ρ/n^2 is mostly unchanged. Here we use a previous value (0.558) acquired from biological tissue as an approximation for the organ of Corti.
Results: It is worth noting that the high-frequency longitudinal modulus M^' measured by Brillouin microscope is different from the quasi-static Young's modulus E measured by conventional test such as atomic force microscopy; their definitions imply much higher (GPa) values of M^'compared to E (kPa). In the cochlea measured, the longitudinal modulus of the tectorial membrane, outer hair cells, and basilar membrane were 2.52 GPa, 2.48 GPa, and 2.28 GPa, respectively.
Conclusions: We believe this is the first time the mechanical properties of these components in the organ of Corti were measured using a non-contact method. The feasibility of using Brillouin microscopy to measure the biomechanics of the organ of Corti was proven. We plan to use this technology to image the mechanical properties of fresh cochleae.

SA132. The Mechanical Behavior of the Guinea Pig and Gerbil Cochlear Apex Compared Using Optical Coherence Tomography

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Category: Inner Ear: Cochlear Mechanics
Background: Low frequency sound is often central to mammalian perception of vocalization and environment. There are biological limitations of neural responses to high frequency sounds, such as membrane time constant. Yet these are not a selection pressure which shapes how low frequency sound mechanotransduction mechanisms have evolved. As a result, the cochlear apex and base appear to function differently in some species, notably in the guinea pig as we recently showed (Burwood, G., Hakizimana, P., Nuttall, A.L. and Fridberger, A., 2022. Best frequencies and temporal delays are similar across the low-frequency regions of the guinea pig cochlea. Science Advances, 8(38), p.eabq2773.)
Methods: In order to assess whether the mechanical properties and behaviors of the guinea pig apical cochlear partition are shared with other species, we employed another model of low frequency hearing, the Mongolian gerbil. By adapting our minimally invasive mirror preparation, we measured acoustically evoked transverse vibrations in the cochlear apex of the gerbil using optical coherence tomography.
Results: We compared the responses of components of the cochlear partition between the two species in terms of tuning, relative phase delay and amplification.
Conclusions: The results provide comparative insight into the region of the inner ear that is dedicated to frequency bands often used in communication.

SA133. A Computational Model of the Gerbil Auditory Periphery for Studies of Sensorineural Hearing-Impairment

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Category: Inner Ear: Cochlear Mechanics
Background: Understanding how specific damage to the cochlear structures translates to altered auditory brainstem responses (ABRs) and otoacoustic emissions (OAEs) is important with a view on developing sensitive, non-invasive methods for sensorineural hearing loss (SNHL) quantification. Realistic computational models of the auditory periphery and generators of OAEs/ABRs are an important tool in this pursuit. While human model parameters can be calibrated based on OAE/ABR recordings, the corresponding animal models have the benefit that direct pathophysiological SNHL data is available as well. Here, we wish to combine the respective benefits of Gerbil and Human experimental SNHL data by developing a Gerbil auditory model that can easily be translated to a human model based on the principle of cochlear scaling symmetry across species.
Methods: Gerbil basilar-membrane velocity was simulated for characteristic frequency (CF) varying between 140 Hz and 50.4 kHz using a time-domain implementation of the Zweig (1990) cochlear impedance description earlier adopted in a human cochlear transmission-line model (Verhulst et al., 2018). Cochlear

tuning and level-dependence was fit to the 4-kHz Gerbil cochlear region and the corresponding double-pole of the cochlear admittance was extrapolated across all CFs adhering experimentally observed Gerbil Qerb changes with CF (Muller, 1995 and Charaziak and Shera, 2021). Afterwards, the cochlear filters were made stimulus-level-dependent using a saturating nonlinear function and the scaling parameters for the auditory-nerve, cochlear nucleus and inferior nucleus stages were set to match the Gerbil ABR amplitudes reported in Burkard and Voigt (1989).
Results: After calibration, we simulated several key features of cochlear and auditory-nerve processing and compared our simulations to literature data. It was possible to maintain a stable cochlear model solution up to CFs of 25 kHz where the Qerb was 30 for low stimulation levels. The model simulated realistic ABR wave I, II and V peaks as well as their level-dependent behavior. To evaluate the SNHL prediction capabilities of the model, we first modified the models' SNHL parameters (cochlear gain loss and different synaptopathy degrees) and simulated distortion-product OAEs and ABRs collected in Gerbils that were either normal hearing or treated with Kainic Acid to cause different degrees of cochlear synaptopathy.
Conclusions: The principles of cochlear scaling symmetry across species can be used to generate a Gerbil version of an auditory periphery model that simulates realistic cochlear tuning, its level dependence and ABR waveforms. In the future, the brainstem pathways of the model can be updated based on Gerbil histopathology and brainstem response recordings, after which these brainstem model stages can be incorporated within the human auditory periphery model to ultimately improve the SNHL prediction power of human auditory evoked potential recordings.
Work supported by ERA-NET Neuron grant CoSySpeech (G0H6420N), FWO grant (G068621N) and Department of Defense grant W81XWH2120027.

SA134. Experimental Investigation of the Relationship Between Temporal Bone 3D Motion and Intracochlear Pressure

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Category: Inner Ear: Cochlear Mechanics
Background: The temporal bone, including the otic capsule, undergoes a complex 3D motion pattern that depends on the frequency of the BC stimulation. The correlation between the 3D motion of the surrounding bone and the intracochlear pressure difference across the cochlear partition is not yet known and is to be investigated.
Methods: Measurements were conducted in 3 fresh frozen cadaver heads, where both medial and lateral bone surfaces of both temporal bones have been exposed, resulting in a total of 6 samples. The skull bone was mechanically excited in the frequency range of 0.1- 20 kHz via the actuator of a bone conduction hearing aid (BCHA). Stimulation was applied sequentially to the ipsilateral mastoid and the classical BAHA location via a conventional transcutaneous (5-N steel headband) and percutaneous coupling. Three-dimensional motions were monitored, across the lateral and medial (intracranial) surfaces of the skull, the ipsilateral temporal bone, the skull base, as well as the promontory and stapes. Each test condition consisted of 130-200 measurement points (~5-10 mm pitch) across the skull surface. Additionally, intracochlear pressure in the scala tympani and scala vestibuli was measured via a custom-made intracochlear acoustic receiver.
Results: The temporal bone surface, surrounding the otic capsule, was mostly rigid up to 10 kHz, in contrast to the skull base, which deforms above 1-2 kHz, with an onset of deformation near the stimulation location already at 0.5 kHz. This is in contrast to the relative similarity of the average motion magnitude across these regions. Above 1 kHz, the ratio, between the differential intracochlear pressure and the promontory motion, is relatively independent of coupling and stimulation location.
Conclusions: The area around the otic capsule appears rigid up to significantly higher frequencies than the rest of the skull surface, resulting in primarily inertial loading of the cochlear fluid.

SA135. Rate Dependence in Outer Hair Cell Mediated Active Processes: Determining Prestin's Speed Limit

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Category: Inner Ear: Cochlear Mechanics

Background: The electromotility of the outer hair cell (OHC) contributes to the sensitivity of the mammalian cochlear by amplifying the traveling waves on the basilar membrane through electrical-to-mechanical energy conversion. Rate-dependent effects, including viscous damping, transmembrane electrical impedance, and state-dependent conformal transition, hold the potential to attenuate OHC-mediated active processes at high frequencies, leading to an active debate surrounding the ability to perform cycle-by-cycle amplification. In this study, we will build a simplified OHC model to explore the influence of the rate dependence on active force generation and power deposition. Further, we propose an efficient experimental approach to determine the rate constants of the conformal changes along with the other OHC parameters consistently according to this modeling.
Methods: In the study, the OHC is modeled as a prestin-based motile element in series with a viscoelastic element. For the motile element, the Boltzmann distribution and absolute-rate Eyring equation are employed to simulate the charge transfer and length change. Since the operating conditions of the OHC experience small variations in vivo, the motile element model is linearized around the resting condition, showing the influence of a state dependent, rate, τ_{nl}, on charge transfer and length change in the frequency domain. The viscoelastic element is characterized by an elastic spring and a viscous contribution with its associated rate constant τ_e. Further, to consider a more general loading case in vitro experiment, the OHC loaded with a mechanical impedance to simulate the responses under experimental loading conditions.
Results: We find that our OHC model is able to fit isolated, in vitro experimental electromotility, force, and charge results. The fitting shows the low-pass filtering of the electromotility and nonlinear capacitance is not affected by the rate τ_e. However, the frequency dependence of the OHC generated electromechanical force exhibits low-pass the depends on viscoelastic rate constant, becoming low-pass when $\tau_e=0$, all-pass when $\tau_e=\tau_{nl}$, or band-pass when τ_e is between 0 and τ_{nl}. Therefore, both τ_e and τ_{nl} can play an important role in electromechanical force generation and electrical-to-mechanical power conversion. However, there is not enough experimental evidence to estimate the value of τ_e. By characterizing the nonlinear charge and electromotility responses, we propose an experimental approach to determine the rate-dependent parameters as well as the other parameters without exposition of the detailed nonlinear constitutive behavior.
Conclusions: Through the investigations, we show the importance of rate dependence in regulating the electrical-to-mechanical power conversion, and propose an experimental approach to estimating the rate-dependent parameters through measured OHC responses.

SA136. Large Quadratic Distortion Products in Vibrations of the Mouse Cochlear Apex

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Category: Inner Ear: Cochlear Mechanics
Background: The cochlea's mechanical nonlinearity leads to the generation of significant distortion products (DPs) in its vibratory response. When stimulated with two tones at frequencies f1 and f2, the odd-order, cubic 2f1-f2 DP is strongest perceptually and has thus been most widely studied. However, there is also physiological and psychophysical evidence that the even-order, quadratic DP at f2-f1 is generated and perceived. It is therefore curious that vibratory measurements from high-frequency cochlear regions indicate that this DP is small or nonexistent on the basilar membrane (BM), and may only be detected in motions of the outer hair cells (OHCs), the presumed source of the DPs. Characterizing the behavior and relative magnitudes of even- and odd-order DPs in cochlear mechanics is important for understanding the form of the underlying nonlinearity and how DPs influence perception. To address this, the present study examined both cubic and quadratic DPs in vibrations of the 9 kHz region of the mouse cochlea.
Methods: Optical coherence tomography was used to image and measure vibrations from the apical turn in adult CBA/CaJ mice in vivo. Various two-tone stimulus paradigms were used to elicit DPs in vibrations of the OHC region, BM, and overlying tectorial membrane (TM). To characterize locally generated DPs, the f2 tone was fixed at the characteristic frequency of the measurement site while f1 was varied. The levels of the two tones (L1 and L2) were either equal and co-varied or varied separately. Postmortem measurements confirmed the absence of artifactual distortions in the in vivo measurements.
Results: The quadratic DP was prominent in OHC region vibrations, typically being much larger than the cubic DP and sometimes exceeding the response to the stimuli. With L2 fixed and L1 varied, quadratic and cubic DP magnitudes grew ~1 and 2 dB per dB increase in L1, and then peaked and declined as the response to the f1 tone exceeded that at f2. The nonmonotonic growth patterns of the DPs could be replicated by the output of a first-order Boltzmann function. Both DPs were also measurable on the BM and TM, though their

magnitudes appeared to be strongly shaped by the frequency responses of these structures. Use of higher-frequency stimuli revealed that both DPs propagated apically to the location tuned to the DP frequency.
Conclusions: The quadratic DP is a significant, often dominant DP in vibratory responses to two tones in the mouse cochlear apex. The presence of strong even-order DPs confirms that an asymmetric output is generated by the underlying nonlinearity, which is commonly attributed to the mechanotransduction process. The functional role and perceptual impact of quadratic DPs requires further examination.
This work was supported by a Hearing Health Foundation Emerging Research Grant and NIH/NIDCD R21 DC019209.

SA137. Higher-Order Mode Vibrations in the Organ of Corti

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Category: Inner Ear: Cochlear Mechanics
Background: The basilar membrane (BM) is the foundation of cochlear mechanics. The longitudinal pattern of BM vibrations which forms the traveling waves has been better investigated as compared to its radial vibration pattern. Previous studies have assumed the fundamental vibration mode for BM radial vibrations. Accordingly, BM motion was reduced to a single degree-of-freedom at a radial section. We examined whether a radial section of the organ of Corti (OoC) carries higher-order mode vibrations. Besides the BM, the radial vibration patterns of the reticular lamina (RL) and the tectorial membrane (TM) were measured and analyzed.
Methods: Cochleas were acutely excised from young Mongolian gerbils (15-30 days old, both sexes). After being reduced to a single turn between 50 and 80-percentile location from the basal end, the excised cochlea was placed in a custom-designed chamber. Mechanical and electrical stimulations were applied to the tissue at different frequencies to evoke the passive and the active vibrations, respectively. Resulting vibrations were measured by an optical coherence tomography system. The optical plane was aligned so that the target structure (BM, TM, or RL) was aligned normal to the optical axis. Forty to sixty M-scans were acquired across the OoC span (2-5 μm span between M-scans).
Results: Higher-order mode vibrations were observed in the BM and the RL radial patterns, but not in the TM. When mechanically stimulated, the primary mode prevailed throughout physiological frequency range of the measurement location, but higher-mode vibrations appeared near or above the best frequency (BF) at the location. On the contrary, when electrically stimulated, the BM and the RL vibrations showed apparent higher-order mode vibrations despite simulating frequencies. TM radial pattern, however, does not show higher-order mode within physiological frequency range for both passive and active cases. Our finite element model analysis reproduced similar vibration patterns depending on stimulation types.
Conclusions: Our measurements suggest that outer hair cell electromotility promotes the shift from the primary to a higher mode of the BM and RL vibrations. There are two possible implications of our findings. First, our results may provide a partial explanation for level-dependent frequency shift. Second, our observations explain how the BM acts as the mechanical reference for active outer hair cells.
This study was supported by NIH NIDCD R01 DC014685.

SA138. Intracochlear Vibrations Measured From Multiple Directions Reveal Outer Hair Cell Motion That is Predominantly in the Longitudinal Direction

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Category: Inner Ear: Cochlear Mechanics
Background: Recent observations of sound-evoked vibrations of the cochlea's sensory organ of Corti (ooC) using optical coherence tomography (OCT) have revealed unanticipated and complex motions. Interpreting these results in terms of the micromechanical inner-ear processes that precede hair-cell transduction is not trivial because motion directions are unknown. In reality, the OCT only measures a projection of the true motion, which may include transverse and longitudinal displacements.
Methods: An OCT system was used for non-invasive measurements of sound-evoked vibrations of the BM and ooC in the middle turn of adult gerbil cochleae in vivo (n=6; female). We measured vibrations from the BM and within the ooC from different "viewing angles" (i.e., the angle between the OCT beam and the BM in the longitudinal direction) to determine if longitudinal motion occurs. The viewing angle was varied utilizing the cochlea's natural curvature. That is, variation of the recording location along the longitudinal

axis changes viewing angle. We calculated the relative phase between ooC responses and the BM, which dramatically depends on this angle if longitudinal motion occurs. Vibrations were evoked with multitone acoustic stimuli (30–70 dB SPL/frequency component) that were delivered to the ear-canal in a closed-field sound configuration.
Results: Sound-evoked responses of the BM and within the ooC all had typical characteristics. Response amplitudes were tuned with best frequencies (BF) that systematically changed with longitudinal location, matching the place-frequency map in gerbil. Phase agreed with the presence of a traveling wave (TW) that decelerated when approaching BF: its wavelength was ~3 and ~1 mm for low-frequency and BF tones, respectively. Throughout the ooC we only found that the OHC-BM phase difference systematically and dramatically varied with viewing angle, switching from an OHC lead for negative viewing angles to an OHC lag for positive angles. This dependence agrees well with OHC motion that was largely in the longitudinal direction.
Conclusions: Our findings confirm the existence intracochlear longitudinal motion that is associated with the BM-supported TW. It was restricted to the OHC, perhaps due to its proximity to the BM and/or anatomical degree-of-freedom to move in and out of the transverse plane. Results also strongly indicate that measured relative motion involving OHC must be interpreted with care. Although it may be tempting to attribute difference to relative up-and-down (transverse) motion, our data suggest it is more likely to signify motions/rotations in the longitudinal plane.
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SA139. Targeted and Untargeted Quantitative Mass Spectrometry Approaches to Analysis of the Inner Ear

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Category: Inner Ear: Damage and Protection

Background: Although 15% of American adults suffer from some type of hearing loss, many of the mechanisms of disease and drug behaviors are not fully understood. In order to improve targeted drug treatment for hearing loss, it is essential to establish pharmacokinetics of drug compounds to identify and compare effective treatments. Additionally, the biochemical profiles of various types of hearing loss must be elucidated to identify biomarkers and novel drug targets. Thus far, this has proved challenging, as the inner ear is difficult to access in most organisms. Though it is possible to extract the perilymph from the inner ear without causing permanent structural damage, due to the small volume available, sample size is limited. Current liquid chromatography-mass spectrometry (LC-MS) techniques do not require large sample sizes and are incredibly sensitive to a variety of analytes, including many small molecules and proteins/peptides. We outline a targeted and untargeted approach in which quantitative mass spectrometry can be utilized to enhance understanding of the inner ear and hearing loss.
Methods: In the targeted approach, a Q Exactive™ hybrid Quadrupole-Orbitrap mass spectrometer coupled to a Vanquish™ Flex Binary UHPLC will be used to target the mass to charge ratio (m/z) of a specific analyte. On a case-by-case basis, LC-MS conditions will be optimized to produce the most robust signal for a single analyte.
Results: Preliminary targeted methods indicate both gentamicin and fractalkine standards were detected and quantitated. Following optimization, analytes will be quantified either absolutely or relatively. For absolute concentration, such as gentamicin, analyte abundance in the samples will be compared to a standard curve of known concentrations. Alternatively, the amount of fractalkine will be determined relatively, by comparing the abundance of fractalkine in treated and untreated samples. Similar techniques will be applied to other analytes in the future. In the untargeted approach, LC-MS conditions are instead optimized to identify as many analytes (proteins, lipids, etc.) as possible by scanning an entire mass range (150-2000 m/z). A comparison of treated and untreated samples will identify analytes that may play a role in the disease mechanism and help establish proteomic or metabolomic profiles of specific types of hearing loss.
Conclusions: LC-MS is a powerful tool that has, thus far, been under-utilized for studies of the inner ear. Here, we propose both a targeted and untargeted use for LC-MS in order to advance understanding of drug

behavior once in the inner ear and mechanisms of specific types of hearing loss. We have shown the versatility of our platform to detect various types of analytes. Future endeavors include testing in biological samples and expanding our method repertoire to include other types of analytes.

SA140. Open Board

SA141. Psychophysical and Electrophysiological Measures of Intensity Coding in Carboplatin Treated Chinchillas

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Category: Inner Ear: Damage and Protection

Background: Auditory temporal processing is essential for perceiving and coding complex acoustic stimuli, such as speech. These signals are considered complex in part due to rapid fluctuations along the intensity and temporal domains. Temporal processing abilities are thought to be negatively impacted by age-related or noise-induced hearing loss. It has been suggested that deficits in temporal coding or intensity detection ability may contribute to speech processing deficits in damaged ears. The current study aimed to evaluate chinchilla sensitivity to intensity differences with an intensity increment detection (IID) psychophysical task, as well as amplitude modulated electrophysiological responses measured with an envelope following response (EFR). Assessments were made before and after carboplatin treatment, a drug known to produce selective inner hair cell (IHC) loss in this species.

Methods: Free-feeding young adult chinchillas were used for this study. Distortion product otoacoustic emissions (DPOAEs) and pure tone thresholds in quiet were used to assess cochlear nonlinearity and overall hearing sensitivity. Chinchillas were conditioned using shock avoidance to respond to intermittent changes in intensity to an otherwise continuous reference narrowband noise. IID performance was assessed at 1, 2, 4, 8, and 12 kHz center frequencies and at three continuous reference noise levels, low (20 dB SPL), moderate (50 dB SPL), and high (70 dB SPL). The low-level noise was initially increased by 20 dB SPL, the moderate-level noise initially increased by 15 dB SPL, and the high-level noise initially increased by 10 dB SPL. An automated method of limits procedure was used to determine IID threshold as intensity decreased by 0.5 dB SPL for correct responses and increased by 1 dB SPL for incorrect responses until the lowest intensity at which the animal achieved 66% correct was obtained. EFR was assessed using amplitude modulated (AM) steady-state stimuli which were modulated using AM depths at 100%, 80%, and 20%. Following baseline testing, chinchillas received a single dose of 75 mg/kg of carboplatin (i.p.). DPOAEs, pure tone thresholds, IID thresholds, and EFR were re-assessed after a three-week recovery period.

Results: Following carboplatin treatment there were no significant elevations of pure tone thresholds and no significant changes to DPOAE; results suggesting that hearing sensitivity had not changed. Chinchilla IID thresholds were slightly elevated following carboplatin treatment. Significant differences were observed as a function of AM depths.

Conclusions: These results suggest that carboplatin may impact sensitivity to intensity changes and that this model could be used to study the effects of cochlear pathologies involving IHC.

SA142. Degradation of Perineuronal-Net-Like Complexes Around Inner Hair Cells Coincides With Synapse Loss Following Noise or Mild Traumatic Brain Injury

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Category: Inner Ear: Damage and Protection

Background: Perineuronal nets (PNNs) are unique complexes of extracellular matrix (ECM) proteins surrounding neuronal cell bodies and neurites that are known to have roles in synaptic plasticity and in the maintenance of synapses. PNN proteins have been shown to be present around the fibers that innervate cochlear hair cells and developmental deletion of a key PNN protein, brevican, leads to increased incidence of mispairing between pre- and post- synaptic components. Recent studies suggest that insults to the inner ear can cause dramatic losses in the numbers of properly paired inner hair cell synapses and that this cochlear “synaptopathy” may cause auditory impairments such as reduced hearing in noise. The goal here is to investigate the degradation of PNN structure following synaptopathic noise or trauma as a first step toward determining whether ECM remodeling may play a mechanistic role in synapse loss and subsequent recovery.

Methods: To investigate the effects of synaptopathic insults on cochlear tissues, 4-month old CBA/CaJ mice were subjected to 100 dB noise (8-16kHz) for 2 hours and cochleae were collected at 1-day, 7-days and 14-days post-insult. In a separate cohort, 4-month old C57Bl/6 mice underwent mild traumatic brain injury (mTBI). Tissues were immunolabeled using antibodies against known PNN component proteins: brevican, aggrecan, haplan4, and tenascin-R. Volumetric analysis (using IMARIS software) was performed in order to test whether otic insult causes changes in the amount of brevican or other PNN components around the inner hair cells. Tissues were immunolabeled with presynaptic marker CtBP2 and post synaptic marker GluR2 to measure synapse loss upon noise injury and colabelled with brevican to investigate any changes in PNN components.
Results: Noise exposure caused significant decreases in brevican volumes around inner hair cells as compared to non-noise exposed, age-matched controls. Similarly, the volume of brevican around inner hair cells showed differences between samples from mice that underwent mTBI and age-matched, unimpacted controls. Immunolabeling of aggrecan, haplan4, and other PNN components suggest similar reductions in PNNs following synaptopathic insults. Also, the volume of brevican positive PNNs was smallest in the apical turns of the cochlea and highest in the basal turns, with the latter having higher numbers of synapses per inner hair cell.
Conclusions: Overall, the data suggest that PNN volume is tightly and positively correlated with the numbers of synapses that are present on inner hair cells. We are currently investigating which ECM-degrading enzymes may be involved in PNN remodeling following noise or mTBI and whether such changes can be manipulated to prevent or exacerbate PNN degradation. These studies, if successful, will allow us to test whether manipulation of PNNs in the inner ear can help prevent or reverse synaptopathy.

SA143. The Effects of Noise Trauma on Outer Hair Cell Function of Otoferlin Knock-Out Mice
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Category: Inner Ear: Damage and Protection
Background: OTOF gene mutations can result in various forms of hearing impairments (Pangršič, Reisinger, Moser, 2012). Patients with auditory synaptopathy reportedly lose otoacoustic emissions (OAE) within the first two decades of life (Kitao et al., 2019). Recently, degeneration of OAEs was confirmed in Otof knock-out mice, especially at high frequencies (Stalmann et al., 2021). We hypothesize that efferent protection of outer hair cells (OHCs) is missing in deaf Otof-knock-out mice and patients, thus we expect OHCs to be more sensitive to acoustic trauma when compared to normal hearing individuals.
Methods: Auditory brainstem responses (ABRs), elicited by click, noise and tone bursts, were measured, along with different DPOAE recordings from Otof knock-out and wildtype mice. Hearing measurements were performed before and 3-15 days after an acoustic trauma with a noise band of 4-16 kHz, 103 dB, 15 min which was applied to anesthetized animals. Whole mount and 3D-structure analysis with automated hair cell quantification were then performed on ethylcinnamate cleared cochleae. Apical synapse count was performed by whole mount confocal microscopy. Pertaining to the evidence for the protective effect of estrogen in noise trauma, both sexes were compared.
Results: ABR elicited by tone burst stimuli showed a threshold shift up to 24 dB at different frequencies after trauma in wildtype animals. This recovered only partially after 11-15 days, leaving a mild permanent 5-23 dB shift at high frequencies (11,3-45,2 kHz), for both sexes. 2F1-F2 distortion products were comparable for all mice before noise exposure and showed an amplitude reduction at the best frequency of 15-17 db SPL 3 days after trauma in all groups. After 11-15 days, females of both genotypes and male wildtypes showed almost full recovery of OHC function, with a mild permanent 4-6 db SPL amplitude reduction in their distortion products. In contrast, male Otof knockouts displayed hardly any recovery of DPOAEs.
Conclusions: After noise trauma causing a mild permanent ABR threshold shift, OHC function recovered in female mice independent of the genotype, i.e. independent of protective mechanisms activated by auditory input. In male mice, DPOAEs recovered only in wildtype mice, but not in Otof knock-outs. Thus, recovery of OHC function requires auditory evoked protective mechanisms in males, likely auditory activated efferent inhibition, but is independent of these in females.

SA144. Morphological Characterization of Cochlear Macrophages With Ototoxic Challenges

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Category: Inner Ear: Damage and Protection
Background: Resident tissue macrophages are typically originated from the bone marrow via circulating monocytes, while in the inner ear, the majority of resident cochlear macrophages are self-maintained and behave independently. Cochlear macrophages that resemble microglia in the central nervous system, are the centerpiece of a variety of immune responses to middle ear infection, ototoxic damage, noise-induced hearing loss, and so on. Upon sensing immune disturbances, including topical and systemic ototoxic drug injections, cochlear macrophages become reactive, produce inflammatory cytokines, and alter their cytoplasmic composition and cytoskeletal formation to transform from dendritic to circular shapes. Presumably, the spatiotemporal progression of morphological changes determines the surveillance, inflammatory, and phagocytic functions of the cochlear macrophages. In the present study, we used the ionized calcium-binding adapter molecule 1 (Iba-1) marker to identify microglia/macrophages in the sensory epithelium after certain ototoxic challenges.
Methods: Six- to eight-week-old CBA/CaJ and C57BL/6 mice were selected. In experiment one, both mouse strains received 1 mg/ml intratympanic lipopolysaccharide (LPS) to assess the acute response of cochlear macrophages to topical bacterial toxins. In experiment two, mice were injected intraperitoneally with 400 mg/g gentamicin followed by 200 mg/g furosemide (G/F) to distinguish ototoxic modifications from the systemic insult of the same native immune cell population. Cochleae were collected, micro-dissected, and immunolabeled either 48-hour (LPS and G/F) or 15-day posttreatment (G/F). Iba-1 positive cochlear macrophages were quantified and morphologically characterized, using a confocal microscope (Olympus FV3000), along the cochlear sensory epithelium.
Results: Iba-1-positive cells in the sensory epithelium were sorted into five categories; dendritic, hypertrophic, bushy, long, and round. Control cochlea phenotype distribution in the apical turn was most abundantly dendritic, long in the middle, but both long and round phenotypes dominated in the basal turn. LPS treatment increased the number of round-type cells in the middle and basal turns of the cochlea at 48-hour posttreatment in contrast to the G/F treatment, which elevated the number of round-shaped cells only at 15-day posttreatment in C57BL/6 mice. Interestingly, for both ototoxic challenges, we observed fewer dendritic but more bushy cells in the apical turn of CBA/CaJ mice 48-hour and 15-day post insult. No significant disparity (2-way ANOVA) between morphological phenotypes was achieved in the middle or basal turns in CBA/CaJ cochlea.
Conclusions: Cochlear macrophages can be effectively activated in an otitis media mouse model, i.e., intratympanic LPS. The activation of the native immune response was likely through direct chemokine signaling, manifested by a swift morphological alteration of cochlear macrophages. In contrast, systemic ototoxicants such as gentamicin triggered the macrophages indirectly, after the ototoxic stress in the sensory epithelium. Differentially activated macrophages in the two examined wild-type mouse strains potentially promote disparate neuroprotection, or neurodegeneration, in the cochlear sensory epithelium.

SA145. The New Otoprotective Indication of a Safe FDA-Approved Drug: Mitigating Cisplatin-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection
Background: Cisplatin is a first-line chemotherapy prescribed to 20% of all cancer patients. However, more than 80% of patients receiving cisplatin treatment developed permanent hearing loss. Despite this debilitating side effect, currently, there is only one FDA-approved drug (sodium thiosulfate or STS) to prevent cisplatin ototoxicity among pediatric cancer patients.
Methods: High-throughput computational screening was performed using our proprietary data science approaches to identify FDA-approved drugs that mitigate the risks of cisplatin-induced hearing loss among cancer patients. A top-ranked drug (DXU1445) was selected and validated by longitudinal clinical data cohort analyses. DXU1445 was further examined using gene expression profiling. The otoprotective efficacy was measured in in-vivo zebrafish lateral line hair cells and in vivo rodent ABR/DPOAE assays. A549 cell assays were performed to test its effect on cisplatin anti-tumor activity.

Results: Our in vivo experimental results show that DXU1445 offers substantial protection against cisplatin ototoxicity and it does not attenuate cisplatin anti-tumor effect in vitro. Most importantly, the new otoprotective indication of DXU1445 is evident in our retrospective patient data analyses, a strong support of its clinical translatability.
Conclusions: We have demonstrated the potential preclinical and clinical efficacy of DXU1445 in our investigations, particularly through retrospective patient data. A pilot prospective human subject study is underway. Given the excellent safety profile of DXU1445, it holds the promise to be a safe and effective alternative to STS for cisplatin ototoxicity prevention. A special formulation will be developed to further improve its otoprotective efficacy.

SA146. Mastoidectomy-Induced Acoustic Trauma Measured Through Intracochlear Pressure Changes

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Category: Inner Ear: Damage and Protection
Background: Mastoidectomy is a common procedure performed for many otologic conditions, including chronic middle ear disease, cholesteatoma, vertigo, and hearing loss. While sensorineural hearing loss (SNHL) has been reported after mastoidectomy, it is considered rare and often attributed to technical or inflammatory sources. Whether there is a potential risk of inducing SNHL from acoustic trauma caused by elevated intracochlear acoustic pressures with high speed (up to 80,000 RPM) otologic drill use is unclear. This study examined the potential for cochlear acoustic trauma from mastoidectomy resulting from pressure changes within the cochlea.
Methods: Intracochlear acoustic pressures were measured in cadaveric hemicrania via transcanal fiber-optic pressure sensors. A tympanomeatal flap was elevated to create cochleostomies over scala tympani and vestibuli. Once the probes were placed in their respective cochlear cavities, the cochleostomies were sealed with dental impression material, fixed to the cochlear promontory and canal wall with cyanoacrylate adhesive, and the tympanomeatal flap was secured in its original position, restoring normal anatomy. The ear canal was then occluded with a foam earplug. The cochlear pressures are measured during: (1) raising of the flap, (2) removal of the outer cortex, (3) exoneration of mastoid air cells, (4) opening of the facial recess, and (5) no activity (control).
Results: Intracochlear pressure changes in the scala tympani and vestibuli were noted throughout simulated mastoidectomy. When compared to controls, the intracochlear acoustic pressure was substantial and persistent during drill contact with the skull. Drill contact generated a broadband noise exposure, and when these pressures were compared to equivalent sounds presented in the ear canal, the equivalent sound pressure levels were well in excess of 100 dB sound pressure level (SPL).
Conclusions: There are significant changes in the intracochlear pressures with otologic drilling during routine mastoidectomy, with potential to induce acoustic trauma. Further investigation is needed to determine if the relatively short and intermittent exposure times related to drilling results in injury to the auditory periphery, or whether an unidentified protective mechanism is responsible for the low rate of observed SNHL associated with routine mastoidectomy.

SA147. 3-MA Co-Treatment Alleviates Cisplatin's Cytotoxicity in HEI-OC1 Cells through Mitochondrial Rescue but Increases Toxicity to H460 Cells

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Category: Inner Ear: Damage and Protection
Background: The chemotherapeutic drug cisplatin is ototoxic partially through the excessive induction of ROS in cochlear hair cells. These cells increase autophagic flux as a protective mechanism until the stressor is removed, or alternatively, the cells die from apoptosis after overwhelming autophagy. Several autophagy-inducing drugs have been found protective against cisplatin-mediated ototoxicity. Additionally, pretreatment with autophagy inhibitor 3-MA had been found to increase cisplatin's toxicity in hair cells. Here, using cell viability assays and immunofluorescence microscopy, we studied the effects of 3-MA/cisplatin cotreatment on the cellular and mitochondrial viability of HEI-OC1 and H460 cells.
Methods: HEI-OC1 and H460 cells were each treated with 3-MA (at 0, 0.3, 1, 3, and 10 mM) for 24 hours with and without 30 μM cisplatin before being assessed for viability via MTT assay. Cells from each line were also prepared for confocal immunofluorescent study after treatment with 0, 1, and 10 mM of 3-MA for

24 hours with and without cisplatin. After fixing, cells were treated with DAPI, mitotracker, and anti-LC3 antibodies and then imaged. Individual focal planes were then selected, standardized, and analyzed via ImageJ.
Results: 3-MA treatment of HEI-OC1 cells slightly reduced viability without cisplatin treatment at 10 mM while rescuing viability of cells when treated with cisplatin between 3 and 10 mM. However, 3-MA treatment of H460 cells reduced viability with each increase in concentration both with and without cisplatin. In the IF microscopy experiments, cisplatin treatment caused reduced mitochondrial staining in both cell lines. However, H460 cells retained distinct organelle staining while HEI-OC1 cells showed indistinct cytoplasmic staining with cisplatin treatment. Treatment with both 1 and 10 mM 3-MA enhanced the distinct mitochondrial staining in HEI-OC1 cells. Cisplatin co-treatment with 10 mM 3-MA increased mitochondrial staining in both cell lines and rescued distinct mitochondrial staining for HEI-OC1 cells.
Conclusions: At the appropriate dose, 3-MA alleviates the cytotoxic effect of cisplatin on HEI-OC1 cells but exhibits an additive toxicity on H460 cells. Cisplatin treatment of HEI-OC1 cells causes a loss of mitochondrial viability that is partially rescued by 3-MA co-treatment. However, this rescue is absent in H460 cells due to their lesser mitochondrial vulnerability. Thus, 3-MA reduces cisplatin's ototoxicity by inhibiting the loss of mitochondrial viability in HEI-OC1 cells, likely through its action as an autophagy inhibitor. These findings suggest 3-MA as an otoprotective co-therapeutic of the antineoplastic cisplatin.

SA148. Cisplatin-Induced Ototoxicity in PGAM5 Constitutive Knockout Mice

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Category: Inner Ear: Damage and Protection
Background: Phosphoglycerate mutase 5 (PGAM5) is a mitochondrial phosphatase critical for mitochondrial fission. PGAM5-/- mice have age-dependent increased mitochondrial fusion and decreased mitochondrial turnover resulting in increased cellular ATP, reactive oxygen species (ROS), and inflammatory signaling. Cisplatin chemotherapy causes significant and permanent hearing loss in mice and humans. The mechanism for cisplatin's ototoxic effects is believed to be through DNA cross-linking and ROS production. Cisplatin is retained in the cochlea indefinitely, with highest accumulation within the stria vascularis, organ of corti, and spiral ganglion. PGAM5's role in hearing loss has not been described.
Methods: PGAM5 constitutive knockout (KO) mice and wild-type (WT) controls were given intraperitoneal cisplatin or saline. Cellular toxicity following single-dose injection of 15mg/kg cisplatin was determined via whole-mount preparations of the organ of corti using the proapoptotic marker cleaved caspases-3 and cell marker antibodies Myo7A and SOX2. Baseline auditory brainstem responses (ABR) of 8-week-old KO mice and matched WT controls were obtained using 4, 8, 12, 16, 24, and 32 kHz tone pips. Hearing loss was evaluated following chronic exposure consisting of three cycles of 3.5 mg/kg cisplatin daily for 4 days and 10 days of recovery. Post-exposure ABR were obtained one day after the final injection and again 9 days later. PGAM5 RNA localization in WT mice was determined via in-situ hybridization of mid-modiolar cochlear cross-sections.
Results: A greater number of Claudius cells undergo apoptotic pathway activation 24-hours after cisplatin injection – translating to increased cell death four days after injection – in KO mice than WT. Cytochochleogram seven days after injection shows an increased loss of outer hair cells (OHC) in KO mice than WT, particularly in the basal area. PGAM5 KO mice had larger ABR threshold shifts at 8, 12, 16, and 24 kHz one-day post-cisplatin exposure than WT. KO and WT mice had similar ABR threshold shifts 10 days post-exposure. KO and WT mice injected with saline had no significant ABR threshold shifts. In-situ hybridization in WT mice shows PGAM5 is widely expressed in the cochlea with highest concentrations noted in the supporting cells of the outer hair cells (OHC), spiral ligament, and outer sulcus regions.
Conclusions: Mitochondrial homeostasis may play a role in susceptibility to cisplatin-induced hearing loss. Increased apoptosis in Claudius cells of KO mice corresponds to a location of cisplatin accumulation demonstrated in previous studies as well as a site of increased PGAM5 RNA expression seen in WT mice. A larger ABR threshold shift in KO mice 24 hours post-cisplatin exposure suggests that the apoptotic pathway activation in Claudius cells may contribute to accelerated hearing loss. ABR threshold shifts equalize between KO and WT mice by 10 days post-exposure despite increased OHC loss in KO mice suggesting an unclear compensatory mechanism.

SA149. Impact of CCR2 Deletion on Outer Hair Cells in Chronic Suppurative Otitis Media

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Category: Inner Ear: Damage and Protection

Background: Chronic Suppurative Otitis Media (CSOM) is one of the most common causes of permanent hearing loss among children in the developing world. It is characterized by chronically draining middle ear, with no effective cure. We have shown that CSOM induces an inflammatory macrophage response in the inner ear, associated with hair cell damage. We have also revealed that CCL-2, an important member of the monocyte chemoattractant protein (MCP) family, is elevated over time following middle ear infections. MCP receptor CCR2 has been implicated in many neurodegenerative disorders. In our current study, we investigate the role of CCR2 on hair cell damage in CSOM. Our goal is to compare CCR2-/- mice to the control mice on cochlear damage in CSOM.

Methods: PCR genotyping was done to isolate CCR2-/-, CCR2+/-, and CCR2 +/+ mice. We inoculated Pseudomonas bacteria to the mouse middle ear cavity for generating CSOM and monitored them at 7 and 14 days after middle ear infection, time points before and after hair cell damage occurs in our model. We dissected the cochlea to assess hair cell damage with whole mount specimens and evaluated macrophages within cross sections.

Results: Our results measure the outer hair cell survival number, with Myosin VIIa immunostaining, in the cochlear basal, middle, and apical turns in CCR2 -/-, CCR2 +/-, and CCR2 +/+ mice at 7 and 14 days. We also measured the number of F4/80 macrophages with F4/80 immunostaining in the cochlear turns and compared them among the three groups.

Conclusions: In the future, we will continue to learn more about the mechanism in which CCR2 influences the immune response in the inner ear and whether it plays a protective or harmful role on hair cells in CSOM.

SA150. A Deafening Experiment in the Egyptian Fruit-Bat: Groundwork to Establish a Mammalian Model for Vocal Learning

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Category: Inner Ear: Damage and Protection

Background: Vocal learning, the ability to go beyond fixed motor programs for the production of vocalizations, is a trait that is believed to be shared by only a few clades throughout the animal kingdom. Similar to how humans learn to speak, cetaceans, elephants, some bird species and bats can also learn to produce species-specific vocalizations. While we have substantial knowledge of how vocal production learning is implemented in a songbird brain, we lack a mammalian model to investigate the same mechanisms at the same cellular resolution in mammals. Recent behavioral experiments in several bat species have pointed to vocal learning abilities in this clade. To test this ability in the Egyptian fruit bat, we conducted a deafening experiment in pups and evaluated the vocal deficits incurred. Early deafening prevents both the ability to form auditory templates of the species-specific repertoire and to correct one's vocal production via auditory feedback, which are the main prerequisites for the ability to change innate vocal motor programs.

Methods: Young pups (4 males and 6 females) were each isolated from the colony with their mother from birth. They were injected with kanamycin (2 males and 3 females) to achieve complete deafness by 3 weeks of age or saline (2 males and 3 females) for control conditions. The extent of deafening was assessed by recording auditory brain responses (ABRs) at the adult stage and histological imaging of the cochlea post-mortem. Cochleae were stained with phalloidin, dissected as whole-mounts and analyzed with epifluorescence.

Results: The expected species ABR could be recorded in saline injected bats, but kanamycin injected individuals did not show any ABR responses in the frequency range of 1-90 kHz with stimulation up to 110 dB SPL. Histological analysis of cochleae from kanamycin bats further demonstrated that the hair cells were absent, replaced by a flat epithelium throughout the organ of Corti in males and that only the most apical area of the organ of Corti (low frequency area) retained a few surviving hair cells in females. The control individuals exhibited a normal organ of Corti.

Conclusions: These results demonstrate that we could achieve profound deafness in a bat species. Acoustical analysis of the vocalizations produced by these animals will reveal the extent to which auditory perception and feedback are necessary to produce the species-typical vocal repertoire.

SA151. Music Festivals: The Effect of Recreational Noise Exposure on Young Adults Hearing

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Category: Inner Ear: Damage and Protection

Background: Noise exposure in young adults mainly takes place during leisure time activities, of which concerts and festivals have been reported as the loudest. A possible result of excessive noise exposure is a temporary threshold shift or 'TTS'. While TTSs caused by noise exposure were previously believed to be innocuous, animal studies revealed that TTSs can produce a permanent loss of up to 50% of synapses between cochlear inner hair cells and auditory nerve fibers, without widespread hair cell loss. This synapse loss has been named cochlear synaptopathy, and potentially contributes to the origin of tinnitus and hyperacusis. Suprathreshold auditory evoked potentials are a promising technique to diagnose cochlear synaptopathy in humans, as high-threshold auditory nerve fibers have been shown to be most vulnerable to noise damage.
The objective of the current study was to evaluate the effects of recreational noise exposure on young adults hearing, by evaluating the subjects hearing status before and after attending a music festival.

Methods: A group of 42 young adults with normal audiometric thresholds, attended a music festival in summer 2022. Personal exposure was objectively monitored using dosimeters, and auditory status was evaluated before going to the music venue and again at one, and three days after the event. Every session, the subject completed a questionnaire and a test battery of (extended high frequency) audiometry, distortion product otoacoustic emissions, and auditory evoked potential measurements, comprising auditory brainstem responses (ABR) and envelope following responses (EFR).

Results: Exposure on the festivals reached equivalent continuous sound pressure levels (LAeq) and peak sound pressure levels (LCPeak) up to 104.8 dBA and 143.5 dBC, respectively. The mean exposure duration was nine hours and 31 minutes. Although a TTS, as defined by OSHA-standards, was found in only one subject, threshold elevations of 10 dB or more at individual frequencies were found in several subjects. Overall, taking into account the mean and standard deviation of each individual measurement, we observed significant deteriorations in EFR-strength in 20% and 27% of the subjects on the frequencies 4 and 6 kHz, respectively, one day after the exposure. More extensive data analysis, including the DPOAEs and ABRs before and after the music festivals, were performed and relationships with the noise exposure level (and use of hearing protection) were evaluated.

Conclusions: This study makes a valuable contribution by including a range of early markers of hearing damage after a detailed monitoring of leisure noise exposure levels. We observed persistent deteriorations in several of our study participants, and our results could hence inform future exposure guidelines. Secondly, this study provides important contributions on the individual level where EFR or TTS monitoring could help sensitize the festival attendee about the possible consequences of noise exposure at such events.

SA152. Characterizing the Effects of Noise Exposure and Cochlear Implantation Trauma in a Longitudinal Rat Model

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Category: Inner Ear: Damage and Protection

Background: Chronic, hazardous acoustic overexposure causes irreversible mechanical and structural damage to the inner ear and is associated with severe-to-profound sensorineural hearing loss (SNHL). Veterans and other service members, such as firefighters and policemen, are at an increased risk of SNHL due to occupational noise exposure. The most common treatment modality for patients with severe-to-

profound SNHL is cochlear implantation (CI). However, CI outcomes vary significantly and often perform poorly in this group of patients with residual hearing loss post-implantation. Here, we developed a novel rodent double-insult model with noise exposure that mimics occupational SNHL and subsequent CI surgical trauma to characterize the mechanisms and patterns of residual hearing loss post-CI.
Methods: The University of Miami Institutional Animal Care and Use Committee approved all procedures. Brown Norway rats were exposed to broadband (4-16 kHz) noise at 110 dB for 1 hour to induce SNHL. CI was performed 3 months post-noise exposure. Auditory brainstem responses (ABR) post-noise exposure and subsequent CI trauma at different time points (up to 84 days post CI) were compared and characterized across the following groups: 1) Low damage noise + CI, 2) high damage noise + CI, and 3) CI only controls (n = 5 each). The contralateral non-implanted ears provided controls for a paired comparison. At 84 days post-CI, cochleae were harvested and dissected for histological studies.
Results: Functional measurements showed a permanent threshold shift (PTS) post-noise trauma in both noise groups mimicking SNHL in patients. Additionally, significant shifts in ABR threshold were observed in both low and high noise damage groups with CI compared to the CI only group. As expected, immunohistology revealed significant damage in the basal to middle sections of the cochleae in all the groups receiving CI. Additional outer hair cell (OHC) loss was observed at apical regions for both noise-exposed groups.
Conclusions: Taken together, our results highlight patterns of sensorineural damage with prior noise exposures that could explain poor CI outcomes in patient populations with extensive noise history. With this double-insult model, we were able to localize the permanent damage within specific cochlear regions and characterize patterns of hearing loss post-implantation. The model further enables considerations for neurotherapeutic techniques, including mild therapeutic hypothermia post-CI which broadly counteracts inflammation, apoptosis, and cell death mechanisms within the inner ear.

SA153. Statins Protect Mice From Noise-Induced Hearing Loss
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Category: Inner Ear: Damage and Protection
Background: Previously, we demonstrated in guinea pigs that the HMG-CoA reductase inhibitor fluvastatin protects against noise-induced hearing loss (NIHL) when delivered directly to the contralateral cochlea. Guinea pigs exhibit a known variability in responses to noise. For this reason, and for future genetic experiments to study the mechanisms of statin protection, we move to an inbred mouse model, CBA/CaJ, which has been more consistent in noise induced auditory brain stem response (ABR) thresholds. We tested three hypotheses: (1) changes in threshold after high decibel noise exposure are stable about one week after exposure, (2) fluvastatin delivered directly to the cochlea protects the contralateral cochlea from (NIHL), and (3) orally given lovastatin protects against NIHL.
Methods: To determine when ABR thresholds become stable, we exposed the mice for 2 hours to bandpass filtered noise (8-16 kHz) at levels between 90-110 dB SPL. To study the protective effect of stains, we developed a laser method to cleanly and accurately create a cochleostomy for insertion of a cannula from a miniosmotic pump. Hearing damage was achieved through bandpass filtered noise (8-16 kHz) exposure at 110dB SPL for 2 hrs. Fluvastatin delivery was initiated at time of noise exposure. We compared the threshold shifts at 2 weeks after noise exposure between fluvastatin+vehicle (50 µM in the pump), n=12, vehicle alone treated (n=8); noise exposure alone (n=5); and unexposed mice (n=11). We tested oral lovastatin (n=13), protection against NIHL. Lovastatin (60mg/kg or 120 mg/kg) was delivered several days before, the day of and 1 day after noise. Statistics - ANOVA with Tukey posttest or two-tailed t-test.
Results: ABR threshold shifts were stable 1 week after noise. Intracochlear delivered fluvastatin protects against NIHL in mice. ABR threshold shifts: Unexposed animals (n=11), did not vary more than 1dB over 4 weeks. At two weeks after noise, ABR threshold shifts for vehicle alone vs fluvastatin+vehicle (in dB) were: 32kHz, 39.4 +/-15.9 vs 12.3 =/- 8.9 (p= 0.0002); 16 kHz, 29.4 +/- 6.8 vs 11.9 +/- 11.7, (p=0.0002); 8 kHz, 16.9 +/- 11.3 vs 5.7 +/-9.5 (p=0.0118). Fluvastatin does not protect against degeneration of synapses. As expected, at 2 weeks past noise, animals given oral carrier alone, had ABR threshold shifts over 30 dB at 8, 16 and 32 kHz. Oral lovastatin (60 and 120 mg/kg) protects against NIHL. ABR threshold shifts (in dB) of the carrier (n=4) vs lovastatin (n=13) were: 32 kHz: 31.8+/-7.5 vs 28.5 +/-11.6 (p=0.271); 16 kHz: 46.3 +/-7.5 vs 25.4 +/-18.0 (p=0.042); 8 kHz: 37.5+/- 10 vs 14.2 +/- 13.7 (p=.0072).

Conclusions: Fluvastatin delivered directly to the mouse cochlea protects against NIHL in the contralateral cochlea. Protection by oral lovastatin is promising for future studies of drugs for hearing loss.
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SA154. Investigation of Modified Hyaluronan Conjugates as Hearing Loss Prophylactics
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Category: Inner Ear: Damage and Protection
Background: Mechanistically, inflammation and oxidative stress are major factors in hearing loss regardless of etiology. Therefore, targeting inflammation and reactive oxygen species seems a viable prophylactic approach. However, due to the complexity of the ear anatomy, treatment and prophylaxis of hearing loss are difficult and extremely limited. Experimental approaches are often focused on invasive topical drug delivery methods or systemic drug delivery leading to damage of ear structures, off target effects, and low concentration reaching the cochlea. Our group has previously investigated several hyaluronan (HA) conjugates with antioxidants that show promise in accessing the inner ear structures and providing protection to inner ear cells against oxidative damage. In this study we explore a new series of drug conjugated HAs, for increased protective effects and potential development as a minimally or non-invasive prophylactic for sensorineural hearing loss (SNHL).
Methods: HA intermediates of varying molecular weights and chemical moieties were first synthesized and then characterized via 1H-NMR and HPLC. The cellular internalization of the intermediates into HEI-OC1 and SV-k1 murine cochlear cells as well as their permeation through round window membrane (RWM)-mimicking tissue models and tympanic membrane (TM) mimicking tissue models were assessed via HA ELISA kits. HA-(drug) agent conjugates (HACs) were prepared by chemical conjugation of HA intermediates with antioxidant or anti-inflammatory molecules. HACs were characterized via 1H-NMR and HPLC. Cytocompatibility was assessed with MTS colorimetric assay as well as CyQuant Cellular proliferation assay. The oxidative protection properties of HACs post-menadione stressing were assessed with MTS colorimetric assay in in vitro assays. Cellular internalization and membrane mimicking permeation was also assessed for the HACs in the same manner as the HA intermediates mentioned above.
Results: The optimal HA molecular weight for cellular internalization as well as anatomical membrane permeation was determined. This was used to inform synthesis parameters for conjugation of anti-inflammatory and/or antioxidant molecules onto HA to yield HACs. Various HACs were subsequently synthesized and tested. These HACs were cytocompatible and provided oxidative protection to stressed HEI-OC1 and SV-k1 cells. Our additional data indicate that the conjugation of the agent to HA helps with internalization of the drug into cochlear cells as well as RWM permeation and TM permeation when compared to the anti-inflammatory or antioxidant agents alone.
Conclusions: Our data so far highlights the practicality of chemically conjugating HA with antioxidants and/or anti-inflammatory agents for oto-therapeutic purposes. Such therapeutics have the potential to be further developed into a new generation of biocompatible and biointegrating topically deliverable agents against hearing loss.

SA155. Lateral Olivocochlear Efferents Protect the Auditory System From Traumatic Noise Exposure
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Category: Inner Ear: Damage and Protection
Background: The auditory system is tasked with interpreting acoustic information from our immediate environment, which in turn helps us navigate the world and communicate with others. When traumatically loud noise damages the cochlea, this crucial information is degraded or lost. The central nervous system likely offers protection from noise-induced hearing loss (NIHL) by utilizing multiple efferent systems that project to the cochlea from the brainstem. One of these systems, comprised of lateral olivocochlear (LOC) neurons, directly innervates the auditory nerve and is implicated in multiple roles related to audition—including protecting the auditory system from NIHL. To directly test this, we specifically and bilaterally ablated intrinsic LOC neurons in mice and then assayed their cochlear function after traumatic noise exposure (NE).
Methods: As LOC neurons are the only subset of auditory efferents that express both the transcription factor Gata3 and the peptide urocortin (Ucn), they were genetically isolated using Gata3-FlpO^(+/-)::Ucn-Cre^(+/-

) mice. This enabled the generation of experimental animals that harbored a Cre- and Flp-dependent diphtheria toxin (DTX) receptor selectively in LOC neurons. This receptor is required for DTX entry into cells, which initiates cell death. Four- to 8-week old mice received two intraperitoneal DTX injections (50µg / kg body weight) at least 7 days before they were exposed to 8-16 kHz noise for two hours at 97-97.5 dB SPL. Two categories of control mice were used: some that received the toxin but lacked the DTX receptor, and others that expressed the receptor but were injected with saline. Additionally, all three conditions were compared to mice that did not receive NE. Peripheral auditory function was monitored 1- and 14-days after NE by evaluating the mice's auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAEs) in response to pure tones.
Results: We found that our approach ablates ~70% of LOC neuron somata in the brainstem, corresponding to a qualitative reduction of their fibers in the cochlea. Mice lacking LOC neurons had elevated ABR thresholds after NE and a reduction in wave 1 amplitude compared to animals with an intact LOC system. Two weeks after NE the ABR thresholds of these same mice recovered at higher frequencies (32 – 45 kHz) but remained elevated at lower frequencies (8 - 22.6 kHz). Thresholds for DPOAEs initially elevated and then recovered similarly to control animals at lower frequencies, but not completely at higher frequencies. Last, we detected an ototoxic effect of DTX on inner hair cells which markedly reduced the amplitude of ABR wave 1 while having little effect on threshold, even in mice lacking the DTX receptor.
Conclusions: Overall, our results demonstrate that LOC neurons protect the peripheral auditory system from noise-induced damage, even within 24 hours of NE. However, the mechanism underlying this protective effect remains unknown.

SA156. Examining the Therapeutic Potential of Cell-Derived Exosomes in Hair Cell Protection

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Category: Inner Ear: Damage and Protection
Background: Exosomes are small (~50-150 nm) extracellular vesicles that mediate intercellular communication. They are generated by most cell types and deliver proteins, metabolites, and nucleic acids to recipient cells to induce functional changes. We have recently shown that exosomes derived from heat-shocked utricles protect against neomycin-induced hair cell (HC) death in cultured utricles from adult mice. Our data suggest that exosomes carry cargo from supporting cells to HCs to activate a pro-survival response. Mass spectrometry analyses of exosomes derived from heat-shocked utricles identified 235 unique proteins, including HSP70, which was critical for the protective effect. The goal of this study is to determine key cargo molecules in addition to HSP70 that are required to mediate HC protection under ototoxic stress. Since exosome yield from inner ear tissue is very low, we have expanded our studies to immortalized cell lines, which release large numbers of exosomes containing HSP70. We found that some cell lines produce protective exosomes while others produce non-protective exosomes. Examining the proteomes of both exosome types is important for identifying key exosomal factors that prevent HC death in addition to HSP70.
Methods: Whole-organ cultures of utricles from adult mice were used as a model system, and multiple cell lines were analyzed for exosome secretion under control and heat shock conditions. Exosomes from cell lines were purified via centrifugation filtration and characterized using nanoparticle tracking analysis and Western blot analysis. Isolated exosomes were applied to utricles in the absence or presence of neomycin. Quantitative mass spectroscopy will be used to examine the proteomes of both protective and non-protective exosomes. The identified peptides will be compared between the two groups (protective vs. non-protective) as well as to our existing data on protein families associated with protective exosomes from heat shocked utricles.
Results: Exosomes derived from a colorectal carcinoma cell line (CT26) contained HSP70 under control conditions, and heat shock significantly upregulated HSP70 in the exosomes. Exosomes released by CT26 cells were applied to utricles at increasing concentrations in the absence of neomycin to verify that they do not elicit a cytotoxic effect on HCs. In the presence of neomycin, isolated CT26-derived exosomes showed promise in inhibiting HC death. Together, our data suggest that the CT26-derived exosomes can be leveraged to identify key exosomal-derived otoprotective factors.
Conclusions: This study examines whether cell lines produce exosomes that protect against neomycin-induced HC death and investigates which key factors are required for the protective effect of exosomes in the inner ear. We can then begin to make deductions about the exosome proteome, understand what is

necessary for protection, and potentially manipulate and amplify the effect with the long-term goal of using exosomes for inner ear therapeutics.
This work was supported by the NIDCD Division of Intramural Research.

SA157. Intratympanic Single Dosage of Dexamethasone Formulated as SPT-2101 Protects Against LPS-Induced Alteration of the Blood-Labyrinth Barrier

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Category: Inner Ear: Damage and Protection
Background: The blood-labyrinth barrier (BLB) separates the inner ear from blood and regulates stria vascularis permeability, which is critical for the maintenance of ionic homeostasis and the prevention of the entry of deleterious substances. BLB dynamics are altered upon bacterial infection and its disruption, or altered permeability, has been associated with hearing pathologies such as autoimmune inner ear disease, acoustic trauma, and presbycusis. Clinically dexamethasone has been used to treat several of these disorders, administered via frequent repeated intratympanic or systemic administration which is associated with adverse secondary effects.
Methods: Here we have used intraperitoneal lipopolysaccharide (LPS) injection to induce BLB alterations to further characterize the molecular and cellular mechanisms involved in inflammatory breakdown of the BLB and to test a new formulation of dexamethasone, SPT-2101. SPT-2101 is an in situ forming gel formulation with sustained middle ear residence, which enables a single local dose treatment. BLB permeability was evaluated by gadolinium dynamic contrast-enhanced 7-Tesla magnetic resonance imaging (Gd-MRI) and Evans blue (EB) incorporation into the cochlea.
Results: LPS-treated rats showed increased cochlear signal enhancement in Gd-MRI, as well as higher cochlear EB concentration than controls. LPS injections increased the expression of LPS receptors Tlr2 and Cd14, pro-inflammatory cytokines as Il1b, its receptors as ll1lr, and injury mediators Nfkb and iNos with specific time course patterns. A single intratympanic injection of SPT-2101 administered 72 hours prior to LPS was sufficient to protect BLB permeability as assessed by Gd-MRI. A similar effect was seen with repeated doses of systemic dexamethasone, but not with a single systemic dose. Furthermore, SPT-2101 was able to reduce cochlear inflammation and normalize gene expression.
Conclusions: Our data indicate that repeated injections of LPS altered the BLB permeability mimicking BLB alterations secondary to infection by inducing LPS TL receptors and increasing Il1β levels and downstream signaling. A single intratympanic injection of SPT-2101, a novel in situ forming gel formulation of dexamethasone that allows slow and sustained drug release, was able to protect BLB function.
This work was supported by a grant from Spiral Therapeutics Inc. (South San Francisco, CA, USA)

SA158. Down- and Up-Regulation of Cochlear Synaptic Immunostaining After Noise: Implications for Analysis of Synaptic Regeneration

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Category: Inner Ear: Damage and Protection
Background: Confocal analysis within our lab has shown minimal recovery of inner hair cell synaptic counts in CBA/CaJ mice from 0 hrs to 2 yrs after noise exposure, whereas others have reported significant post-exposure recovery in C57Bl/6. However, confocal counts of pre-and post-synaptic puncta can be reduced or increased by down- or up-regulation of synaptic proteins. Differentiating reversible expression changes from frank degeneration and regeneration of auditory nerve dendrites is critical for our understanding of synaptopathy. To clarify these issues, we are comparing noise-induced synaptopathy in the two strains, using strictly controlled immunostaining and imaging parameters to allow quantitative inferences as to the expression levels of synaptic proteins.
Methods: We exposed mice to noise (8-16 kHz) at 90, 94, or 98 dB SPL for 2 hr, and sacrificed them at 0 hrs, 24 hrs, 2 wks, or 8 wks post-exposure, after recording ABRs and DPOAEs. Cochlear whole mounts were microdissected and batch-immunostained for CtBP2 (pre-synaptic ribbons) and GluR2 (post-synaptic receptor patches). Variations in immunostaining were controlled by including unexposed and exposed ears

in each batch, imaging the entire batch with the same parameters, and normalizing staining intensity in the exposed ears to the unexposed controls.
Results: In C57Bl/6 exposed to 94 dB noise, CtBP2 immunostaining intensity decreased slightly at 24 hrs across all cochlear regions, then rebounded at 2 wks to levels higher than control, and dropped back to control levels at 8 wks. GluR2 intensity decreased dramatically at 0 and 24 hrs post-exposure, especially in the synaptopathic region, and rebounded at 2 wks. Synaptic loss from 94 dB noise in C57Bl/6 peaked at 24 hrs, and showed a 50% recovery by 8 wks. At 98 dB, the immediate reduction in synapse counts in C57Bl/6 was greater than in CBA/CaJ, but recovery was also greater: i.e. about 30% increase by 2 wks. At 90 dB, there was no synaptic loss in either strain.
Conclusions: Our study is ongoing. Although there are hints that C57Bl/6 shows more synaptic recovery than CBA/CaJ, synaptic counts are complicated by the transient down-regulation of GluR2 expression and up-regulation of CtBP2 expression after noise exposure. Moreover, synaptopathy studies in C57Bl/6 have exposed animals at 6 wks, whereas the CBA/CaJ studies of long post-exposure survivals exposed mice at 16 wks of age.

SA159. On the Interdependence of Cochlear Implant Insertion Forces, Insertion Speed and Lubrication
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Category: Inner Ear: Damage and Protection
Background: During the insertion of cochlear implant (CI) arrays, forces occur which may cause trauma and poorer hearing outcomes. Unfortunately, research groups investigating factors which influence insertion forces come to contradicting results, especially regarding insertion speed. Furthermore, results are difficult to compare as there is no standardized approach for testing with insertion phantoms, e.g. regarding geometry, manufacturing process, material and lubrication. This study was conducted to determine how different testing conditions influence experimental findings.
Methods: An anatomically correct mean scala tympani (ST) insertion phantom was developed: segmentations of human cochlear cross sections were conducted in n=15 micro-CT datasets. In order to derive the mean shape of these segmentations, an averaging procedure was developed and applied which preserves as many common anatomical features as possible. The resulting ST model was then placed inside an insertion phantom in a predefined manner, which represents a combination of the cochlear consensus coordinate system and a previously proposed method for describing insertion trajectories based on the curvature of the ST. The phantom further comprises an idealized cochlear opening and a 1mm pressure release hole in the apex. It was produced by an Aiglista 3D printer (Keyence, Osaka, Japan) with a resolution of 15 µm step size.
Repeated, automated insertions with three different FLEX28 arrays (MED-EL, Innsbruck, Austria) were performed into the newly developed ST phantom. The testing protocol included variations in insertion speed (v = 0.1 - 2.0 mm/s) and lubrication (90%, 50%, and 10% liquid soap solution), which resulted in a total of 153 insertions. Evaluations were conducted in Matlab and included the investigation of the change in electrode behavior over time, maximal insertion force, insertion work and stick-slip-behavior.
Results: The test setup and protocol allowed for repeatable insertions with only minimal change in electrode array behavior. Strong but varying dependencies of the maximal insertion forces and work were found regarding both lubrication and speed: work-speed dependency, for instance, is constant for the 10% lubricant, negative for the 50% lubricant and positive for the 90% lubricant. Stick-slip behavior became more noticeable with increasing speed and less viscous lubrication.
Conclusions: The test setup, which includes guidance of the electrode prior to insertion, allows for conducting repeatable and comparable insertion studies. Our results can explain part of the contradicting results found within previous studies as measured bulk forces are likely influenced by generated friction forces and viscous damping, which are strongly dependent on insertion speed and lubrication. The developed insertion phantom and proposed testing setup are an important step towards repeatable and comparable CI array testing when studying relative effects between insertion parameters or the impact of single anatomical features (e.g. A, B, H values) of the ST onto insertion forces.

SA160. In Vivo Testing Potassium Channel Blockers to Attenuate Noise-Induced Hearing Loss and Cochlear Synaptopathy
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Category: Inner Ear: Damage and Protection
Background: Noise can induce inner ear ribbon synapse degeneration leading to hidden hearing loss (HHL) and other hearing dysfunctions. Our previous study revealed that excess extracellular K+ elevation, which is a physiological consequence following noise exposure since hair cell and auditory nerve extra-activity, plays a critical role in the ribbon synapse degeneration (Zhao et al., 2021, PMID: 33398038). We also evidenced that in vitro application of K channel blockers can attenuate K+ induced ribbon synapse degeneration. In this study, we continually tested whether in vivo administration of K channel blockers can attenuate noise-induced hearing loss and cochlear synaptopathy.
Methods: Adult CBA/CaJ mice were exposed to 95-98 dB SPL white noise for 2 hr, one time. K channel blockers were administrated by intraperitoneal injection (i.p.) before or after noise exposure. Hearing function was assessed by ABR, DPOAE, and other hearing function tests. After one month of noise exposure, the cochlea was collected and hair cell degeneration and ribbon synapse degeneration were examined by immunofluorescent staining. The ribbon synapses under inner hair cells (IHCs) and outer hair cells (OHCs) were quantified under confocal microscopy.
Results: First, as observed in vitro study, administration of K channel blockers in vivo before noise exposure could significantly attenuate noise-induced hearing loss and ribbon synapse degeneration. Second, administration of K channel blockers after noise exposure also significantly reduced noise-induced hearing loss and cochlear synaptopathy. Finally, because ATP purinergic P2x receptors play a critical role in the recycling K+ to enter the cells in the cochlear supporting cells (Zhu and Zhao, 2010, PMID: 20806014), we further tested if the administration of agonist of P2x receptors could attenuate noise-induced hearing loss and cochlear synaptopathy.
Conclusions: The data demonstrated that administration of K channel blockers in vivo can attenuate noise-induced hearing loss and cochlear synaptopathy. The data further support our previous report that noise-induced K+ excitotoxicity is a major contributor for the noise-induced cochlear synaptopathy. This study also reveals that K channel blockers are potential agents for prevention and treatment of noise-induced cochlear synaptopathy and hearing loss.
Supported by NIH R01 DC 017025 to HBZ

SA161. Supporting Cells in Ears Devoid of Hair Cells
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Category: Inner Ear: Drug Delivery
Background: The auditory epithelium contains sensory cells (hair cells) and several sub-types of non-sensory cells. The development of the non-sensory cells and their molecular characteristics have been characterized in normal cochleae but the changes they undergo after hair cell loss are less well understood. The non-sensory cells in deaf ears can serve as a substrate for several therapeutic approaches such as neurotrophin gene therapy or transdifferentiation therapy to induce hair cell regeneration. Therefore, it is important to characterize the molecular markers, morphology, and ability to express viral mediated transgenes in the non-sensory epithelium after the degeneration of hair cells. Effects of adenoviral vectors are of special interest because they are efficient at infecting the non-sensory cells in mature cochleae.
Methods: The experiments used mature Pou4f3-DTR mice and guinea pigs. We used adenoviral vectors because they efficiently infect non-sensory cells when injected into the endolymph in normal (intact) cochleae. DTR mice were injected with an adenoviral vector at the same time as diphtheria toxin (DT), or 1 or 3 months following DT. For histology, we used whole-mount preparations and plastic sections, and assessed reporter gene expression, hair cell survival and non-sensory cell structure. Guinea pigs were injected with neomycin into scala tympani and whole-mounts of their cochleae were assessed for hair cell loss and expression of Sox2, a non-sensory cell marker, 2 weeks later. Data were compared to previous results for normal ears.
Results: In DTR mouse ears, non-sensory cells remained tall until 3 months following DT. Adenoviral vectors expressed reporter genes when they were injected at the same time as DT or several months later, but gene expression was absent when the virus was injected 1 to 4 weeks after DT. In guinea pigs deafened by neomycin, flat epithelium was seen 2 weeks later in the base to mid-3rd turns. In the upper 3rd turn and

the apex, non-sensory cells were not flat and some hair cells survived. Adenoviral vectors could infect the flat epithelium. The flat epithelium cells lost expression of Sox2 by 2 weeks after neomycin treatment.
Conclusions: The loss of hair cells leads to morphological and molecular changes in non-sensory cochlear cells. It is unclear why the nonsensory cells of the deaf DTR mouse become refractory to adenovirus gene expression. It is also unclear why the guinea pig flat epithelium cells lose Sox2 expression. To better apply molecular therapies to non-sensory cells in deaf ear, it is necessary to better characterize how the loss of hair cells affects their molecular composition, and to distinguish between direct effects of the deafening agent (DT or aminoglycosides) versus consequences of the loss of the hair cells.
Supported by the HRP and NIH-NIDCD grant R01-DC014832

SA162. Open Board

SA163. Proof of Concept of a New Technique of Intracochlear Administration by Laser-Assisted Bioprinting
Manon Jaffredo*[1], Océane Duchamp[2], Nicolas Touya[3], Didier Dulon[4], Raphaël Devillard[5], Damien Bonnard[5]
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Category: Inner Ear: Drug Delivery
Background: Transtympanic administration is used clinically for the injection of gentamicin and/or corticosteroids. This atraumatic route is based on passive diffusion through the round window membrane. The main limitation of this method is related to the clearance through the Eustachian tube, making the concentration of the therapeutic agent at the intracochlear level uncertain and limited. Moreover, this technique remains unsuitable for molecules of high molecular weight or in the case of gene therapies. The goal is to study a new technique of intracochlear administration in an atraumatic, direct and controlled manner by laser-assisted bioprinting.
Methods: Laser-assisted bioprinting is based on a pulsed laser source directed on a quartz slide coated with a thin absorbing layer of gold and a layer of ink (donor slide). The interaction of the laser on the gold layer triggered the formation of droplets from the ink to the substrate. The ink is composed of thermo-reversible poloxamer 407, dexamethasone phosphate and fluorescent microspheres (to localize the droplets). The regularity and homogeneity of the pattern and the quantity of fluorescent microspheres were analyzed in vitro to validate the bioprinting parameters. Then, bioprinting was performed in vivo on anesthetized mice on which the round window was surgically exposed. The diffusion in vivo of the dexamethasone into the perilymph has been assessed by ELISA. Auditory function after bioprinting was evaluated by recording the auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) in 3 groups of mice: control (unoperated mice), sham (surgery only) and laser assisted-bioprinting (mice having undergone the surgery and bioprinting).
Results: The droplets of ink were well bioprinted on the round window in anesthetized mice and the ELISA analysis confirmed the diffusion of dexamethasone in the perilymph after one hour application (27.6±16.8 µg/mL, N=6). ABR recordings showed no statistical difference in the click threshold at 5, 12 or 19 days post-operation between the 3 groups (on average threshold less than 35 dB SPL, N=3 for each group) suggesting that bioprinting does not induce significant cochlear damage. The wave I latency was similar between the 3 groups for all time points. DPOAE were still present after bioprinting, similar to controls at 5 and 19 days post-operation, but slightly reduced at 12 days post-operation.
Conclusions: In conclusion, these results confirm that the laser-assisted bioprinting can be used to deliver a substance of interest in intracochlear compartment in an atraumatic and controlled way. Overall, this study is very encouraging to pursue further our experiments to develop medical device that can be used in humans.

SA164. In Vivo Assessment of Dexamethasone (DXM) Infused and Coated Poly(lactic-Co-Glycolic) Acid (PLGA) Microneedles as an Intracochlear Drug Delivery System
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Category: Inner Ear: Drug Delivery

Background: Drug delivery to the inner ear is challenging because of the complex cochlear anatomy that hinders successful molecular transportation. The use of biodegradable drug-infused microneedles inserted through the round window membrane (RWM) to deliver drugs directly to the cochlea is a promising approach for drug delivery to the inner ear at a constant rate overtime.
Methods: Microneedles were engineered in an elastomer mold created via photolithography as described in our published manuscript. Poly lactic-coglycolic acid (PLGA) copolymer was dissolved in dimethyl sulfoxide (DMSO) with or without dexamethasone (DXM), a corticosteroid used to minimize hair cell (HC) loss from inner ear trauma. To assess the success of microneedle insertion and corresponding drug delivery, DXM microneedles were introduced into the scala tympani through the RWM via a unilateral retroauricular approach of adult rats previously exposed to an ototoxic drug (ethacrynic acid) via intratympanic injection. Bilateral hearing was assessed using auditory brainstem responses (ABRs) to pure-tone stimuli (1, 4, 8, 16, and 32 kHz) pre-operatively and at post-operative days 3, 7, 14, 21, and 28. Animals were euthanized 28 days after microneedle insertion for cochlear harvesting and histologic analysis under confocal microscopy (hair cell counts).
Results: ABR data revealed an initial increase in hearing thresholds on the side of microneedle insertion, particularly at higher frequencies (i.e. 16 and 32 kHz) as compared to the contralateral control ear. However, hearing thresholds of those ear receiving the microneedle blended and coated with DXM significantly improved over the course of 28 days when compared to those animals undergoing insertion of pure PLGA microneedles. Similarly, histologic analysis demonstrated a decrease loss of hair cells in those ears exposed to DXM-infused and coated microneedles.
Conclusions: Biopolymeric microneedles are safe to use in the inner ear. DXM-infused microneedles may protect against insertion trauma, with hearing thresholds significantly improving over 28 days after surgical treatment. Biodegradable polymeric microneedles represent a novel strategy for inner ear DXM delivery without introduction of toxic foreign agents.

SA165. Microneedle-Mediated Injection of Gadodiamide Through the Round Window Membrane to Support Diagnosis of Ménière's Disease Using Magnetic Resonance Imaging

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Category: Inner Ear: Drug Delivery
Background: Ménière's disease is characterized by endolymphatic hydrops, which can only be definitively diagnosed via postmortem histopathological analysis. Clinical diagnosis is based on criteria including vertigo, sensorineural hearing loss, tinnitus, and other aural symptoms. More recently, magnetic resonance imaging (MRI) has been proposed as a method for the diagnosis of endolymphatic hydrops. In this study, we investigate the use of microneedle-mediated intracochlear injection of gadodiamide, followed by MRI, as a means to delineate the endolymphatic space in a guinea pig model.
Methods: 100 µm-diameter microneedles were synthesized using two-photon polymerization lithography and mounted onto 30-gauge blunt Hamilton needles. The tympanic bulla of a Hartley guinea pig was opened to expose the RWM. A microneedle was advanced into the tympanic bulla to perforate the RWM. After perforation, 1.0 µl of 10 mg/mL gadodiamide (Omniscan) in artificial perilymph was injected into the cochlea at a rate of 1.0 µl/min. The animal was euthanized after injection and transported to a Bruker Biospin 9.4 T small animal MRI. Multiple scans were conducted using a T1-weighted contrast-enhanced MRI over the course of 4 hours, with the first scan beginning at 90 minutes post-injection. The animal was positioned on its left lateral side throughout surgery and scanning to minimize reflux of gadodiamide from the inner ear. The contralateral cochlea was used as a control.
Results: Gadodiamide contrast reached the basal and middle turns of the cochlea within 90 minutes after injection through the RWM and reached the apex of the cochlea within 5 hours after injection. The perilymphatic spaces (i.e. scala tympani and scala vestibuli) were contrast-enhanced and could be distinguished from the non-enhanced endolympatic space (i.e. scala media) as early as 90 minutes after injection.
Conclusions: Microneedle-mediated intracochlear injection of gadodiamide contrast may be useful to delineate the endolymphatic space from the perilymphatic space. Thus, this method may be useful for the

detection of endolymphatic hydrops, which can then be used to inform a diagnosis of Ménière's disease. Injection of contrast directly into the inner ear may decrease the lead time from contrast administration to scan and make MRI more feasible as a diagnostic test for endolymphatic hydrops.

SA166. Differential Analysis of Perilymph, CSF and Vestibular Schwannoma Proteome in Patients Grouped by Hearing Function

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Category: Inner Ear: Membranes and Fluids

Background: Surgical treatment of sporadic vestibular schwannoma (VS) threatening the brainstem is one of the rare situations when samples of the inner ear fluids can be safely collected in vivo from patients. Protein content of perilymph was in the 1970 reported as increased in VS patients and later temporal bone studies reported that a VS may cause degeneration of the cochlear nerve ganglion, fewer axons to the hair cells, loss of stereocilia and degeneration of stria vascularis and spiral ligament. A perioperative electroacoustic study reported the site of hearing impairment in VS patients to be in 33% of the patient's pure cochlear impairment, 13% pure retrocochlear impairment and 54% both. These findings indicate a tumour effect on the microenvironment in the cochlea.
The aim of this study was to explore the protein composition of perilymph, cerebrospinal fluid (CSF) and tumour tissue in patients with normal to mild hearing loss in contrast to patients with moderate to severe hearing loss.

Methods: We included 32 patients recommended translabyrinthine surgery with no previous radiation therapy and post-operative histopathological confirmation of vestibular schwannoma. To exclude other causes of hearing loss were patients with known sensorineural hearing loss in their contralateral ear excluded, yielding 22 patients in the analysis. Samples were analysed in a Liquid chromatography tandem mass spectrometry (LC-MS/MS) and database search was done in MaxQuant using the UniProt database. NormalyzerDE was used to calculate differential expression using Limma powers differential expression ant to correct for possible batch effects was LC-MS/MS batch used as covariate.

Results: We found the expression profile differed in 26 proteins in the perilymph. In the moderate to profound hearing loss group were seven perilymph proteins enriched. Three were immunoglobulins and two complement C3 and C8. The other two were calcium binding protein S100-A6 and actin binding tropomyosin α4 chain. Protein profile in CSF and VS are under analysis.

Conclusions: The results indicate a higher expression level of immunoglobulins and complement activation in perilymph among vestibular schwannoma patients with moderate to severe hearing loss. This raises the question whether tumour induced inflammation in the cochlea affects the hearing function in patients with sporadic VS. These findings may direct future research and pave the way into possible hearing preservation therapies.

SA167. Recurrent Circuits Amplify Corticofugal Signals and Drive Feed-Forward Inhibition in the Inferior Colliculus

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Category: Midbrain: Structure and Function

Background: Descending auditory cortical projections target the inferior colliculus (IC) and are thought to provide top-down control of ascending acoustic signals. This corticofugal activity often leads to a net inhibitory effect, with implications such as sharper tuning or sparser neuronal activity. However, auditory cortico-collicular neurons are predominately glutamatergic (Feliciano and Potashner, 1995; Saint Marie, 1996), and anatomy data show that they primarily target the local IC glutamate neurons (Nakamoto et al., 2013; Chen et al., 2018). Thus, the mechanisms via which the auditory cortex generates inhibition in the IC are unclear.

Methods: We combined in vivo and in vitro patch-clamp electrophysiology, transgenic mice, optogenetics and pharmacology to determine how auditory corticofugal inputs drive IC inhibition. We applied in vivo whole-cell recordings to test how mouse IC neurons respond to optogenetic (n=21 cells) or electric (n=7 cells) stimulation of auditory cortex. We next crossed VGAT-ires-cre and Ai14 mouse lines to target whole-

cell patch clamp recordings from GABAergic (VGAT+) and presumptive glutamatergic (VGAT-) neurons in the dorsal shell region of the IC. We expressed the optogenetic activator Chronos in auditory cortex and optogenetically activated corticofugal fibers in IC brain slices using single light flashes and train stimuli. This approach enabled us to compare the amplitude and dynamics of corticofugal activity in specific IC neurons.
Results: In vivo recordings revealed some IC neurons respond with a brief excitatory post-synaptic potential (EPSP) followed by an inhibitory post-synaptic potential (IPSP) after optogenetic (n=6/21 cells) and electric (n=4/7 cells) auditory cortex stimulation, similar to previous studies (Mitani et al., 1983; Syka and Popelář, 1984). In vitro, optogenetic stimulation of auditory cortico-collicular fibers with single light flashes generated larger amplitude EPSPs in IC VGAT- neurons compared to VGAT+ neurons. These results agree with anatomy data suggesting that corticofugal projections primarily target glutamatergic IC neurons. Interestingly, train stimulation of corticofugal projections drove spikes in a subset of presumptive glutamatergic neurons and could triggered large amplitude, disynaptic EPSPs in 24/44 of GABA neurons. These data suggest that GABA neurons pool excitatory input from glutamatergic IC neurons with local axon collaterals. This prediction was further tested via circuit mapping experiments and pharmacological dissection.
Conclusions: We suggest that corticofugal driven inhibition of IC neurons is supported in part by local IC glutamate neurons that provide feedforward excitation onto GABAergic IC neurons during repetitive corticofugal activity. This novel circuit mechanism may contribute to classic in vivo observations that auditory cortex activity sharpens the selectivity of IC neurons to a variety of acoustic features (Zhou and Jen, 2007).

SA168. The Ventral Tectal Longitudinal Column: A Midbrain Nucleus for Modulation of Auditory Processing in the Cochlear Nucleus, Superior Olive and Inferior Colliculus

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Category: Midbrain: Structure and Function
Background: Saldaña, Fitzpatrick and colleagues identified a ventral tectal longitudinal column (TLCv) that receives input from the superior olivary complex (SOC) and the inferior colliculus (IC) and projects to the SOC (doi.org/10.1523/JNEUROSCI.1892-07.2007). TLCv cells respond to acoustic stimuli, consistent with a role in auditory feedback to modulate acoustic processing in the SOC. Cytoarchitectural evidence suggests TLCv may be present across mammals, but connectional and physiological data have been obtained only in rats. Here, we identify a TLCv in mice and show connections like those described in rats plus newly discovered TLCv projections to the cochlear nucleus (CN) and the IC.
Methods: We used adult mice of either sex (C57BL/6J or C57/CBACaJ mixed background). In the first experiment, we injected fluorescent retrograde tracers into the IC, CN or SOC, then identified retrogradely-labeled cells. In a second experiment, we injected AAV2-hsyn-EYFP into the IC to label IC axons. After 5-7 days for retrograde experiments or 4 weeks for AAV experiments, brains were fixed by perfusion and sectioned for fluorescence microscopy.
Results: Retrograde labeling from the SOC or the CN revealed a column of cells from near the midline just dorsal to periaqueductal gray and extending rostro-caudally through the levels of the IC and the SC. In both sets of experiments, the cell labeling in TLCv was bilateral with a strong ipsilateral predominance. A third series of experiments, with retrograde tracer deposited in the IC, also labeled TLCv cells. The cells in these experiments were fewer than after SOC or CN tracer deposits, but once again showed a bilateral distribution with an ipsilateral dominance. Thus, our retrograde results show a TLCv projection to the SOC like that in rats and additional projections to both the CN and the IC.
Anterograde labeling revealed inputs to the TLC from the IC and from the SOC. The projections from both sources terminate throughout the ipsilateral TLCv, and less so in contralateral TLCv.
Conclusions: Anatomical connections confirm the presence of a TLCv in mice. A prominent projection to the SOC as well as inputs from the IC and the SOC suggest strong similarities between mice and rats. Our discovery of additional outputs from the TLCv to the CN and to the IC substantially broaden our view of the likely impact of TLCv projections. Together, these results suggest a broad role for the TLCv in integration of ascending auditory information and feedback modulation of auditory processing in the cochlear nucleus, superior olivary complex and the inferior colliculus.
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SA169. Microglia Refine Early Multisensory Circuits in the Lateral Cortex of the Inferior Colliculus in a Complement-Dependent Manner

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Category: Midbrain: Structure and Function

Background: Microglia cells (MGCs), the resident macrophage of the brain, play a pivotal role in synaptic pruning of newly established circuits during early critical periods. Classical complement cascade signaling enables MGCs to identify unnecessary or underutilized contacts and specifically target them for removal and subsequent degradation. Such recognition and engulfment behaviors require receptor-ligand interactions, whereby "eat me" tags (complement component 3, C3) bind their cognate receptor (complement receptor 3, CR3) expressed on MGCs. Compromised complement signaling in other systems often results in under-pruning or unrefined map configurations, and is thought to underlie certain autism-related behavioral phenotypes (e.g. sensory hypersensitivities, decreased social interaction, increased repetitive behaviors). The lateral cortex of the inferior colliculus (LCIC) is a compartmentalized midbrain center that receives segregated inputs from different sensory modalities. Somatosensory afferents target a series of modular domains, while auditory afferents terminate throughout an encompassing matrix. Despite the fact that auditory and multisensory processing deficits are some of the most reliable early indicators and severity predictors of autistic symptoms and communication difficulties, the role of MGCs and complement signaling in sculpting multisensory circuits has yet to be addressed. Thus, the present study aims to: (1) characterize the time course of C3 expression in the developing LCIC, (2) correlate CR3/CD11b-positive MGC patterns with any observed changes in C3 staining, and (3) determine if mice incapable of complement signaling (CR3KO) exhibit any abnormalities in the described expression patterns.

Methods: C3 and CD11b (a subunit of the heterodimeric CR3) immunostaining were performed in GAD67-GFP knock-in and CR3KO mice at developmental stages throughout the established LCIC critical period (P0, P4, P8, P12) and thereafter (P36). Double-labeling experiments in GAD-67 GFP mice facilitated characterization of expression patterns with respect to emerging LCIC compartments (i.e. GAD-positive modules). C3 staining was combined with GAD immunocytochemistry in CR3KO mice to determine whether observed changes in C3 expression were complement-dependent. Widefield epifluorescence imaging was performed on a Nikon Eclipse Ti-2 microscope. An extended depth of focus (EDF) algorithm was used for two-dimensional renderings of acquired Z-stacks.

Results: Both C3 and CD11b are expressed in the LCIC during its critical period and downregulated shortly thereafter. C3 expression is initially homogeneous with some CD11b-positive MGCs already present in the LCIC. At P8, CD11b-expressing MGCs aggregate within modular domains, coincident with the appearance of spatially matched voids in C3 labeling. As CD11b-positive microglia occupy the surrounding matrix by P12, compartmental-specific disappearance of C3 is again observed. CR3 mutants with compromised signaling lack the timely and reliable progression of C3 loss, suggesting compartmental LCIC pruning is complement-dependent.

Conclusions: These findings implicate MGCs as potential key players in sculpting early LCIC circuits and that deficits in complement pathway signaling may yield unrefined multisensory network maps.

SA170. The Effects of the Candidate Tumor Suppressor ECRG4 on Middle Ear Immunomodulation

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Category: Middle and External Ear

Background: Otitis media (OM) is the most common pediatric disease. 10-15% of children exhibit chronic illness, indicating a failure of the response to infection and persistence of pathophysiology. We used in vivo mouse models to understand the molecular regulation of immunity in the middle ear (ME).

Methods: The MEs of mice were infected with the human otopathogen nontypeable Haemophilus influenzae (NTHi) to induce OM. ME responses were characterized by histology, qPCR, immunoprecipitation, and Western blotting. To assess changes in gene expression at various time points following infection, Affymetrix mRNA microarray analysis and single-cell RNA-Seq were used. Potential intergene relationships were evaluated by STRING enrichment analysis.

Results: Analysis of the murine ME transcriptomes during OM showed strong downregulation of the tumor suppressor gene Ecrg4 which was inversely related to mucosal hyperplasia, and identified stromal cells as

the primary ECRG4 source. The reduction in Ecrg4 gene expression coincided with the cleavage of ECRG4 protein to release the extracellular fragment augurin, which may mediate effects on the epithelium. The duration of mucosal hyperplasia during OM was greater in Ecrg4-/- KO mice, the number of infiltrating macrophages was enhanced and ME infection cleared more rapidly. ECRG4-null macrophages showed increased bacterial phagocytosis. ME expression of genes related to mucosal growth were increased in the KO, while those related to innate immunity were broadly decreased. In particular, Hbegf and Ereg were dramatically more upregulated in ME epithelial cells 6 hours after NTHi bacterial infection in the Ecrg4-/- KO, indicating that ECRG4 normally acts to suppress expression of these growth genes. In contrast, genes related to innate immune receptors, cytokines, and the synthetic enzymes of the lipid leukocyte chemotactic factor LTB4 were significantly lower at 6 hours in the KO, suggesting that ECRG4 normally stimulates their expression during infection.
Conclusions: Taken together, these studies suggest that ECRG4 is a major regulator of the ME response to infection, inhibiting mucosal hyperplasia during infection, but stimulating innate immunity. Because Ecrg4 expression is primarily limited to stromal cells, its effects on other tissues is likely to be mediated by augurin released in response to infection. Modulating ECRG4 or its effectors could be useful in the treatment of chronic OM.

SA171. Efficacy of Topical Intranasal Corticosteroids in Relieving Clinical Signs of Eustachian Tube Dysfunction: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

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Category: Middle and External Ear
Background: Eustachian tube dysfunction (ETD) is a highly prevalent condition both in children and adults and is widely accepted as the underlying cause of common clinical signs and symptoms such as negative middle ear pressure (NMEP), otitis media with effusion (OME), hearing loss, and otalgia. Tympanometry has very high sensitivity (84-93%) and is an objective measure for diagnosis of ETD. Treatment for this condition is especially important in young children, for whom persistence can interfere with learning and development. The use of topical intranasal corticosteroids (INCS) for ETD therapy remains controversial given conflicting evidence. Current clinical guidelines instead recommend watchful waiting until the need arises for invasive, costly procedures, such as tympanostomy tube placement. The aim of this study is to 1) systematically review randomized controlled trials (RCTs) assessing the efficacy of topical INCS for ETD treatment, and 2) conduct a meta-analysis of available tympanometry data.
Methods: Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, a standardized search query was conducted in the following electronic databases: PubMed, EMBASE, Web of Science, and The Cochrane Library. Criteria for inclusion: RCTs assessing the effect of topical INCS in adults and children of any age, clinically diagnosed with ETD or OME. No restrictions were set for control treatment. Screening was performed independently through Rayyan by two reviewers. A modified version of Cochrane's data collection form for RCTs was used for data extraction. A standardized critical appraisal tool (Cochrane's RoB 2) was used for risk of bias assessment. A meta-analysis of proportions was conducted for available data on the binary outcome measure of tympanogram normalization.
Results: Initial pooled results (n=330) underwent title/abstract screening, and eligible papers (n=21) underwent full text review to yield the final included RCTs (n=8). Studies ranged in size from 59 to 217 participants, with mean age ranging between 3.8 – 41.7 years. INCS sprays assessed were mometasone (n=4), beclomethasone (n=3), and triamcinolone acetonide (n=1). In a meta-analysis of 512 pooled ears, there was no significant difference in the overall proportion that recovered from baseline Type B/C to Type A tympanogram (OR 1.22, 95% CI 0.80–1.84) between ETD patients receiving INCS and those receiving control treatment.
Conclusions: Study results do not provide supportive evidence for the use of INCS in ETD. However, data obtained through this systematic review was small in quantity and on average low in quality. This reveals a need for larger, higher quality randomized controlled trials with thorough subgroup analyses to more rigorously address this contention in ETD medical management. Until more sufficient data is obtained,

current clinical recommendations of avoiding INCS for treatment of ETD sequela remain acceptable and should be more strictly enforced.

SA172. Rat Model of Otitis media: Comparison Between the Transtympanic and Transbullar Methods for Bacteria Inoculation

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Category: Middle and External Ear

Background: Literature regarding animal models of otitis media is scarce. Animal models such as cat, monkey, and chinchilla have been successfully used. However, due to regulatory issues, the use of these models has been discouraged. We aimed to evaluate the use of a rat model for creating acute otitis media and to compare the transtympanic with the transbullar methods for bacterial inoculation.

Methods: 12 rats were assigned to two groups (transtympanic, n=6; transbullar, n=6). Animals were anesthetized with ketamine HCL (100mg/kg) and xylazine (5mg/kg). In the transtympanic group, a tuberculin syringe with a 27-gauge needle was used to inoculate 50µl of 107 colony forming units (CFUs) of S. pneumoniae type 3 in both ears of the animals. In the intrabullar group, a post-auricular incision was made with a scalpel with a 15 blade, and bullae was exposed by blunt dissection. Bullae was identified by digital palpation. A 27-gauge needle was used to inoculate 50µl of 107 CFUs of S. pneumoniae type 3 in both ears. Post-procedure analgesia was performed using buprenorphine (0.01mg/kg) once daily. Half of the animals in both groups (n=3) were sacrificed 48h post inoculation, and the remaining animals (n=3) at the 96h timepoint. Following euthanasia, temporal bones were harvested, and bullar effusions were collected using a 27-gauge syringe. The middle ear effusions were then put on ice, then evaluated for number of CFUs.

Results: Two rats died throughout the study - one (transbullar group) during anesthesia and one (transtympanic group) 24h following injection. At the 48h-timepoint, all animals presented opaque tympanic membranes at examination. As two animals deceased in the first 48 hours, only 2 animals were sacrificed per group. We could not collect sufficient effusion from any of the bullae to allow counting the CFUs. At the 96h-timepoint, we observed clear signs of otitis media (red, bulging tympanic membranes) in 5 of 6 ears (83%) of the transtympanic group versus 4 of 6 ears (66.6%) in the transbullar group. Middle ear effusion was successfully collected from all 12 ears. There were no differences in the number of CFUs in the effusion from both groups (transtympanic, 3.97E+05; transbullar, 3.12E+0.5; P=0.25).

Conclusions: Both methods were effective for creating acute otitis media in rats. Effusion started to accumulate after the 48h-timepoint, being present in all ears at the 96h-timepoint. The transbullar injection is technically more challenging: the bullae of the rats are small and difficult to identify through direct inspection and palpation. On the other hand, the transtympanic approach is advantageous as (1) does not require a skin incision, reducing the risks of postoperative infection of the incision site; and (2) allows direct visualization of the penetration of bacteria in the middle ear through the transparent tympanic membrane.

SA173. Open Board

SA174. Chronic In-Vivo Test of a Stent Developed for the Human Eustachian Tube

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Category: Middle and External Ear

Background: Eustachian tube dysfunction (ETD) is a common disorder characterized by the impairment of, or permanent damage to, at least one of the following main functions: pressure equalization, mucus transport, and protection from ascending pathogens. Chronic ETD has a direct effect on middle ear health and can cause discomfort, severe pain and, in some patients, loss of hearing which requires long-term treatment. To address these issues, a nitinol stent (with its shape adapted to fit the human Eustachian tube (ET)) was developed and tested in vivo in Blackface sheep.

Methods: Three groups of N=8 adult, female Blackface sheep (with observation periods of 3, 6 and 12 months respectively) were unilaterally implanted with a stent (3-5 mm x 14 mm). Stent insertion was completed via a transnasal approach, using a specially developed application tool under constant endoscopic

control. Tympanometry was used prior to stent insertion to confirm normal ET function. Weekly tympanometric measurements were performed throughout the study period to observe the status of the middle ear. All animals underwent endoscopic examinations at the halfway point and at the end of the follow-up period to monitor the eardrum and the pharyngeal opening of the ET. As a final step, cone-beam computed tomography (CBCT) and histological analysis were conducted.
Results: None of the animals showed signs of discomfort or specific deviations in their clinical health status. Stent dislocation during the study period occurred in 1/24 cases. Tympanogram results recorded for the 3-month group were: 94% type A, 2% type B and 4% type C curves, while controls (without stent) produced 80%, 13% and 7% respectively. Results for the 6-month group were: 56% type A, 28% type B and 16% type C, with results for control ears recorded as 84%, 14% and 2% respectively. Within the 12-month study group, the results were recorded as: 61% type A, 31% type B and 8% type C, while control ears produced 91.55%, 0.25% and 8.21% respectively. Endoscopic investigations showed mild secretion from the pharyngeal ET opening in some animals. CBCT data revealed that all stents except one (incorrect placement) were fully opened and exhibited a close to circular shape. Initial histology data show epithelialization of the stent but also granulation tissue formation resulting in reduced stent lumen in some cases.
Conclusions: The stent appears to be suitable as a future treatment option for ETD. However, training is crucial in order to achieve correct placement of the stent in the ET.

SA175. The oZ Method to Measure Wide-Band Reflectance in Less Than Two Seconds

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Category: Middle and External Ear
Background: Wide band reflectance (WBR) has emerged as a highly discriminating test of middle ear function and conductive hearing loss (CHL), but remains primarily a research tool that has not been adopted as the standard of care. One challenge has been the time and expertise required to calibrate and hermetically seal the probe into the ear canal. To avoid this, we developed a new method to measure WBR that does not require routine calibration or sealing of the probe into the ear canal. Measurements are made in an open ear canal using a device resembling an otoscope, but with miniature microphones arranged to facilitate mathematical separation of the forward acoustic stimulus from the reverse reflection/ emission. The ratio of the two provides the reflection coefficient, from which the input impedance and length of the ear canal can be determined. We call the measurement oZ, with reference to otoacoustic impedance.
Methods: WBR can be measured using the oZ approach in the time or frequency domain. The method works by measuring the pressure inside a speculum probe inserted into the ear canal and calculating the forward and reverse traveling waves based on the pressure distribution (forward-reverse decomposition, FRD). The frequency-domain calculation is similar to the standard 2-microphone methods used to test sound absorption of acoustic materials (e.g. ISO 10534-1), but based on 3 microphones. Frequency-domain oZ measurements were done using a variety of broad-band stimuli to measure WBR from 100Hz-16kHz in less than 2 seconds without sealing the canal. Time-domain FRD was done using a novel algorithm based on time-dependent pressure differences between the three microphones. Use of 3 microphones renders both the time-domain and frequency-domain equations overdetermined, which we take advantage of to improve signal-to-noise and bandwidth. Additional methods were developed based on acoustics of the speculum-ear canal system to estimate impedance at the tympanic membrane. Preliminary data was collected in normal hearing adults and compared to measurements using alternative approaches.
Results: For sinusoidal stimuli, both the time- and frequency-domain methods provide the same forward wave, reverse wave and WBR. WBR results below 6 kHz fall within the range of normal hearing results measured by the standing wave method, the Thévenin equivalent method, and the acoustic intensity method. Below ~6kHz in adults, measurements in the speculum provide a good estimate of impedance at the tympanic membrane. Above 6kHz, the impedance miss-match at the speculum tip and subject-specific ear canal morphology influence the reflectance measured.
Conclusions: The oZ method provides ultrafast (<2 sec) WBR measurements from 100Hz to 16kHz, with results substantially equivalent to alternative methods below 6kHz in adults. The approach also provides a direct method to quantify the acoustic input to the ear that is valid over the entire frequency range of hearing range.

SA176. Temporal Course of Visual Modulation Revealed by Auditory Evoked Potentials in the Cat

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Category: Multisensory Processing/Interactions

Background: Visual modulation of the auditory system not only serves multisensory processing in hearing individuals, but may also underlie the cross-modal plasticity identified in deaf individuals, where auditory cortical areas no longer receiving acoustic inputs are re-purposed for visual functions. Event-related potentials (ERPs) studies in humans have provided evidence of multiple-stage audiovisual interactions, ranging from tens to hundreds of milliseconds following the presentation of stimuli. In addition, the temporal disparity, or stimulus delay, of the two modalities has also been shown to affect the behavioral and neural measurements of audiovisual processing in a complex and non-monotonous way. The periodicity in the temporal course of visual modulation derived from such delay dependency suggested a "phase resetting" hypothesis, which was proposed based on extracellular recordings in non-human primate primary auditory cortex. However, it is still unknown if the temporal course of visual modulation in the auditory system can be characterized in animal models using ERPs.

Methods: EEG signals were recorded in a total of 14 cats under dexmedetomidine sedation. The auditory stimuli (clicks) and visual stimuli (flashes) were timed by two independent Poison processes and were presented either simultaneously or alone. EEG signal was recorded from subdermal needle electrodes. Before the averaging process, two off-line band-pass digital filters (1-30 Hz and 10-300 Hz) were applied for long-latency and middle latency responses respectively. The ERPs from the visual-only condition were subtracted from the audiovisual condition before the comparison to the ERPs from the auditory-only condition.

Results: Grand-averaged ERPs showed significant visual facilitation in the amplitudes of the P1 component at the 1-30 Hz band and the P22 components at the 10-300 Hz band. In individual subjects, significant visual modulation of both directions were found in N1 and N40 components. Further analysis demonstrated SOA-dependent temporal patterns for the visual modulation in different components. The largest facilitation in the P1 component was associated with a visual-to-auditory delay of ~250-ms. Finally, our preliminary spectral analysis revealed a periodic visual effect at a rate of 8-15 Hz.

Conclusions: Visual modulation of auditory processing can be successfully evaluated in cats under dexmedetomidine sedation using auditory evoked potentials, although a large individual variation was observed in some peak components. The periodic temporal pattern can be interpreted with "phase resetting" hypothesis. Future studies can further characterize the roles played by visual modulation in the deaf and the restored auditory system.

SA177. Interdisciplinary Sensory Disorders Program: Development of an Innovative Clinical and Research Model for Precision Medicine

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Category: Multisensory Processing/Interactions

Background: Untreated sensory disorders significantly impact quality of life and have been linked to worsening of medical illnesses and impaired immunity. Sensory disorders often serve as early manifestations of many neurological diseases such as Parkinson's, Alzheimer's, and COVID-19. The University of Miami Interdisciplinary Sensory Disorders Program (UMISDP) was established to provide high quality patient care, top-caliber academic training, and translational research. This multidisciplinary team incorporates otolaryngology, psychiatry, psychology, neurology, ophthalmology, genetics, molecular/cellular physiology, and epidemiology/public health to focus on the advancement of clinical care for individuals with sensory disorders, and translational research to improve outcomes in this population. This abstract describes our service delivery model and innovative research protocol for our newly established UMISDP.

Methods: We have a highly interactive team that individually represent unique strengths in many areas of sensory disorders, ranging from basic sciences to patient care. The large multi-ethnic and racially diverse South Florida population is ideal for the initial development of treatment and intervention initiatives which can scale globally. Patients complete intake questionnaires followed by smell, taste, and vision testing, and

cognitive evaluation. Patients also undergo a comprehensive audiovestibular evaluation. All results are reviewed by a research nurse and/or physician in combination with a physical exam to determine if outside referrals are needed. Lastly, blood is drawn for genetic testing, which supports our clinical and research databases.
Results: The UMISDP was established through collaborations with several departments, including Otolaryngology, Bascom Palmer Eye Institute, Public Health, and the Center for Cognitive Neuroscience and Aging (CNSA). The multi-tiered approach for management of patients with sensory disorders will be presented. Our experienced team are trained in management of sensory disorders and have developed referral pathways to collaborating departments. Our UMISDP protocol ensures that we are screening patient's mental health, quality of life, and sleep, as they have been linked to poorer outcomes. Additionally, we will present our collaborative research model for individuals with cognitive impairments and associated sensory disorders. This partnership has led to several NIH funded research studies evaluating sensory impairments and cognitive decline. The UMISDP has established a minority focused Miami SDP Repository in older adults with mild Alzheimer's disease (AD), mild cognitive impairment (MCI), common and rare genetic hearing loss including Usher syndrome, age-related audiovestibular disorders, and cognitively normal adults (NC), a comprehensive MiamiOtogenomic screening pipeline including patient DNA/cell lines/inner ear tissue repositories, and a database of genomic variation and phenotypes.
Conclusions: Our established sensory disorder program within the Department of Otolaryngology and CNSA provides comprehensive and multidisciplinary care to underserved clinical populations. This successful program highlights the benefits of collaboration between researchers and clinical departments. The UMISDP aims to support the efficacy of similar programs in providing high quality care to patients with a range of sensory impairments.

SA178. Early Auditory- And Visual-Evoked Cortical Potentials Predict Auditory Speech-In-Noise Identification and Lipreading Ability in Older and Younger Adults

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Category: Multisensory Processing/Interactions
Background: Sensory loss can lead to the recruitment of more information by the other senses. As examples, cross-sensory recruitment is demonstrated when deaf individuals exhibit better lipreading ability and blind individuals exhibit more acute auditory spatial perception compared to normal hearing and sighted individuals, respectively. We have found that cross-sensory recruitment can even compensate for subtle variability in the early cortical processing of auditory information in younger normal-hearing adults, such that smaller auditory-evoked P1 amplitudes are related to poorer auditory speech-in-noise recognition and better lipreading. Older adults exhibit well documented deficits in auditory and visual neural processing and perception. Age-related deficits in auditory and visual neural processing may interact with auditory and visual perceptual outcomes, extending our past results to explain individual differences in auditory and visual perception in older adults.
Methods: A group of 37 younger adults (mean age = 23 years, SE=0.566) with normal hearing and a group of 37 older adults (mean age = 67 years, SE=1.158) with clinically normal hearing or mild-to-moderate sensorineural hearing loss participated in this study. All participants completed an auditory-visual speech identification task, identifying bisyllabic words either auditory alone (AO), visual alone (lipreading, VO), or audiovisually (AV). Stimuli were presented in three levels of Gaussian noise (-5, 0, and +5 dB SNR relative to auditory stimulus intensity). In a subset of these participants, P1, N1, and P2 peak amplitudes and latencies were measured from click-induced auditory-evoked potentials (AEPs). In another overlapping subset of participants, C1, P1, N1, and P2 peak amplitudes and latencies were measured for sine-gradient-induced visual-evoked potentials (VEPs). Linear mixed-effects regression (LMER) models were then used to determine how variability in speech identification was accounted for by auditory and visual neural processing.
Results: Across age groups, smaller auditory-evoked P1 peak amplitudes predicted poorer auditory speech identification in the most difficult listening conditions (β=0.676, SE=0.266, t=2.539, p=0.014) and better lipreading ability (β=-0.413, SE=0.202, t=-2.041, p=0.046). Across age-groups, larger visual-evoked C1 peak amplitudes predicted better lipreading ability (β=-0.378, SE=0.116, t=-3.261, p=0.002) and larger visual-evoked P1 peak amplitudes predicted better auditory speech identification.

Conclusions: We replicate our previous finding in younger adults that smaller auditory-evoked neural responses predict poorer speech-in-noise identification and better lipreading ability, but extend our previous results to now include older adults. These findings suggest that the young and aging brain can recruit information from across sensory modalities to accommodate for variability in auditory neural processing. Larger visual-evoked neural responses appear to positively relate to both lipreading ability and auditory speech-in-noise identification across age groups. These results will be discussed in relation to AV speech processing, age-related changes in neural plasticity, and age-related loss of inhibition.

SA179. The Impact of Temporally Coherent Multisensory Cues on Speech Detection and Recognition in Noisy Environments

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Category: Multisensory Processing/Interactions
Background: The inputs delivered to different sensory organs provide us with complementary information about the environment. A series of our previous studies demonstrated that presenting abstract visual information of speech envelopes substantially improves speech perception ability in normal hearing (NH) listeners (Yuan et al., 2020; 2021a; 2021b). The purpose of this study was to expand this audiovisual speech perception to the tactile domain. We tested a hypothesis that by utilizing either visual or vibrotactile input that is temporally synchronized with the speech envelope, we can enhance the listener's speech perception performance.
Methods: Twenty young NH adults (age 20-24 years) participated in measurements including two different levels of speech perception processing (detection and recognition) in four different sensory modalities (AO: auditory-only; AV: auditory-visual; AT: audio-tactile; AVT: audio-visual-tactile). For the detection test, the target speech level was fixed at 65 dB SPL, and the masker levels were adaptively varied to find masked thresholds. For the recognition test, the targets were fixed at 65 dB SPL and presented in various levels of the masker from -7 to 1 dB SNR. The amplitudes of both visual and vibrotactile stimuli were temporally synchronized and non-synchronized with the target speech envelope for comparison. Subjects were instructed to pay attention to the target sentences and make their answer. Sentence detection/recognition results in the multisensory stimulus conditions (AV, AT, and AVT) were compared to those in the auditory-only (AO) condition.
Results: Average results showed that adding temporally-synchronized multimodal (visual and/or tactile) cues to the auditory signal did provide significant improvements in speech detection (2 to 4 dB) and recognition (2 to 25%) abilities compared to audio-only stimulation. These multisensory speech perception benefits were reduced when the cross-modal temporal coherence characteristics were eliminated. Another interesting finding in this study is that visual and tactile stimuli additively stack in their influence on both speech detection and recognition performance, especially when their cues are temporally synchronized.
Conclusions: Our findings suggest that multisensory interactions are fundamentally important for different levels of speech perception processing in NH listeners. The outcome of this multisensory speech processing highly depends on temporal coherence characteristics between multimodal sensory inputs. This suggests that a multisensory integration process in speech perception requires salient temporal cues to enhance speech perception ability in noisy environments. Amplitude envelope, serving as a reliable temporal cue source, can be applied through different sensory modalities (i.e., visual and/or tactile) when the auditory ability is compromised.

SA180. Visual Motion Profile Has Minimal Effect on Visual-Vestibular Multisensory Integration

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Category: Multisensory Processing/Interactions
Background: Visual motion is ambiguous in that it can either represent external motion or self-motion through a fixed environment. Visual-vestibular integration is most advantageous during self-motion. It has previously been shown that visual-inertial integration occurs only when they have a consistent direction and good temporal alignment. The current experiment tests the hypothesis that the visual motion needs to have a motion profile consistent with the experienced inertial motion. To test this we use visual and inertial stimuli that could have different directions.

Methods: Ten healthy human subjects (mean age 20 ± 4 years, 7 female) experienced 2 seconds of translation which could be left or right of center. A 6-degree-of-freedom (6-DOF) motion platform was used to deliver the inertial motion stimuli while a 55" color display delivered the apparent visual motion. Inertial headings were paired with a synchronized 2 s duration visual headings that were presented at relative offsets of 0°, ±45°, ±60°, and ±75°. In some trials the visual motion was consistent with the inertial motion and in other trials it was inverted – it started at the peak velocity, decreased to zero mid stimulus, then accelerated back to the peak velocity.
Subjects judged the direction of the inertial heading as either left or right of midline. Visual-vestibular integration was determined by measuring the bias in inertial heading towards the visual stimulus.
Results: A visual optic flow stimuli biased inertial heading perception in the direction of the visual stimulus. When the velocity profile of the visual stimulus matched the velocity profile of inertial motion the inertial stimulus was biased 8.2 ± 2.1° (mean ± SE) with a 45° visual offset, 7.0 ± 1.7° with a 60° offset and 6.0° ± 2.2 ± with a 75° offset. When the visual stimulus was inverted so it was inconsistent with the inertial motion the respective biases were 5.4 ± 1.4°, 3.9 ± 2.0°, and 2.4 ± 2.0°. Thus, the biases with the inverted stimulus were significantly smaller ($p < 0.05$, t-test across all offsets), although the inverted visual stimulus still demonstrated the known pattern of decreasing influence as the relative offset to inertial heading increased. When the subjects were examined individually, there were three subjects with essentially no difference in the inertial bias towards the visual stimulus (<1°) between the two visual stimuli, while in the most extreme subject the difference was 10.3°. Thus, some subjects seemed to consider the velocity profile of the visual stimulus in multisensory integration while others did not.
Conclusions: The visual stimulus has a greater and more consistent effect when its velocity and acceleration match the inertial stimulus, but the effect was small and not present in all subjects.

SA181. BK Channels as a Therapeutic Target for Auditory Impairments in Neurodevelopmental Disorders: Preclinical Findings From a Mouse Model of Williams-Beuren Syndrome

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Category: Other, Auditory impairments and neurodevelopmental disorders
Background: Auditory impairments, such as hearing loss and hyperacusis (i.e., increased sound sensitivity), are commonly described in patients with neurodevelopmental disorders (NDDs) and often exacerbate the communication and social deficits typical of these pathological conditions. In particular, both hyperacusis and progressive hearing loss are frequently associated with Williams-Beuren syndrome (WBS), a rare genetic NDD, characterized by several neurobehavioral and cardiovascular alterations. However, the cause of auditory dysfunction in WBS is still largely unknown. Big Potassium (BK) channels play a crucial role in multiples aspects of auditory signaling and are known to be quantitatively and functionally altered in several NDDs. Hence, we tested the hypothesis that a reduction in BK channel expression and functionality underlies acoustic dysfunction in WBS and that the pharmacological activation of these channels may therefore represent an effective novel pharmacological approach.
Methods: Our preclinical study was performed on an animal model of WBS recapitulating the complete human chromosomic deletion (i.e., the CD mouse). We evaluated the behavioral (acoustic startle reflex) and electrophysiological (ABR, DPOAE) acoustic responses of CD mice and we analyzed their peripheral auditory structure by immunofluorescence staining. For the pharmacological studies, we selected the FDA-approved BK channel agonist chlorzoxazone (CHLOR) typically prescribed for muscular pathologies, which has been recently proposed also for treating neurodegenerative disorders in animal models. We combined acute and chronic (10 days) intraperitoneal injections of CHLOR with behavioral, electrophysiological and structural analyses. All the experiments were conducted on adult (3 months-old) CD mice and their WT littermates.
Results: CD mice exhibited both behavioral and electrophysiological abnormalities resembling those observed in WBS patients. Mutant mice showed reduced acoustic startle response and increased hearing threshold, indicating hearing loss. DPOAEs were significantly reduced in CD mice suggesting a marked loss of outer hair cells integrity, which was further confirmed by immunofluorescence analysis. We also found a significant reduction in the expression, but not in the functionality, of BK channels on inner hair cells.

Finally, we reported that both acute and chronic CHLOR treatments were able to rescue most of the abnormal auditory phenotypes of CD mice.
Conclusions: Our findings demonstrate that acting on BK channels is a valuable therapeutic strategy to treat acoustic dysfunction in WBS and provide preclinical evidence for future clinical applications of CHLOR in WBS research. They also suggest that peripheral BK channel dysfunction may play a role in the etiopathology of auditory impairments in the context of multiple neurodevelopmental disorders.

SA182. The Hearing Brain in Healthy and Concussed Division 1 Collegiate Athletes

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Category: Other, Auditory Processing
Background: Participating in a sport is a key component in building a healthy mind and body. Yet, sports injuries, particularly concussive and subconcussive head injuries, can be deleterious to the athlete brain. While the positive and negative effects of sports participation are well-documented in musculoskeletal, metabolic, cardiovascular, psychosocial, and cognitive domains, whether the effects of sports participation – both positive and negative – extend to auditory function is uncharted territory.
Methods: Frequency-Following Responses (FFR) to the speech stimulus /da/ were collected from NCAA Division I student-athletes annually: pre-season, post-season, and following a concussion. Data were gathered up to four years of university sports participation. Healthy-athlete data (i.e., pre-season and post-season) were compared to healthy, age-matched, non-athletes and analyzed with respect to the level of contact in their chosen sport. Concussion data were longitudinally compared to the same athlete's healthy data.
The FFR is a neurophysiological potential evoked by a complex sound, that captures micro-second fast processing of discrete sound ingredients, including the fundamental frequency (F0), and harmonics as well as a measure of background neural noise levels. Due to the response's similarity to the evoking stimulus, direct correlation between stimulus and response and assays of stability were also assessed.
Results: Compared to non-athletes, college athletes demonstrate "quieter" brains, explained by a reduction in neural noise that boosts the acoustic representation of speech sounds. In addition, they display better neural response consistency and improved harmonic encoding, especially in females.
When dividing athletes into contact and non-contact groups, we find that male contact athletes have smaller F0 response amplitudes compared to non-contact males and that, furthermore, their response size was inversely related to the number of years they cumulatively participated in a contact sport. This degradation of F0 encoding is similarly observed in acutely concussed athletes.
Conclusions: The auditory system is sensitive to the positive and negative effects of sports participation. Ongoing work is focused on furthering our understanding of these effects by looking across multiple years, sports, and positions within a sport.
Supported by NIH NS102500

SA183. Use of a Humanoid Nao Robot as an Interactive Interface for Auditory Testing With Normal-Hearing Adults and Cochlear-Implanted Children

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Category: Other, Auditory test interface, engagement
Background: Repetitive tasks in auditory testing may sometimes be challenging, potentially resulting in decreased participant engagement and test performance. Human-robot interaction (HRI) could provide an alternative test interface, potentially leading to benefits in engagement due to the novelty effect and collaboration with the robot. We propose the use of a humanoid NAO robot as an alternative interactive interface to conduct auditory tests, which are typically run on a laptop or through a computer and external speaker. We present two studies. In Study 1, we conducted auditory psychophysical tests with normal-hearing adults and analysed test performance and engagement, and in Study 2, speech audiometry tests were run with cochlear-implanted children and their engagement was analysed.

Methods: Study 1 investigated the robot implementation of two tests from the Perception of Indexical Cues in Kids and Adults test battery: voice cue sensitivity and voice gender categorisation. Thirty normal-hearing adults (19–38 years old) participated in the study. Participants performed each test twice, once on the laptop and once on the robot. Study 2 investigated the robot implementation of two speech audiometry tests widely used in the Netherlands: the digits-in-noise test and the Nederlandse Vereniging voor Audiologie phoneme lists in quiet. Both tests are typically scored by a clinician via correctly identified digits, producing a speech reception threshold, and phonemes, producing a percent correct speech recognition, respectively. Twenty-six cochlear-implanted children (4–15 years old) completed at least one of the two tests using the robot during a clinical visit. Interactions in each study were video-recorded by two cameras, one capturing body postures and one focusing on facial expressions. The behavioural analysis of the HRIs was performed by three and two independent coders for Studies 1 and 2, respectively. Engagement was measured through quantification of smile and laughter frequency for Study 1, and smiles and amused facial expressions for Study 2.
Results: Study 1 showed that the results of the two auditory psychophysical tests on both interfaces were comparable, and in-line with data reported in previous studies using similar tests. Behavioural analyses of HRI showed that participants exhibited more frequent smiles and laughs when using the robot in comparison to the laptop. For Study 2, behavioural analyses illustrated a higher number of smiles and amusement facial expressions than other behaviours (possibly denoting disengagement; e.g., boredom and distress) throughout the whole interaction.
Conclusions: A high potential of the NAO robot as an alternative interface for conducting auditory tests was indicated by the overlap in behavioural analyses between both studies. The observed behaviours of both normal-hearing adults and cochlear-implanted children for Studies 1 and 2, respectively, when interacting with the robot during repetitive auditory tests, suggest that a high level of engagement may be maintained throughout such tasks.

SA184. Characterization and Comparison of Standard and Non-Standard Bone Conduction Transducers at Extended High Frequencies

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Category: Other, Bone Conduction
Background: Measurement of bone conduction (BC) hearing thresholds at extended high frequencies (EHF; above 8 kHz) is of clinical interest but is technically complicated by: limitations in standard BC transducer output, a lack of calibration standards and sparse clinical data from human subjects. Herein, we use a novel calibration scheme to characterize and compare acceleration and computed force outputs over the 4-20kHz range for four different BC transducers.
Methods: The outputs of two standard electromagnetic BC transducers: the RadioEar B71 and B81, as well as two non-standard, commercially available BC transducers: the Tascam HP-F200 and the Aftershokz AS400, were measured using a low mass accelerometer interposed between the transducer and an artificial mastoid (B and K 4930) from 4-20kHz. Measures of output growth and harmonic distortion with varied stimulus levels were assessed and compared across devices. At EHF, a maximum force output for each device is determined by setting a maximum linear input voltage based upon variations from linear output growth greater than ±1 dB and total harmonic distortion greater than 5% of the response at the fundamental frequency. Acoustic radiation from each device was evaluated from comparisons of force and acoustic output made for each device.
Results: Within its linear working range, the Tascam BC transducer demonstrates the largest force output per volt stimulus with the least frequency dependence over the 4-20kHz range, where the magnitude of force-per-voltage falls within ± 8dB N/V, while the other three BC transducers show up to 50dB decreases in force output per voltage with increasing frequency over the same frequency range. Both compressive and expansive non-linear growth were observed with each device at different input voltages. Total harmonic distortion (THD) of >5% of the force at the fundamental frequency was observed for the Tascam at the same input voltage for all frequencies. The B71, B81 and AS400 met THD limits per input drive in a frequency dependent fashion. Maximum linear force was greater for the Tascam by 25 to 40dB above 8kHz compared to the other three BC transducers. Acoustic radiation was greatest for the AS400, which behaved more like an air conduction earphone than a force generator.
Conclusions: At EHF, standard and non-standard BC transducers demonstrate non-linearities and distortion at high input voltages. At our defined maximum input voltage, the Tascam generates a relatively flat output

that is 25-40dB greater compared to the other three transducers. Tascam-like devices show promise as potential diagnostic BC transducers at EHF.

SA185. Regional and Cellular Specificity of Nicotinic and Muscarinic Cholinergic Transcript Expression in the Central Auditory Pathway

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Category: Other, Central auditory pathway structure

Background: Cholinergic signaling in the brain is involved in diverse functional processes, including neuromodulation, attention and cognition, learning and memory, development, addiction and reward, aging, and neuropathology. Sources of acetylcholine include neuronal subpopulations in the striatum, basal forebrain, thalamus, midbrain and brainstem. Their projections are topographically distributed to cortical and subcortical regions. Signaling is mediated by two receptor classes: metabotropic muscarinic (mAChRs) and ionotropic nicotinic (nAChRs) receptors. They are widely distributed in the brain, but expression can vary by location and even cell type. To improve the foundation for applied studies, we used multiplexed fluorescent in situ hybridization (FISH) to construct a reference "mini-atlas" of nAChR subunit and mAChR receptor transcript expression in major central auditory structures, delineated by prominent cell classes.

Methods: Coronal sections of brains from adult (8 week) male/female C57BL6/J mice were used. Sections containing cochlear nucleus (CN), inferior colliculus (IC), medial geniculate (MG) and auditory cortex (AC) were assayed with multiplex FISH (12-probe cocktails consisting of: cell-type markers + nAChR subunits or mAChR receptors pre-selected for each brain region)(nAChRs: a2, a3, a4, a5, a6, a7, b2, b3, b4)(mAChRs: M1, M2, M3, M4, M5)(cell-type markers: VGluT1, VGluT2, GAD1/2, GlyT2, Pvalb, Calb2, SOM, CCK, VIP). Sections were tile-imaged for each probe, then registered by alignment to DAPI to create a composite image z-stack. Cells were plotted and tallied by cell type for individual and selected combinations of nAChR or mAChR transcripts.

Results: nAChR and mAChR expression and co-expression patterns are regionally- and cell-type specific. Between brain regions (CN, IC, MG, or AC), expression tended to be mixed, with some transcripts found in all regions (e.g., nAChR b2), and others located in only one or two (e.g., nAChR b4). Within a given brain region, subunit/receptor transcripts may be broadly distributed across its subdivisions, or concentrated in particular loci (e.g., nAChR a4). Transcripts could also be found in multiple cell types, or restricted to particular subtype. Exemplary data from the mini-atlas will be presented for feedback on format and content.

Conclusions: The regional and cellular specificity of nAChR and mAChR expression suggests that cholinergic signaling mechanisms are heterogeneous in the auditory pathway, and that function may vary accordingly. Applications include biomarker discovery and greater specificity of experimental and therapeutic targeting.

SA186. Prevalence and Risk Factors of Olfactory and Gustatory Loss and Alterations in the U.S. During the COVID-19 Pandemic: Results From 2021 National Health Interview Survey

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Category: Other, Epidemiology: smell and taste, COVID-19

Background: The senses of smell (olfaction) and taste (gustation) are frequently impaired in patients infected with COVID-19. The highly transmissible Delta strain became dominant in the last half of 2021 while the even more transmissible Omicron variants emerged the following year. In this report, we estimate the prevalence of perceived olfactory and gustatory impairments associated with the COVID-19 pandemic, and the proportion of affected individuals who sought healthcare advice for their smell or taste problems.

Methods: We examined responses from adults (18+ years old; n=29,482) about their smell and taste problems and COVID-19 infection status in the 2021 National Health Interview Survey (NHIS). The NHIS is a continuously operating, nationally representative sample of the civilian non-institutionalized U.S. population. Prevalence estimates were adjusted to account for the complex random sampling design. We

used survey-weighted logistic regression to estimate risk factors of olfactory and gustatory impairments after adjusting for age, sex, race/ethnicity, educational attainment, body mass index, general health status, and geography (regional and urban-rural classifications).
Results: In 2021, prevalence of report of ever having had COVID-19 was 14.1% (95% CI: 13.5%-14.7%). The prevalence of perceived smell impairment was 19.1% (49.3% for individuals reporting COVID-19; 14.2% without COVID-19). Among individuals with smell impairments: (a) 57.0% reported diminution or loss, (b) 21.2% reported worse ability to smell than when younger, (c) 16.1% reported olfactory alterations or phantoms, and (d) 6.7% reported other combinations of smell problems. The prevalence of taste impairment was 13.0% (40.1% with COVID-19; 8.5% without COVID-19). Among those with taste impairments: (a) 44.7% reported current loss and that their ability to taste was worse than when younger, (b) 33.7% reported current diminution or loss only, (c) 13.9% reported persistent unwanted tastes in the mouth and diminution of ability to taste, and (d) 7.7% reported other combinations of taste problems. Overall prevalence of smell and/or taste impairments was 22.6% (54.4% with COVID-19; 17.5% without COVID-19). Among these individuals, 15.5% reported having ever discussed their smell/taste problems with a healthcare professional (27.1% with COVID-19; 9.6% without COVID-19). In addition to socio-demographic determinants, risk factors for smell/taste impairment included: smoking, hypertension, cardiovascular conditions, asthma, arthritis, dementia, anxiety, depression, fatigue, immunosuppression, difficulty remembering or concentrating, respiratory allergy, food allergy, use of prescription medications, cold or flu lasting longer than a month during past year, and persistent dry mouth (xerostomia). Individuals with smell/taste problems who had not had COVID-19 showed stronger associations with these risk factors.
Conclusions: In 2021, the prevalence of olfactory and gustatory impairments and proportion who discussed their problems with a healthcare provider was nearly 4 times higher among individuals infected with COVID-19. The COVID-19 pandemic has amplified the need for evidence-based guidance for assessment, treatment, and management of smell/taste impairments.

SA187. Development of an Awake Animal Model for Hyperacusis Screening

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Category: Other, Hyperacusis
Background: 2 to 15% of people experience hypersensitivity to everyday sounds, ranging from discomfort to pain; this is called hyperacusis. The pathophysiological mechanisms underlying hyperacusis are not yet characterized, mainly due to a lack of reliable animal models and difficulty to objectively quantify this subjective perception.
Recent studies in animal models suggest that the neural correlates of hyperacusis would be mediated by enhanced sound-evoked responses and neuronal spiking activities, i.e. hyperexcitability, in the central auditory system. Moreover, anesthesia used in most studies is known to dramatically alter the neuron's response (neuronal depression, response inhibition) which could induce bias in results.
The objective of this study is to develop a new awake animal model with a combination of neural biomarkers and behavioural changes reliably characterizing the presence of hyperacusis.
Methods: Male and female CBA mice were randomly divided into two groups: one sham group (unexposed), and one exposed group. Exposed awake animals were submitted to an acoustic trauma (8-16 kHz, 95 dB SPL, 2 hours) leading to temporary hearing loss (Temporary Threshold Shift = TTS), which is known to induce hyperacusis. To perform long-term electrophysiological measurements on vigil animals, we developed a technique for chronically implanting ECoG electrodes on the surface of the auditory cortex and inferior colliculus, enabling repeated in live measures.
ABRs, evoked potentials and EEG were measured at baseline (T0), T+1DAY, T+3-4WEEKS, T+6-8WEEKS and T+14-15WEEKS on awake animals. Behavioural tests, startle reflex intensity, were also analysed at the same time points.
Results: ABR thresholds were significantly increased (+ 30/40dB at high frequencies) in the exposed group at T+1DAY compared to T0 in the same group, but completely recovered from 4 weeks after noise exposure.
The amplitude of evoked potentials increased in the cortex after noise exposure, but not in the thalamus compared to Sham mice.

At T+1DAY the intensity of the startle response increased, and its latency decreased in the exposed group. These results were confirmed and reproduced at T+3-4WEEKS, T+6-8WEEKS and T+14-15WEEKS.
Conclusions: While developing an awake animal model for hyperacusis following TTS acoustic trauma, we observed an increase in sensitivity relative to the intensity of stimulation, for electrophysiological and behavioral measurements.
Taken together, these results, as well as the further analysis of EEG and ABRs, should lead to the identification of neural biomarkers and behavioural changes reliably characterizing the presence of hyperacusis.

SA188. Incidence and Clinical Characteristics of Laryngotracheal Stenosis Post-Acute Respiratory Distress Disorder After SARS-CoV2 Infection

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Category: Other, Laryngology
Background: Laryngotracheal stenosis (LTS) is a frequent complication after airway intubation and mechanical ventilation, with high subsequent morbidity. A peak in the incidence has been reported in the literature after the increasing number of intubation and tracheostomy. As this number has been suggested to have sharply increased after the pandemic, the appropriate calculation of the LTS incidence is needed as this number is still unknown in the present literature. We aim to understand the true incidence of LTS during the pandemic and depict some of the risk factors associated with developing LTS during the SARS-CoV2 global pandemic.
Methods: This is a retrospective cohort of 994 patients with ARDS secondary to SARS-CoV2 infection, who were seen in our emergency, intensive care unit (ICU), and airway surgery department between 2020 and 2022. 11 patients developed LTS. They were further analyzed for risk factors associated with the development of LTS after ADRS due to SARS-CoV2 infection. Subgroup analysis based on the LTS group was performed. The incidence rate of LTS was calculated by using the rate of the exposed population (ADRS patients with mechanical ventilation).
Results: Of the 994 patients in the study, 11/994 developed subsequent LTS. The incidence rate was 1.11 per 100 cases with ARDS due to SARS-CoV2 infection. The risk of LTS in patients with ARDS due to SARS-CoV2 infection was two times greater in those with diabetes mellitus (95% CI, 0.97 – 4.65, p=0.005). Other variables associated with the development of LTS were pronation risk factor of 6.86 (95% CI 1.06 – 44.5, p=0.001) and ventilator associated pneumonia (VAP) risk factor of 9.48 (95% CI 1.46 – 61.47, p=0.001). The clinical characteristics of the LTS population were as follows; mean of mechanical ventilation before tracheostomy 15 days (IQR 10.5 to 17.5 days). The LTS was characterized in most cases as grade II and III according to the Cotton Myer scale. The average length stenotic track was 13.2mm (IQR 10.8 to 15.4mm), and the distance from the stenotic track to the vocal cord was, on average 20 mm (IQR 20 to 22.5mm).
Conclusions: The risk of LTS in ARDS due to SARS-CoV2 was more significant in those with diabetes mellitus, who underwent pronation, and developed VAP during the inpatient treatment. We found an incidence rate of 1.1 per 100 ARDS cases after SARS-CoV2 infection. In addition, the present study report and confirm for the first time the incidence of LTS after SARS-CoV2 infection and the development ARDS, as well as the risk factors associated with the development of LTS compared to the ARDS-exposed population.

SA189. Translational Cyclic Cisplatin Ototoxicity (CIHL) Models in Rats and Mice

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Category: Other, Ototoxicity
Background: Cisplatin, an antineoplastic drug widely used in the treatment of many cancers, causes permanent hearing loss. Cisplatin ototoxicity, which appears in approximately 62% of adults and 61% of pediatric patients treated, is dose related and cumulative.
The severity of ototoxicity could be influenced by age, cumulative doses, and dose administration schedules.

Several animal models of cisplatin-induced hearing loss (CIHL) have been published, mainly with a single high dose and few with cyclic administrations. While acute models can serve as proof of concept, they are not fully translational to human situations, with multi-cycles of cisplatin chemotherapy over several weeks. This study aimed to develop cyclic mouse and rat cisplatin models.
Methods: In both species, cisplatin was administered daily by intraperitoneal injections over 3 consecutive cycles of 4 days, each cycle followed by a 10-day recovery period. The doses were calculated to reflect human doses and literature.
Two doses were administered in male CBA/JRj mice at 2.5 (n=10) and 3 mg/kg/day (n=10) and in male Wistar rats at 1.5 (n=8) and 2 mg/kg/day (n=8), corresponding approximately to 7.5 and 9 mg/m2, and to 9 and 12 mg/m2 in humans, respectively.
From the first day of administration, animals received specific daily care to limit cisplatin induced nephrotoxicity and global health deterioration. Animals were fed twice or three times daily with a Gel Diet Breeding and EmerAid IC Omnivore, and hydrated twice daily with Plasmalyte.
Body weight, clinical signs and general health were monitored daily over the 3 cycles, as well as hearing, using DPOAE and ABR measurements. After the 3rd cycle, cochleae were removed for analysis.
Results: In both species, the highest dose was abandoned due to dramatic weight loss and health issues, leading to death or euthanasia. However, ABR and DPOAE measures after the 2nd cycle in rats demonstrated an onset of HL.
After the third cycle, the rats treated with cisplatin at 1.5 mg/kg/day demonstrated a slight but insignificant increase of ABR thresholds and a decrease of DPOAE amplitudes, showing no CIHL in these experimental conditions.
In mice, after the third cycle, Cisplatin at 2.5 mg/kg/day induced important increases of ABR thresholds and decreases of DPOAE amplitudes, correlated with a substantial OHC loss at the base of the cochlea.
Conclusions: There is a fine line between the cisplatin dose producing hearing loss and animal welfare. In our cyclic models, despite similar clinical dosage regimen and lower doses, CIHL was observed only in mice with important health issues.
These results demonstrated the difficulty of having cisplatin models that reflect human protocol while maintaining animal welfare, and of reproducing published data. The obtained cisplatin model in mice is suitable to evaluate new drug candidates in the prevention of CIHL.

SA190. Neural Stimulating Electrodes Nano and Micro-Structuration and Surface Area on Safe Charge Limits

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Category: Other, Safe Stimulation
Background: The culmination of complex systems such as signal engineering (signal processing, transmission), system architecture (signal allocations, stimulators), electrode/stimulation designing (electrode material and pulse parameters), and bioactivation (neuronal activation, action potential generation) is the prerequisite in developing and proper functioning of the neural/biomedical stimulating implants. For efficient stimulation, electrode-neuron interface, biocompatibility, and biosafety are the most crucial part in mediating the stimulation from the electrode to extracellular space to target neurons. The electrode-neuron interface is governed by electrode properties, specifically, the topological structure and biosafety are regulated by electrode corrosion, pulse polarity, amplitude, and pulse width. In this work, we have studied the electrochemical properties of nano/microscopic topographic structures of stimulating electrode and their influence on safe stimulation strategies.
Methods: For this study, Oticon Medical EVO electrodes were used. The electrodes were characterized for both physical and electrochemical properties using SEM (Sorbonne University) and AFM (Faculty of Medicine, University of Cote D'Azur), whereas Princeton Applied Research Parstat MC potentiostat with a three-electrode system in Artificial Perilymph (AP) electrolyte set-up for electrochemical characterization and stimulation safety investigations. Electrode corrosion was evaluated employing ICP and further understanding of the stimulation-induced corrosion mechanism was studied using atomic emission spectroelectrochemistry (AESEC, Sorbonne University).
Results: In this work, we have studied the electrochemical properties of nano/microscopic topographic structure of EVO electrode and their influence on safe stimulation strategies. Scanning electron imaging

analysis of the nano/microstructures morphologies of the electrodes fabricated by different manufacturing processes were investigated. The influence of topographic variation was studied by understanding the electrochemical surface properties (radial and planar diffusion). The charge storage capacity, CSC, and charge density were calculated for the geometric and electrochemical surface area of the electrodes. The evaluation of the biosafety was explored by calculating the concentration of the Pt corrosion, with respect to the anodic-first charge balanced asymmetric (passive discharge) stimulation pulse. Furthermore, understanding the impact of stimulation parameters on electrode corrosion mechanism was carried out by comparing anodic-first charge balanced symmetric (active discharge) and asymmetric (passive discharge) stimulation pulses using AESEC.
Conclusions: From this study, it can be concluded that electrode nano/microstructures have a direct influence on altering the requirements of the safe limits of the stimulation parameters. It is also substantiating the discrepancies in determining the safe limit (Shannon's limit) depending on the outlook of the surface property (radial vs. planar diffusion) and electrode area (geometric vs. electrochemical). This study also provides a preliminary and explicit approach to understanding the impact of stimulation (symmetric vs. asymmetric) on corrosion behaviour mechanism. This information is vital for building on novel stimulation strategies and electrodes for better, safe, and efficient neuronal activation.

SA191. Central Gain: A Closer Fit to Hyperacusis Than to Tinnitus?

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Category: Other, Young Investigator Symposium "Putting Tinnitus Theories to the Test"
Background: Central gain refers to the increase of spontaneous activity observed in hierarchically higher auditory pathway areas after hearing loss induction (Schaette and Kempter, 2006). The upregulation of neuronal activity in central auditory regions is interpreted as a homeostatic plasticity response to decreased peripheral input. In the context of hyperacusis (Auerbach et al., 2014; Diehl and Schaette, 2015) and tinnitus (Norena, 2011; Schaette and McAlpine, 2011), the central gain framework has been extended to include sound-evoked activation. Sound-evoked activation is of particular interest in the context of hyperacusis which is characterized by an increase in the perceived loudness of sounds, unlike tinnitus which reflects the perception of a phantom sound. Whereas tinnitus is the most extensively studied condition co-occurring with hearing loss, 59% of those with hyperacusis have co-occurring hearing loss (Paulin et al., 2016), and the majority of those with hyperacusis also report tinnitus (Anari et al., 1999; Dauman and Bouscau-Faure, 2005; Schecklmann et al., 2014). Even though hyperacusis frequently co-occurs with hearing loss and tinnitus, it is often neglected in experimental studies, hampering adequate characterization of the neural signatures of these conditions.
Methods: The aim of this study was to characterize the neuroimaging signatures relating to hyperacusis in subcortical and cortical auditory regions in humans. In a functional Magnetic Resonance Imaging (fMRI) study, we investigated the Blood Oxygenation Level Dependency (BOLD)-responses in a group (n=35) that often reports hyperacusis: individuals with hearing loss and tinnitus. Hyperacusis was indicated by a cut-off score of 22 on the Hyperacusis Questionnaire (HQ), and a sparse-sampling paradigm was used to minimize interference of scanner sound on the presented loudness-matched pure-tones. The link between loudness and fMRI response amplitude is well established, making it an ideal method to investigate markers of the increased loudness reported in hyperacusis. Additionally, we characterized the frequency tuning of cortical voxels in the primary auditory cortex of those with and without hyperacusis.
Results: In the group with hyperacusis, sound-evoked activity was higher in both cortical and subcortical auditory structures. This increase in responsivity extended to frequencies not affected by hearing loss and appears to be a marker of hyperacusis. The observed higher subcortical and cortical activity in response to sound is in agreement with the perceived increased loudness of sounds reported by individuals with hyperacusis. The frequency tuning of auditory cortical voxels was not significantly different in those with hyperacusis, indicating that the increased perceived loudness does not relate to a loss of cortical frequency specificity. In contrast, the auditory cortex BOLD signal was reduced in response to the presentation of the tinnitus frequency in those with higher hyperacusis scores.
Conclusions: Overall, the heightened subcortical and cortical activity aligns with an increase in neural gain along the auditory pathway reflecting hyperacusis.

SA192. Latencies of Short-Pulse Distortion-Product Otoacoustic Emissions and Their Test-Retest Reliability

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Category: Otoacoustic Emissions

Background: The processing of sound in the auditory system is accompanied by frequency-dependent delays that originate predominantly from the propagation and active, nonlinear amplification of traveling waves in the cochlea. Cochlear amplification provides high sensitivity, large dynamic range, and sharp frequency tuning of the auditory system. As a by-product of nonlinear amplification, distortion-product otoacoustic emissions (DPOAEs) arise in response to the simultaneous stimulation of the cochlea with two sinusoidal waves of frequencies f1 and f2 (typically, f2/f1 ~ 1.2).
Latencies of DPOAEs may be used to assess changes in the function of the cochlear amplifier, e.g., in ototoxicity monitoring programs, and to facilitate differential diagnosis of hearing disorders in combination with latencies of auditory brain stem responses. Here, we present latencies and their test-retest reliability for the nonlinear-distortion component of short-pulse DPOAEs extracted directly in the time domain from ten normal-hearing adults.
Methods: Short-pulse DPOAEs were recorded for ten stimulus-level pairs at 14 frequencies with f2 = 1 – 14 kHz (f2/f1 = 1.2) at seven visits over three months in 20 ears of ten normal-hearing adults. L2 was 25 – 80 dB SPL (5-dB steps) and L1 was selected dependent on L2, and derived from individual optimal-path parameters. Primary-tone phase variation enabled the determination of latencies of the nonlinear-distortion component directly from short-pulse DPOAE time signals by onset decomposition.
Results: The DPOAE latency decreased with increasing stimulus frequency and level from 16.42 +/- 7.20 ms at f2 = 1 kHz and L2 = 25 dB SPL to 1.50 +/- 0.58 ms at f2 = 14 kHz and L2 = 80 dB SPL. The median DPOAE latency pooled over stimulus levels ranged from 11.39 periods at f2 = 1 kHz to 23.17 periods at f2 = 14 kHz.
DPOAE latencies at low frequencies demonstrated higher test-retest reliability than those at mid or high frequencies, with median absolute differences ranging from 0.78 to 1.14 periods for f2 = 1.5 – 4 kHz and from 1.68 to 3.92 periods for f2 = 5 – 14 kHz, respectively. The test-retest reliability depended significantly on the stimulus level and frequency, the stimulus-level ratio, and the signal-to-noise ratio (SNR). The test-retest reliability improved with decreasing stimulus frequency and increasing SNR.
Conclusions: Short-pulse DPOAEs enable the determination of DPOAE latency directly in the time domain. The remarkably high test-retest reliability of the extracted DPOAE latency, especially at low frequencies, demonstrates that short-pulse DPOAE latencies are a promising tool for evaluating and tracking the functional state of the cochlear amplifier, e.g., for monitoring ototoxicity during cancer treatment or the impact of occupational noise exposure. Further studies in hearing-impaired patients could help establish DPOAE latency as a parameter valuable for clinical applications.

SA193. Does Near-Frequency Suppression Extract the Complete Stimulus Frequency Otoacoustic Emission?

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Category: Otoacoustic Emissions

Background: Stimulus-frequency otoacoustic emissions (SFOAEs) are used to study cochlear amplification, but interpreting SFOAEs requires understanding where they originate along the cochlea. With a tone stimulus, an ear-canal microphone records the sound pressure from the tone source (the "source tone") plus the SFOAE from the cochlea. The SFOAE is separated out of this overall response by making a separate source-tone alone measurement with a manipulation done to remove the SFOAE. For example, the source-tone alone is typically measured (1) using a near-frequency, high-level tone to suppress the SFOAE and computationally removing the "suppressor" sound by filtering (or by subtracting a separate suppressor-tone measurement), or (2) by scaling a measurement from a high-level probe-frequency tone in which the SFOAE is small compared to the probe tone and becomes negligible after the high-level response is scaled down to estimate the source tone. However, these methods will not yield the SFOAE if the high-level tone

produces new, additional probe-frequency components. Here we measured SFOAEs with a standard high-level, near-frequency, suppressor-tone extraction paradigm, and also using a pharmacologic reduction of cochlear amplification that did not require a high-level tone that might evoke new probe-frequency SFOAE-like residuals.
Methods: SFOAEs were measured from guinea pigs using near-frequency suppression, before, during, and after eliminating outer-hair-cell (OHC) function and SFOAE amplification with salicylate or KCl solutions perfused into the cochlear apex and slowly driven toward the cochlear aqueduct in the base. After the perfusion had blocked OHC function throughout the cochlea, the source-tone pressure (without any SFOAE) was measured directly from the probe-alone pressure (Pend). SFOAEs were calculated by subtracting Pend from the probe-alone pressure measurements made before (Pstart) or during (Pduring) the perfusion. Measurements were adjusted to account for drift.
Results: The spatial location along the cochlear length of SFOAEs extracted with pharmacologic blocking differed little from the SFOAEs extracted using a near-frequency high-level suppressor, but were, on average, a few dB higher in amplitude.
Conclusions: Extraction with sound suppression reveals most, but not all, of the SFOAE. Perhaps the typical use of a suppressor 20 dB higher than the probe is not enough to suppress all of the SFOAE. Our data provide no evidence that new SFOAE-frequency residuals are produced by a near-frequency suppressor. After the apically-injected-solution front continued one octave or more basally past the probe-tone CF region, there was no further reduction of the SFOAE amplitude (with both methods), which indicates that no SFOAE components originate from this far-basal region. Thus, probe-frequency residuals that appear with a high-frequency (more than an octave above the SFOAE frequency) second tone are due to the 2nd tone and are not there when the probe-tone is the only stimulus.
Supported by NIDCD/NIH R01 DC014997 (JTL).

SA194. Relationship Between Distortion Product Otoacoustic Emissions and Audiometric Thresholds in the Extended High-Frequency Range

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Category: Otoacoustic Emissions
Background: Clinical audiometry is typically limited to frequencies between 125 and 8000 Hz; however, thresholds at frequencies above 8000 Hz appear to be most susceptible to ototoxic insult, aging, and possibly acoustic overexposure. Extended high-frequency distortion product otoacoustic (DPOAE) measurements offer an alternative metric of assessing cochlear health and may be sensitive to the effects of cochlear damage before they are apparent on audiometric testing. Furthermore, DPOAEs are common in audiology clinics and have practical benefits compared to audiometry such as reduced test duration and no need for patient participation. DPOAEs in the extended high frequencies, however, have proven to be less reliable due to the influence of standing waves in the ear canal. To reduce the test-retest variability of these measures, forward-pressure-level (FPL) and emitted-pressure-level (EPL) calibrations for the stimulus and response, respectively, are recommended. The purpose of this study was to assess the correlation between behavioral audiometry and DPOAEs in the high-frequency and extended high-frequency ranges when using these more precise calibration techniques.
Methods: We collected data from 165 individuals with normal hearing as defined by traditional clinical audiometry. Thresholds above 8 kHz were allowed to vary. Following individual ear FPL calibrations, we measured swept DPOAEs from 2-16 kHz. Audiometric thresholds were assessed from 250-16 kHz. Frequency specific audiometric thresholds and DPOAE amplitudes were each averaged across the high-frequency (3-8 kHz) and the extended high-frequency (9-16 kHz) ranges.
Results: Extended high-frequency thresholds were negatively correlated with extended high-frequency DPOAE amplitudes. Age was correlated with audiometric thresholds and DPOAEs in both the high and extended high-frequency ranges. The strongest correlation was between age and extended high-frequency audiometry. Though also highly correlated with age, extended high-frequency DPOAE amplitudes fall to the noise floor for many subjects over the age of 40. High-frequency DPOAEs were not tightly correlated with high-frequency audiometric thresholds, but this could be an artifact of the narrow range of thresholds in that frequency range (< 25 dB HL).
Conclusions: Preliminary results suggest that with appropriate calibration methods, extended high-frequency DPOAEs are sensitive to reductions in audibility within the same frequency range. These

findings support the use of DPOAEs in clinical applications such as ototoxicity monitoring and hearing conservation programs.

SA195. Orbitofrontal Cortex Shapes Auditory Cortical and Perceptual Sensitivity

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Category: Primary Auditory Cortex

Background: Sensory acuity can benefit from practice, through which we can improve our ability to see, hear, smell, and taste – a process termed perceptual learning. In the auditory system, perceptual learning supports the development of speech and musicality and improves speech recognition in the hearing-impaired. Non-sensory processes, like attention or reward, make critical contributions to perceptual learning, but the neural circuits and mechanisms that mediate their involvement are poorly understood. The orbitofrontal cortex (OFC) is a prefrontal cortical region with established roles in signaling reward and in transmitting state-dependent feedback via direct projections to sensory (including auditory) cortices, and is therefore a promising candidate to explore. We hypothesized that OFC neurons provide non-sensory input to the auditory cortex that mediates auditory perceptual learning.

Methods: If OFC transmits a non-sensory signal to the auditory cortex that shapes auditory cortical and perceptual sensitivity, then silencing OFC activity should disrupt auditory cortical activity, and impair behavioral sound detection. To test this prediction, we used muscimol (GABAa agonist) to inactivate bilateral OFC, and simultaneously recorded extracellular responses from auditory cortical neurons in freely moving Mongolian gerbils as they performed an amplitude modulation (AM) detection task. Next, we asked whether OFC neurons exhibit learning-related changes in activity by using chronically implanted electrode arrays to record from individual OFC neurons as gerbils trained on an auditory perceptual learning task with progressively more challenging AM stimuli. Finally, to determine whether learning affects the specific subpopulation of OFC neurons that innervate the auditory cortex, we used fiber photometry to record calcium signals from just the OFC neurons that project to the auditory cortex as gerbils underwent auditory perceptual learning on the same task.

Results: We found that inactivation of bilateral OFC significantly impaired AM detection. OFC inactivation also impaired task-dependent modulation of auditory cortical activity. During perceptual learning, a subset of OFC neurons gradually increased their firing rates, which significantly correlated with the degree to which perceptual thresholds improved. This finding was recapitulated in the subpopulation of OFC neurons that project to the auditory cortex: calcium signals in these cells grew larger as perceptual thresholds improved, suggesting that OFC neurons send progressively stronger signals to auditory cortex over the course of perceptual learning.

Conclusions: Our findings suggest that the OFC provides non-sensory input to the auditory cortex that (I) supports AM detection, (II) modulates auditory cortical activity, and (III) becomes stronger with perceptual training. These results support the hypothesis that the OFC facilitates practice-dependent improvements in perceptual and auditory cortical sensitivity via a direct projection to auditory cortex.

SA196. Neuronal Types Support Hybrid Temporal Encoding Strategies in Auditory Cortex

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Category: Primary Auditory Cortex

Background: Neurons in auditory cortex of the awake marmoset (Callithrix jacchus) encode temporal information with either stimulus-synchronized or nonsynchronized responses (reviewed in Wang, 2018, Annu Rev Neurosci). It is unclear to what extent cell type diversity might contribute to this functional diversity.

Methods: We recorded single unit responses to a variety of synthetic and natural auditory stimuli primarily in core regions of the marmoset auditory cortex. We then used electrophysiological features such as spike timing and waveform to classify single-unit recordings with either a criteria-based or an unsupervised classification method into regular-spiking, fast-spiking, and bursting units. A majority of, but not all, recorded units could be classified with confidence, and there was high consistency between the two methods.

Results: A subset of intrinsically bursting neurons formed the most highly synchronized group, with strong phase-locking to sinusoidal amplitude modulation (SAM) that extended well above 20 Hz. These bursting

neurons fired primarily on the rising phase of SAM or the onset of unmodulated stimuli, and preferred rapid stimulus onset rates. Such differentiating behavior has been previously reported in bursting neuron models (Kepecs et al., 2002, J Neurosci) and may reflect specializations for detection of acoustic edges. These units responded to vocalization stimuli with brief and precise spiking at particular time points that could be decoded with high temporal stringency. Regular-spiking units better reflected the shape of slow modulations and their overall firing rate was more selective for vocalization identity. A relatively small pool of bursting units was sufficient to achieve high population decoding performance when temporally binned firing rate was used. These may be similar to a group of temporally precise and highly informative units previously reported in macaque auditory cortex (Ince et al., 2013, J Neurosci).
Conclusions: Our results support the notion that auditory cortex uses divergent transformations in different neuronal types to accomplish both rate and temporal encoding.

SA197. Effects of Stimulus Rate and Periodicity on Auditory Cortical Entrainment and Their Relation to Speech Rhythms

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Category: Primary Auditory Cortex
Background: The neural mechanisms underlying rapid exogenous coding and the neural entrainment to auditory stimuli have been the subject of controversy. Using inter-trial phase-locking (ITPL) and phase-locking value (PLV) analyses applied to high-density human electroencephalogram (EEG) data, this dissertation investigated the degree to which the brain entrains to speech and non-speech (i.e., click) sounds and how changes in the rate and periodicity of the ongoing streams alter brain oscillation patterns.
Methods: Temporal processing of cortico-acoustic tracking was investigated in N=24 normal young adults utilizing EEG time-frequency and source analyses that isolated neural activity stemming from both auditory temporal cortices. We manipulated the rate and periodicity of repetitive and continuous speech and click stimuli parametrically to investigate how the speed and jitter in ongoing auditory stimuli affect neural entrainment. Brain-behavior associations were investigated using analogous perceptual tests—temporal modulation transfer functions (TMTFs) and computerized adaptive beat alignment test (CA-BAT)—to tap listeners' behavioral rate and periodicity sensitivity, respectively.
Results: Both stimulus domains (speech/non-speech) showed rightward hemisphere asymmetry in phase-locking strength; stronger response at the nominal speech syllable rate than at faster rates; and longer latency for speech vs. click. PLV in speech demonstrated a stark improvement in neuronal synchronization at 4.5 Hz that degraded at higher rates but with an opposite pattern for click.
Conclusions: ITPL periodicity had stronger phase-locking in the right hemisphere for both stimulus types but longer latencies for speech. PLV, though, showed decreased phase-locking to speech with increasing jitter, yet entrainment to speech was still superior to that of clicks. However, click responses were not affected by periodicity. Even though the interpretation of 'periodic speech preference' can't really be drawn from the presentation of only a syllable train with no comprehension task but overall, speech is more sensitive to changes in rhythm and periodicity in passive listening, maybe as a result of the greater neuronal entrainment it elicits in the brain. These findings explain fundamental response characteristics that will be critical for clinical and research applications of neural entrainment.

SA198. Ecological Momentary Assessment and Environment-Specific Hearing Aid Benefit by Auditory Perceptual Profile

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Category: Psychoacoustics
Background: Individuals with similar pure tone thresholds, identified as audiometric "twins", may exhibit different auditory perceptual and cognitive abilities. Because these individuals may receive fairly similar hearing aid (HA) gain settings that were validated and fine-tuned with real-ear measures, some could perceive benefit in speech understanding, while others may not. This suggests the presence of inter-individual differences not taken into consideration. Hearing loss may result in degradations in auditory perceptual and cognitive abilities to different extents across individuals, which may be important factors affecting HA benefit. The goal of this study was to identify "auditory perceptual profiles" based on individual differences in auditory perceptual and cognitive abilities, and to determine any associations with

subsequent HA benefit from clinical and real-time measures. A long-term goal, with precision audiology, is to leverage perceptual profiles to target compromised perceptual abilities in hearing aid fittings.
Methods: A condensed test battery using the Portable Automated Rapid Testing (PART) platform was used to assess the auditory perceptual and cognitive abilities of twenty older adults with mild to moderately-severe hearing loss. Assessments included measures of frequency selectivity, spectro-temporal processing, temporal fine structure (TFS) and binaural processing, temporal envelope perception, spatial release from masking (SRM), and measures of working memory and fluid intelligence. A step-up design was applied in which listeners were evaluated unaided for two weeks followed by three weeks aided. Ecological momentary assessment (EMA) surveys reflecting real day-to-day listening experiences were collected four times per day for the first (unaided) and last (aided) two weeks. An objective hearing in noise test and standard HA questionnaires were also administered prior to and after HA use.
Results: Cluster analyses identified three auditory perceptual profiles from individuals' auditory perceptual and cognitive abilities. One profile (A) indexed poor performance on all perceptual and cognitive tasks and was associated with minimal HA benefit. Profiles B and C were associated with good perceptual performance and greater HA benefit. Profile B differed from C in which measures of HA benefit were significant. Working memory and SRM were major factors separating the "best" profile (B) from the other two (A and C); whereas the other two profiles were separated by performance on frequency selectivity, spectro-temporal sensitivity, and TFS processing.
Conclusions: Auditory perceptual profiles were associated with different degrees of HA benefit, especially as measured under ecological momentary assessment. Auditory perceptual abilities may be important precursors in identifying realistic expectations, and in the long-term, may be used to guide aural rehabilitations strategies.

SA199. Weighting and Noise Efficiency of Musicians and Non-Musicians in Multi-Talker Speech Segregation

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Category: Psychoacoustics
Background: Studies have shown benefits of musical training in auditory tasks requiring the segregation of speech of simultaneous talkers - the so-called "cocktail-party effect" [Coffey et al. (2017). Hear Res 352:49-69.). The present study reports two possible components of the benefit: (1) weighting efficiency reflecting the degree to which the listener's reliance on segregation cues approximates the ideal observer; (2) noise efficiency measuring the degree to which the listener's response is veridical with the stimulus from trial to trial [Lutfi et al. (2020). JASA 148(6):4014–4024].
Methods: 28 Listeners participated (20-31 years old): 10 musicians with 10+ years of music training (7 females) and 18 non-musicians with no musical training (15 females). All listeners were USF students with audiometric thresholds of less 25 dB HL between 0.25-4kHz. The task was two-talker speech segregation as described by Lutfi et al. (2020). A random sequence of synthesized English vowels was presented on each trial and listeners were asked to judge whether the vowels were produced by one or two talkers. Talkers were distinguished by average voice fundamental frequency (F0=120 vs. 150 Hz) and mean azimuthal location (θ=0 vs. 30 deg, simulated using KEMAR HRTFs). Random perturbations in F0 and θ (σ = 10 Hz and 10 deg) were added independently for each talker on each trial. Decision weights (reliance) on the values of F0 and θ for each listener were estimated from regression coefficients in a general additive model where the perturbations were predictor variables for the listener's trial-by-trial response. For each listener weighting efficiency (the degree to which the listener's response is tied to the stimulus) and noise efficiency (the degree to which internal noise decorrelates the listener's response from the stimulus) were estimated using COSS analysis [Berg (1990). JASA 88: 149–158].
Results: Musicians performed better as a group than did non-musicians, though there was large variation within each group. Both individual and group differences in performance were near exclusively due to estimates of noise efficiency.
Conclusions: The results are consistent with previous studies showing noise efficiency to be the component of performance having the strongest relation to the individual variation in performance in simulated cocktail-party listening tasks [cf. Lutfi et al. (2022). JASA 152(3):1804–1813].

SA200. Simulating Physiological and Psychoacoustic Forward Masking in a Subcortical Model With Efferent Gain Control

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Category: Psychoacoustics

Background: Forward masking is a classic psychoacoustic paradigm in which detection thresholds for a short probe tone are elevated by the presence of a preceding sound. A previous physiological study (Nelson et al., 2009, JNeuro 2553:2562) demonstrated that forward masking in the inferior colliculus (IC) of awake animals may explain the range over which forward masking increases with masker level in psychophysical results (referred to as growth-of-masking, GOM). Various attempts have been made to explain how forward-masking emerges in the IC; however, a physiologically realistic model that accounts for the emergence of GOM at that stage has not been reported. The present study evaluated the alternative hypothesis that physiological and psychophysical GOM does not emerge in the IC, but originates in the periphery due to the medial-olivocochlear (MOC) efferents. Reduced GOM in anesthetized recordings of the auditory-nerve (AN) may then be explained by the suppression of the efferent system under anesthesia (Boyev et al., 2022, JARO, 362:373). The MOC efferent mechanism was also tested as an explanation for a psychophysical result that is difficult to explain using an energy detector model of forward masking (Jesteadt et al., 2005, JASA, 325:337).

Methods: An updated version of the efferent-system model of Farhadi et al. (2021, ICASSP, 291:295) and an updated version of the IC model of Nelson and Carney (2004; JASA, 2173:2186) were used together to simulate AN and IC responses in awake mammals. Models of the AN and IC that do not incorporate the efferent system were also tested. All physiological and behavioral thresholds were estimated using stimuli matched to those in the original studies but using a two-interval, method of constant stimuli procedure. For each trial, the interval yielding a higher maximum rate in a time window surrounding the probe was selected as the target. Percent correct was calculated across several trials and probe tone levels, and a logistic curve was fit to determine threshold.

Results: With efferent mediated cochlear gain control, both AN and IC model simulations explained the ~20 dB decrease in masked thresholds observed in awake midbrain recordings for probe delays that increased from 0 to 150 ms after the masker offset. Model simulations also explained the ~0.5 dB/dB GOM found in both awake midbrain recordings and behavioral results. In good agreement with Jesteadt et al., simulations incorporating between-interval random variation in masker level were robust to this random variation. Computational models for neural responses without efferent-mediated cochlear gain control were not able to simulate the same set of results.

Conclusions: Model simulations suggest that MOC efferent cochlear gain control can explain physiological forward masking with tone maskers, as well as key features of psychophysical forward masking.

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SA201. Contribution of Listening Effort and Cognitive Processing to Performance on Perceptual Tasks Among Normal-Hearing Young Adults

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Category: Psychoacoustics

Background: Hearing and listening are dependent on auditory encoding and processing as well as cognitive abilities such as memory and attention. Cognitive processes are often measured using standardized tests of memory, attention, reasoning, and processing speed. During task performance, physiological measures such as blink rate and pupillometry reflect cognition and engagement: Previous studies have shown that the blink rate tends to decrease and the pupil size tends to increase when participants are more engaged in a task, indicating higher levels of listening effort (i.e., autonomic arousal). This project uses both standardized cognitive testing and physiological measures to evaluate the contribution of various cognitive processes to performance on auditory tasks among young adults.

Methods: The participants were normal-hearing 18- to 23-year-olds with no known language, learning, or attentional issues. A battery of standardized audiometric, cognitive, and language tests was administered to

assess hearing, speech perception in noise, attention, working memory, processing speed, verbal reasoning, and non-verbal reasoning. In addition, an eye-tracker measured the number of blinks and pupil size while the method of constant stimuli was used to measure performance on frequency discrimination, temporal interval discrimination, and gap detection tasks.
Results: Preliminary data suggest that the relationship between pupil size, number of blinks per trial, and whether a trial was correct or incorrect varied across conditions. This outcome suggests that poorer performance may be more attributable to arousal or engagement on some conditions than others. Blink rate and pupil size contributed to but did not solely account for variance in performance across tasks and between listeners. Once a sufficient number of participants have been tested, a regression model will be used to evaluate the contribution of various cognitive processes, as measured with standardized tests, to psychometric functions on each condition will also be examined and discussed.
Conclusions: These findings are consistent with previous work indicating that cognition contributes to hearing and listening performance. Future work will examine the contribution of auditory encoding to this performance using electrophysiology (the acoustic change complex). The results will also provide a strong basis for comparison with younger and older listeners to establish patterns across the lifespan.

SA202. Masking Effects of Amplitude Modulation on Frequency-Modulated Tones

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Category: Psychoacoustics
Background: Human sensitivity to frequency modulation (FM) is best for low carrier frequencies (fc < ~4-5 kHz) and slow modulation rates (fm < 5-10 Hz) that are most relevant for speech and music. This high fidelity is thought to be afforded by neural phase locking to temporal fine structure (TFS), providing precise temporal information about the stimulus periodicity. At faster rates and higher carriers at slow/fast rates, TFS cues may no longer be viable, with sensitivity to FM instead relying on amplitude modulation (AM) of the temporal envelope, produced by FM-to-AM conversion via cochlear filtering. The AM produced by FM-to-AM conversion differs from traditional AM, in that the sweeping of FM through the tonotopic axis results in AM cues that are out of phase between tonotopic locations with characteristic frequencies above and below the carrier frequency. Imposing AM on an FM carrier has been proposed as a way to disrupt envelope but not TFS coding. Some, but not all studies, have found that performance is most impaired by AM in conditions where temporal-envelope coding is thought to be used (i.e., fast modulation rates and/or high carrier frequencies).
Methods: The present study aimed to test the replicability of the perceptual effects of imposing AM on FM (experiment 1) and assess the effects of an AM masker on “simulated FM tones”, which are composed of out-of-phase AM dyads that do not provide TFS cues (experiment 2). FM detection thresholds were assessed for 1-s tones at two carrier frequencies (1 and 6 kHz) and two modulation rates (2 and 20 Hz) with and without AM imposed on the carrier.
Results: Experiment 1 results from 28 young, normal-hearing listeners showed that FM sensitivity was more affected by AM at the fast than at the slow modulation, but that this pattern was the same for both low and high carrier frequencies. Preliminary results from experiment 2 suggest a similar pattern of results, despite the fact that no TFS cues are available.
Conclusions: Overall, the results do not provide support for the idea that AM interference can be used to distinguish between TFS- and envelope-based codes for FM. Instead, they suggest that the effects of AM on FM sensitivity may be limited by central constraints that apply to envelope-based coding at all combinations of carrier frequencies and modulation rates. [Supported by NIH grants R01 DC005216 (AJO) and R21 DC019409 (KLW).]

SA203. Effect of Masker Duration on Behavioral Estimates of Human Cochlear Tuning

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Category: Psychoacoustics
Background: It has been claimed that behavioral forward-masking thresholds for spectrally notched-noise maskers and a fixed low-level probe tone provide accurate estimates of cochlear tuning. Using that method, it has been further claimed that cochlear tuning is sharper in humans than in other mammals. In making

those claims, however, the potential effect of stimulus duration was disregarded and long, high level maskers can activate peripheral reflexes, which could affect masking thresholds and/or tuning estimates. Here, we investigate the effect of masker duration on behavioral estimates of human cochlear tuning at 500 Hz and 4 kHz.
Methods: Seven listeners (four women) with normal hearing participated in the experiments. Probes were 20-ms sinusoids at 10 dB sensation level (SL). Maskers consisted of noise with a spectral notch symmetrically and asymmetrically placed around the probe frequency. Masker levels at masking threshold were measured in forward masking for various notch widths. For each listener, thresholds were measured for masker durations of 30 and 400 ms with no time gap between the maskers and the probe. Individual notched-noise curves (i.e., plots of threshold masker levels vs notch width) were fitted assuming rounded exponential filter shapes and the power spectrum model of masking, and equivalent rectangular bandwidths (ERBs) were inferred from the fits.
Results: At 4 kHz, mean masker levels were equally higher for the 30-ms than for 400-ms maskers at all notch widths (i.e., notched-noise curves were shifted upwards for the longer maskers) but ERBs were not significantly different for the two masker durations (ERB30ms=280 Hz vs ERB400ms=277 Hz, p=0.904). At 500 Hz, by contrast, notched-noise curves were shallower for the 30-ms than the 400-ms masker, and ERBs were significantly broader for the shorter masker (ERB30ms=118 Hz vs ERB400ms=57 Hz, p=0.016).
Conclusions: At low frequencies, masker duration can affect behavioral estimates of cochlear tuning. Caution should be exercised in asserting sharper tuning of human cochlear filters, at least at low frequencies.
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SA204. Categorisation of Biological Sounds in Pristine soundscapes: A Psychophysical Investigation Based on an Ecologically-Valid Database

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Category: Psychoacoustics
Background: Evolutionary pressures may have produced specialised neural mechanisms that are hardwired to process different categories of perceptible objects, such as living things. Consistent with this theory, electrophysiological and brain-imaging studies have identified neural structures in the human brain that are involved in the categorical perception of biological versus geophysical sounds. These studies have identified several acoustic features that play a key role in the categorical perception of biological sounds, such as high spectral modulations and slow temporal modulations. However, the stimulus sets used in these studies had poor ecological validity due to an over-representation of mammals, whereas pristine soundscapes are usually dominated by birds and insects. Sounds produced by these animals may often be inharmonic or show fast temporal rates. Therefore, the role of high spectral modulations and slow temporal modulations in the identification of biological sounds may have been overestimated. The aim of this research is to characterise the acoustic cues used by humans in the perception and categorisation of biological (versus non-biological) sounds. This is achieved by analysing acoustic samples extracted from pristine soundscapes after they have been identified and categorised by normal-hearing adult subjects.
Methods: Acoustic stimuli correspond to 1-sec samples extracted from a large database of pristine soundscapes recorded in nine distinct terrestrial biomes on five continents during two contrasting seasons and at four moments of the day. The use of recordings made in natural habitats allows a valid comparison between contributions of acoustic cues produced by biotic and abiotic sources because biological and geophysical sounds composing each stimulus are congruent. All subjects are tested binaurally under

headphones at a comfortable level. Each subject performs a categorisation task where they have to select one of the four categories (Biologial sound, Non-biological sound, Unidentifiable sound, or Silence) for each stimulus by pressing the button associated to the chosen category. Acoustic stimuli are then analysed based on subjects' responses. The spectro-temporal modulation power spectra of the stimuli are then calculated and analysed to determine the cues used by the subjects in this task. Additional analyses scrutinize the potential contribution of modulation phase and cross-spectral modulation correlations to categorisation among others.
Results: Preliminary results on few subjects and modulation analyses will be presented and discussed.
Conclusions: Overall, the outcome of this research will help unveil the acoustic cues and mechanisms used by humans when listening to natural soundscapes, and their capacity to monitor biological sounds sources in their close environment through their auditory brain.
Acknowledgements: This project was supported by ANR grants HEARBIODIV and ANR-17-EURE-0017.

SA205. Behavioral Measurements of Gap Detection and Frequency Difference Limens in CBA/CaJ Mice Following Noise Exposure

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Category: Psychoacoustics
Background: Damaging noise exposure is associated with accelerated hearing loss and consequently diminished speech comprehension in humans. The temporal and pitch resolutions of the auditory system play an important role in the processing of complex acoustic signals such as speech. Mice are frequently used to study and model noise-induced hearing loss due to similarities in human and mouse cochleae and in genetic makeup. Although the effects of noise exposure on the detection of simple sounds is well studied in mice, the influence of damaging noise on temporal and spectral resolutions are largely unknown. In the present study, we measured gap detection and frequency difference thresholds and threshold shifts in young and old mice before and after a noise exposure. In addition, we examined behavioral performance stability in mice across the lifespan before and after noise exposure. We hypothesized that temporal and spectral resolution abilities, as well as behavioral stability, would deteriorate in mice following a noise exposure.
Methods: Young and old mice were trained and tested using operant conditioning procedures and positive reinforcement on gap detection and frequency difference limen (FDL) tasks. Once baseline thresholds were obtained, mice were exposed to 8-16 kHz narrow band noise at 100 dB SPL for 2 hours. Following noise exposure, thresholds and threshold shifts were calculated daily to examine recovery at 7, 14, 28, and 40+ days after noise exposure. To assess behavioral stability for both experiments, coefficients of variation (CV) of thresholds were calculated before and after noise exposure.
Results: The results revealed that gap detection thresholds are shorter in young than in old mice. Gap detection measurements were longer in the first week following a noise exposure and returned to baseline by 14 days after exposure. CVs for gap detection performance increased with age and noise exposure, returning to baseline by 14 days after noise exposure. FDLs and threshold shifts were not affected by age or noise exposure in mice. Young mice, however, had higher CVs in the FDL task than older mice. CVs for FDL performance also increased for mice of all ages following a noise exposure, returning to baseline 14 days after exposure.
Conclusions: In conclusion, the present experiment revealed that temporal but not spectral resolution abilities deteriorate in mice due to noise exposure and age. Interestingly, young mice showed better behavioral stability for gap detection and worse behavioral stability for the FDL task. Nonetheless, behavioral stability temporarily deteriorated due to noise exposure across both tasks. The results highlight the necessity for behavioral studies of complex measures of hearing in evaluating mouse models of noise-induced hearing loss, as results here differed significantly from physiological and behavioral studies measuring responses to simple pure tones.
This work was supported by NIH DC012302 and DC016641.

SA206. Non-Negative Matrix Factorization Improves the Efficiency of Recording Frequency-Following Responses in Normal-Hearing Adults and Neonates

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Category: Psychoacoustics
Background: One challenge in extracting the scalp-recorded frequency-following response (FFR) is related to its inherently small amplitude, which means that the response cannot be identified with confidence when only a relatively small number of recording sweeps are included in the averaging procedure.
Methods: This study examined how the non-negative matrix factorization (NMF) algorithm with a source separation constraint could be applied to improve the efficiency of FFR recordings. Conventional FFRs elicited by an English vowel /i/ with a rising frequency contour were collected. Fifteen normal-hearing adults and 15 normal-hearing neonates were recruited.
Results: The improvements of FFR recordings, defined as the correlation coefficient and root-mean-square differences across a sweep series of amplitude spectrograms before and after the application of the source separation NMF (SSNMF) algorithm, were characterized through an exponential curve fitting model. Statistical analysis of variance indicated that the SSNMF algorithm was able to enhance the FFRs recorded in both groups of participants.
Conclusions: Such improvements enabled FFR extractions in a relatively small number of recording sweeps, and opened a new window to better understand how speech sounds are processed in the human brain.

SA207. An Anamniote Specific Cyclin D is Required for Sensory Hair Cell Progenitor Proliferation in Zebrafish

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Category: Regeneration
Background: Tissue regeneration is limited in mammals but can be robust in other vertebrates. A long-studied goal of regenerative science is to characterize regenerative mechanisms in other species and then apply them therapeutically in people. Zebrafish can regenerate their sensory hair cells in both the ear and lateral line. The mechanosensory lateral line is composed of clusters of cells called neuromasts, which contain mechanosensory hair cells surrounded by different support cell types. Zebrafish regenerate lateral line hair cells via proliferation and differentiation of support cells. In mammals support cells do not proliferate or regenerate in response to hair cell death. There is therefore a need to understand the molecular regulation of regenerative proliferation.
Methods: To uncover genes that are important for hair cell regeneration, our laboratory performed single-cell RNA sequencing analysis of regenerating neuromast cells. For one potentially interesting cell cycle gene we generated a mutant using CRISPR/Cas9 and characterized it with EdU, in situ expression analysis and live in vivo time-lapse imaging.
Results: One gene identified is a cyclin D family member ccndx. Intriguingly ccndx is only found in anamniotes and has been lost in amniotes. In neuromasts ccndx shows highest expression in proliferating hair cell progenitors. EdU analysis and live time-lapse imaging of a CRISPR ccndx mutant shows that hair cell progenitors fail to proliferate but new hair cells can still form through direct differentiation of support cells. This finding is surprising as it was thought that mitosis was required for neuromast hair cell regeneration. The hair cells that form express mature hair cell markers and are polarized, although there is a directional bias. Support cell proliferation is normal in these mutants. To determine how ccndx expression is induced we characterized a 4.3kb upstream region and identified two enhancers. Functional studies show that these enhancers are sufficient to drive neuromast expression. We identified transcription factor motifs in these enhancers that we are currently functionally testing.
Conclusions: These results show that hair cell regeneration in zebrafish is robust and even occurs in the absence of proliferation. However, in mammals proliferation of support cells may be required for successful regeneration, as otherwise their progenitor pool would be depleted. ccndx is an exciting candidate gene to induce proliferative regeneration in the mammalian ear.

SA208. Longer-Term Morphological Changes in Drug-Induced Hair Cell Regeneration Through Manipulation of Pou4f3 and p27Kip1 in Adult Sox2-CreER; Tdtomato Reporter Mice

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Category: Regeneration

Background: Hearing loss is a common condition in today's world, with approximately 1 in 5 people experience some sort of hearing loss. The only current treatments for hearing loss are either behavioral or bionic, with limitations to each approach. Non-mammalian species are able to recover lost hearing by regenerating cochlear hair cells (HC) from their surrounding supporting cells (SC), but this ability is lost in mammals. Thus, development of regenerative therapies would be of great benefit for this condition.
It has previously been shown that Atoh1 and Pou4f3 upregulation in conjunction with p27Kip1 downregulation can induce SCs to transform into a hair cell-like immature form (converting hair cells, cHC). We have previously shown Pou4f3 and p27 manipulation through our high throughput screening-found compounds, Compound 18 (C18) and Alsterpaullone 2-cyanoethyl (A2CE) respectively can induce a phenotype change in wild-type mice. Here we build upon those results using a Sox2-CreER; TdTomato (TdT) reporter mouse line.
Methods: Adult 4-10 week-old Sox2-CreER; TdT mice were induced with tamoxifen IP at P21 and unilaterally injected intra-tympanically with 5µl of a 600µM C18/5mM A2CE cocktail; contralateral uninjected ears served as controls. Mice were euthanized at either 8 or 12 weeks post-injection, and cochleae subsequently extracted for processing and analysis. Cochleae were immunostained with α-Myosin 7a (Myo7a) antibodies (AB) and corresponding Alexa 488 secondary AB; they were also stained with DAPI. Samples were scanned using a Leica SP8 confocal microscope. Analysis was focused on TdT/Myo7a double positive cells corresponding to cHC phenotype. Cell counts were done using Imaris software, and statistical analysis was done using Graphpad Prism.
Results: Both time-points show a robust cHC population in treated cochleae compared to controls; this has a clear basal-to-apical decreasing gradient and based on their location within the organ of Corti involves different supporting cell types, mainly Hensen's and 3rd row Deiters' cells. There is a large variation in cHC cell counts from mouse-to-mouse. There is little morphological difference between both time-points, as measured by both changes in cHC nuclear diameter and cell position.
Conclusions: Here we show further evidence of drug-induced SC-to-HC conversion using our novel C18/A2CE cocktail. The apparent lack of morphological progression (Δ in nuclear diameter, Δ in nuclear position, persistence of Sox2 expression) at these further time-points suggests an arrest in the conversion process and an immature hair-cell endpoint. To explore this phenomenon we will be performing single cell RNA sequencing on tamoxifen-induced, cocktail-treated cochleae. These results represent an important step on the road to developing a pharmaceutical solution to hearing restoration: we've been able to show in vivo that we can induce an SC-to-HC conversion using locally delivered drugs that can occur at least 3 months after induction.

SA209. Selective Targeting TrkC Receptors as a Disease-Modifying Tool for Noise Induced Hearing Loss
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Category: Regeneration
Background: Noise induced hearing loss (NIHL) is caused by neural degeneration characterized by the loss of inner ear hair cells and synaptic connection between cells and their afferent neurons. Neurotrophins (NTs) are a family of growth factors that drive neuronal survival, synaptic maintenance, and function in healthy systems, but can also cause synaptic loss and neuronal death in disease states. This novel paradoxical role of NT receptor signaling in neuronal survival and death makes it a challenging therapeutic target for NT-based neuroprotection strategy. In this study, we focus on characterizing the role of two NT receptor isoforms, TrkC-FL and TrkC.T1, which are both Neurotrophin-3 (NT-3) mediated receptors with opposite roles: full length TrkC-FL regulates survival signals, while truncated version TrkC.T1, pathologically up-regulated, promotes neuronal death. We evaluated both the neuroprotective and neurodegenerative mechanisms of these two NT-3 receptors in health and disease states by using novel TrkC.T1 knockout animal model and patented novel pharmacological tools to treat NIHL.
Methods: We have developed genetic and pharmacological strategies that selectively promote TrkC-FL protective function, while avoiding activating TrkC.T1 toxic function. We have also developed selective TrkC-FL agonists and TrkC.T1 antagonists that are patented and at late preclinical stage as promising drug leads. Our methods include the use of genetically-modified mouse models and selective stimulators of TrkC-FL to activate its neuroprotective mechanism and validate its therapeutic target in amelioration of disease both ex vivo and in vivo. Pharmacological validation of these targets will yield the added benefit of having developed potential drug leads useful for intervention in human diseases.

Results: We found significant upregulation of both isoforms in synaptic puncta and spiral ganglion neurons in response to acoustic insult. Using a genetic approach, we engineered knockout mouse of TrkC.T1 and studied functional hearing loss and synaptic damage of different genotypes (+/+, +/-, -/-). Our results showed that inhibiting expression of TrkC.T1 and increased expression of TrkC-FL protected mice from hearing loss and allowed faster synaptic recovery after NIHL trauma, and the result is validated by pharmacological approach by using specific TrkC-FL antagonist or TrkC.T1 agonist ex vivo.
Conclusions: Our results elucidate biological paradoxes of TrkC receptor isoforms and their role in NIHL, validate the concept and importance of selective targeting of TrkC growth receptor as a promising disease-modifying tool to treat NIHL. It validates the neurotrophin receptors as distinct target for medical therapy of hearing loss. Our pharmacological tools may yield therapeutic drug leads to develop novel activators and inhibitors to translational stage of new drugs to treat NIHL and have the potential to expand to treat other hearing loss models such as age-related, sensorineural and ototoxic induced hearing loss.

SA210. Transplanting ES Cell-Derived Inner Ear Organoids Into the Guineapig Cochlea

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Category: Regeneration

Background: Cochear hair cells are mechanosensitive receptors which convert mechanical sound vibrations into electrical impulses. Hair cell loss is the leading cause of hearing loss. To regenerate hair cells is the key for the therapy of hearing loss. To regenerate hair cells, we transplanted inner ear organoids into the cochlea. We differentiated mouse embryonic stem cells (mESCs) into inner ear organoids. Those organoids contained myosin-7a positive cells. We transplanted them into the guinea pigs' cochlea.
Methods: We differentiated mESCs into inner ear organoids as Jing N, Hashino E, et al. presented in 2017. Briefly, we added BMP-4, TGFβ inhibitor, FGF-2, Bmp inhibitor, and Wnt agonist step by step in 3D culture.
We transplanted those organoids to guinea pigs. Guinea pigs were deafened with kanamycin and furosemide before the transplantation. Transplanting organoids, we used a robot arm UR3 (Universal robots, Denmark). Because using a robot arm, it is possible to transplant organoids in proper position precisely. After animals were anesthetized, we opened the bulla and made a cochleostomy at the basal turn of the cochlea. We made a small hole at the osseus spiral lamina from the scala tympani. Through the hole, we transplanted cochlea organoids near the organ of Corti with a needle. One or four weeks after the transplantation, we collected cochleae. We decalcified them with EDTA. Then we stained them with anti-TuJ1, anti-GFP, and anti-myosin-7a antibody. We examined whether the transplanted cells survived.
We measured ABRs (Auditory Brainstem Responses) before deafferentation and before the transplantation, and every one week after the transplantation of organoids.
Results: About 3 weeks after the differentiation, cells formed organoids, and a part of organoids expressed myosin-7a. Transplanted cells were detected in the cochlea. The number of the remaining cells were limited. Hearing of the transplanted animals was almost no change after the transplantation.
Conclusions: Transplanting inner ear organoids can be a method for regenerating damaged hair cells. To increase the number of surviving cells, we have to find more suitable place and the condition for the transplanted organoids.

SA211. Inhibitor of Mitosis Reduces Supporting Cell Regeneration in the Neonatal Mouse Cochlea

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Category: Regeneration

Background: While the adult mammalian cochlea does not proliferate or regenerate, the non-mammalian cochlea can regenerate following damage by conversion of supporting cells (SCs) to hair cells through either mitotic regeneration or direct transdifferentiation. Prior work has shown that SC subtypes (inner phalangeal cells (IPhCs) and inner border cells) from the neonatal mouse cochlea can regenerate after damage. However, the mechanisms by which SCs are regenerated remain unknown. Here, we utilized a recently described mouse model (Lgr5-DTR) that allows for specific ablation and subsequent regeneration of SCs to assess the mechanisms (mitotic regeneration vs direct transdifferentiation) of SC regeneration.

Methods: In Lgr5DTR/+ mice, the human diphtheria toxin (DT) receptor is expressed specifically in inner pillar cells, inner phalangeal cells (IPhCs) and the third row of Deiters' cells. DT (4ng/g) was administered on postnatal day 1 (P1) to selectively ablate cochlear Lgr5+ SC subtypes. Cochleae were harvested at P4, and P7. Lgr5DTR/+; GLASTCre/+; R26RTdTomato/+ mice were used to fate-map GLAST+ cells in the greater epithelial ridge (GER). Mice were injected with DT and tamoxifen (0.2mg/g) at P1, and EdU at P3, P4, and P5. Lgr5DTR/+; Ki67Cre/+; R26RTdTomato/+ mice (DT at P1 and tamoxifen at P3) were used to fate-map proliferating cells. Additionally, Lgr5DTR/+ and WT littermate cochleae were administered DT at P1, dissected at P2, and cultured as whole organs with EdU for 48hr and 72hr in vitro. Aphidicolin, an inhibitor of mitosis, was added to the cell culture media. Regenerated IPhCs were quantified by counting Fabp7 positive cells after damage.
Results: After DT injection at P1, Lgr5DTR/+ cochleae had significant loss of IPhCs in apical (80.6%±3.9%), middle (79.2%±3.7%), and basal (70.9%±4.1%) turns at P4 in vivo compared to controls. At P7, IPhCs significantly regenerated to control levels in all three turns of the cochlea (81.9%±1.89% in apical, 91.3%±2.1% in middle, and 86.4%±3.6% in base). Fate-mapping studies revealed that many regenerated IPhCs arose from GLAST+ cells in the GER region. In both Lgr5DTR/+; GLASTCre/+; R26RTdTomato/+ and Lgr5DTR/+; Ki67Cre/+; R26RTdTomato/+ mice, ~50% of regenerated IPhCs had undergone proliferation (Edu+, Fabp7+, or Tomato+, Fabp7+ cells, respectively) in both apical and middle turns. In the base, only ~20% of regenerated IPhCs underwent proliferation. Furthermore, in organotypic cultures of P2 Lgr5DTR/+ cochlea, aphidicolin treatment significantly reduced regeneration of IPhCs in the apical and middle turns without impacting IPhC regeneration in the base.
Conclusions: After selective SC damage in the neonatal cochlea, cells in the GER regenerate inner phalangeal cells through mitotic and non-mitotic mechanisms, with the former significantly contributing to and required for regeneration in the apical and middle cochlear turns. Future directions will further investigate the effects of stimulating proliferation in these mouse models.

SA212. Plasticity in Recent Cochlear Implantees' Cortical Response to Speech and to Noise
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Category: Speech Perception
Background: Successful recognition of speech-in-noise (SiN) is critical to communication in a wide range of situations. While young adults with normal hearing (NH) are capable of recognizing SiN even in challenging scenarios, individuals with cochlear implants (CIs) often struggle when it comes to SiN processing. However, there is a large amount of variance in SiN performance that goes beyond explanation by the auditory periphery. Overall speech recognition improves in CI patients due to acclimatization that comes with continued device use, it is unlikely that changes in SiN performance are driven by the same mechanism. CI users may demonstrate plasticity in their neural responses during these challenging listening scenarios. An ideal model of plasticity may result in enhanced cortical responses to task-relevant speech and inhibited cortical responses to task-irrelevant noise. Changes in this internal signal-to-noise (SNR) ratio has been shown to predict SiN performance in NH listeners. Here we ask if the internal SNR of CI users changes as they adapt to the new input from their CI.
Methods: Thirteen recently implanted adult CI users were recruited and measured at both three- and six-months post implantation as part of a larger ongoing study. The task of interest for this study was the identification of isolated monosyllabic words in two different levels of multi-talker babble noise that always started one second earlier than the speech. While completing this task, EEG recordings were completed using a 64-electrode setup to measure cortical responses to the onset of noise and the onset of speech. We present sensor-space results examining changes in the amplitude ratio of cortical responses to speech and noise (internal SNR) between three- and six-months as well as results from source analyses.
Results: Between three- and six-month visits, the auditory evoked potential in response to noise onset decreased significantly on average. Meanwhile, in the same time period, the auditory evoked potential in response to target word onsets increase significantly on average. This results in the composite measure of internal SNR improving significantly on average as well. While there was a great deal of variability in the degree to which internal SNR changed between three- and six-month visits, all but one participant did show an increase in internal SNR. Preliminary source analyses suggest that activity in early auditory areas tend to

increase between three and six months while activity in the left inferior frontal gyrus tends to decrease between three and six months.
Conclusions: CI users do not simply retune their perceptual mapping of speech sounds to adapt to the novel input provided by their device. This plasticity allows CI users to selectively enhance relevant signals while suppressing irrelevant noise. Exploring the variance in patients' internal SNR may provide new options for improving outcomes in the future.

SA213. Effect of Attention and Working Memory Demands on Neural Decoding of Consonant-Vowel Syllables

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Category: Speech Perception
Background: Speech perception requires sustained attention and constant updating and manipulation of information stored which is also referred to as working memory. Working memory plays a critical role in speech comprehension and in complex cognitive tasks such as learning and reasoning. Effective speech perception relies on the interplay of utilization of working memory and attention control. However, little is known about how the brain process different speech information, specifically phonological features in active attentional tasks with working memory demands. Event related potentials (ERP) can be used to assess differential processing of speech sounds in the brain In the present study, we recorded ERPs to syllables presented in an n-back task, which engages sustained attention and working memory. We leveraged recent machine learning approaches to study how and at what timescale the brain decodes phonological information under high and low attention and working memory demands.
Methods: Electroencephalography (EEG) data from 64 channels was recorded while participants performed no-back and 2-back tasks. Twenty-two participants in the age range of 18-26 years listened to an auditory stream of six different CV syllables presented randomly in no-back condition. They were asked to press a button when they heard the same syllable as 2 prior in 2-back condition. So, 2-back condition requires sustained attention while memorizing and retrieving the speech sound constantly and comparing it with what they heard a few tokens before. The continuous EEG was further analyzed offline, downsampling and filtering. Independent component analysis (ICA) was performed to identify and remove the components associated with ocular movement and other artifacts and were epoched separately for different syllables. A linear support vector machine classifier was used to decode the syllables from the clean ERPs. The features used for the classification were the scalp-topographies of ERPs. The classification was performed for each time point in the ERP, to obtain a temporal waveform of classification across time. This enabled us to assess the timescale of the classification of the syllables in two conditions.
Results: Formal analysis is underway. We predict that the syllable decoding for the 2-back condition is stronger than the no-back condition. Further, this higher decoding is expected in the range of 80 -150 ms of the ERP which is attributed to phonological processing.
Conclusions: The effect of sustained attention on the decoding and classification of different syllables by the neural systems would have implications in terms of selective listening and speech perception in noise. The objectivity of the test to predict these effects used in the present study would be helpful in assessing the assess attention and working memory deficits, and also tracing the efficacy of rehabilitation in difficult-to-test populations.

SA214. Are Musical Abilities Related to Speech Prosody Perception? A Meta-Analysis

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Category: Speech Perception
Background: Studies investigating the relationship between musical abilities and speech prosody report that trained musicians show alterations or enhancements in the perception of prosody compared to non-musicians, or report positive correlations between abilities of music perception and prosody perception in populations overall. However, effect sizes differ between studies, and some studies find no such effects. We conducted a meta-analysis of previous research investigating the correlation between musical ability and the perception of speech prosody, with two aims: to assess the size of this effect across studies, and to examine

what factors contributed to the differential results found thus far. We expected a higher correlation for non-native compared to native prosody perception, due to potential ceiling effects in native perception. We also expected a higher correlation for music perception metrics compared to music training metrics, as music perception metrics seem to capture musical abilities more directly.
Methods: Studies of normal-hearing children and adults were systematically collected and relevant measurements were extracted. These included behavioral and electrophysiological measurements of prosody perception in native and non-native languages, including the perception of pitch, duration, and rhythm, with linguistic or emotional function. Measures of musical abilities included music training and music perception metrics. We used a multilevel random effects model to compute a summary effect of the included correlations (k = 472). Next, we added potential moderators as fixed factors to the model.
Results: The summary effect gave a significant positive correlation between musical ability and prosody perception (r = 0.37). However, a bias assessment indicated that effects sizes were skewed towards larger positive effects, possibly due to publication bias. The moderator analysis showed that non-native perception gave a significantly larger effect compared to native perception, and music perception measures showed a larger effect compared to music training, in line with our hypotheses.
Conclusions: Musical ability may especially benefit prosody perception in foreign languages, and moreover supports the use of music perception metrics as opposed to training metrics for empirical studies. Findings generally support the notion of transfer between the domains of music and speech in overlapping neural networks.

SA215. A Novel fNIRS Stimulus Presentation Protocol to Assess Speech Discrimination Ability in Sleeping Infants

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Category: Speech Perception
Background: The ability to differentiate one sound from another is important for normal language development in infants. We have previously shown that speech discrimination ability can be measured in sleeping infants using functional near-infrared spectroscopy (fNIRS) using a habituation/dishabituation stimulus presentation paradigm. However, that paradigm is time-consuming and not suitable for testing individual infants. The present study adopted a "non-silence baseline" paradigm. This paradigm saves time by presenting novel stimuli between a baseline of repeated standard stimuli with no silence periods. The discrimination response is the fNIRS response following the change from standard baseline to novel stimulus block. We hypothesised that fNIRS speech discrimination responses in individual sleeping infants can be elicited using this non-silence baseline paradigm.
Methods: We recorded fNIRS data from 16 sleeping infants with no known hearing loss. Three consonant-vowel (CV) syllables were used as novel speech stimuli, and one CV syllable was used as a standard speech stimulus. For each novel stimulus, a stimulus block was formed by concatenating six novel and six standard stimuli in alternating order. The stimulus block for each novel stimulus was repeated for 20 trials in a pseudo-randomised order yielding a total of 60 trials.
Results: The group average fNIRS response showed the novel stimulus block evoked a positive oxyhaemoglobin (HbO) response that peaked around 7 s and an anti-correlated deoxyhaemoglobin (HbR) response with delayed latency in comparison to the HbO. There was also a systematic change in response morphology with repeated stimulus presentation during the experiment. Analysis of individual infant's data with a novel statistical analysis technique showed that individual accuracy of the test was greater than 90%.
Conclusions: Speech discrimination ability of individual sleeping infants can be measured using fNIRS when presented with the non-silence baseline paradigm. Further work is being conducted to further enhance the accuracy of this paradigm.

SA216. Does Bimodal Stimulation Improve or Interfere With Consonant Perception?

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Category: Speech Perception
Background: Bimodal stimulation, the usage of both a cochlear implant (CI) and hearing aid (HA) in the contralateral ear, can improve speech perception in quiet and in noise, but there is variability in this benefit.

This has not been systematically investigated at the phoneme level. In a previous study of vowel perception, bimodal listeners showed both improvement and interference in the bimodal condition compared to the best monaural condition (Reiss et al 2016). The goal of this study was to investigate whether consonant perception shows improvement, averaging, or interference under bimodal stimulation and how this varies by the consonant.
Methods: Eight adult bimodal listeners were tested under three conditions: Bimodal, CI-only, and HA-only. Two experiments were conducted. The first experiment, using natural consonants, measured the ability to correctly identify 16 consonants. Each was presented in an /aCa/ format, by four different speakers, for a total of 64 stimuli per run, with four repeat runs per condition. Listeners were asked to indicate the consonant sound perceived. Total percent correct and linguistic features such as manner, voicing, and place were analyzed. The second experiment, using synthetic consonants, measured classification along a continuum between consonant pairs, specifically sa-sha and wa-ya. Each continuum consisted of eight consonant-like stimuli transitioning between sa and sha, or wa and ya, with 10 repeats per stimulus for a total of 80 stimuli per run, and two repeat runs per condition. Listeners were instructed to select which of the two consonants they heard. Responses were plotted versus the stimulus number in the continuum.
Results: In experiment 1, the bimodal condition had the greatest mean at 70.01% ± 15.5, followed by CI only at 61.81% ± 16.1, and the HA-only condition with a mean of 55.48% ± 18.7. Confusion matrix patterns indicate that 5/8 benefited from bimodal stimulation, 2/8 showed ear dominance with no benefit of bimodal stimulation over the best ear condition, and 1/8 showed interference with a worse score in the bimodal condition compared to the best ear condition. In experiment 2, response curves were steeper in the bimodal condition compared to the monaural conditions for 3/7 subjects for sa-sha and 2/6 subjects for wa-ya, averaged for 2/7 subjects for sa-sha and 2/6 subjects for wa-ya, and shallower indicating interference in 2/7 subjects for sa-sha and 2/6 for wa-ya. Subject numbers varied by pair since some subjects were not able to discriminate between both pairs.
Conclusions: As seen previously for vowel perception, subjects varied in whether they showed benefit or interference under bimodal stimulation, which varied with the specific consonant. This variability may be due to external factors such as experience, binaural fusion, and programming. Supported by NIH R01DC013307 and Everts-Smith Medical Student Fellowship.

SA217. Differential Benefits of Unmasking Extended High-Frequency Content of Target Vs. Masker Speech

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Category: Speech Perception
Background: Current evidence supports the contribution of extended high frequencies (EHFs; >8 kHz) to speech recognition, especially for speech-in-speech scenarios. However, it is unclear whether the benefit of EHFs is due to phonetic information in the EHF band, talker segregation cues, or both. This study investigated the mechanisms of benefit derived from EHF cues by creating a substantial mismatch in EHF level between target and masker talkers. We also examined the relationship between EHF cues and number of masker talkers in a speech-in-speech recognition task.
Methods: We tested speech recognition in four filtering conditions using full-band (FB) speech and speech low-pass filtered at 8 kHz (LP8k): (1) FB target, LP8k masker; (2) FB target, FB masker; (3) LP8k target, FB masker; (4) LP8k target, LP8k masker. We hypothesized that EHFs provide talker segregation cues, predicting better performance in filter-mismatched conditions (1, 3) than in corresponding filter-matched conditions (2, 4). We also hypothesized that EHFs provide phonetic information, predicting better performance in conditions with the FB target (1, 2) than corresponding conditions with the LP8k target (3, 4). We tested whether performance in these conditions was affected by the number of masker talkers (one vs. two), hypothesizing that listeners would perform better with a single masker talker overall, but effects of EHF cues would be greater for the two-talker masker.
Results: With the one-talker masker, mean speech reception thresholds (SRTs) were similar for the four conditions, with values of -19.0 to -20.8 dB SNR. In contrast, SRTs differed by up to 5.5 dB across conditions with the two-talker masker, with values ranging from –6.6 to –12.1 dB SNR. Performance was best when the target was full-band and the masker was low-pass filtered at 8 kHz, but this effect was more

pronounced with the two-talker masker. Results from a word-by-word analysis of data from the two-talker masker indicated higher probability of recognition with increasing EHF energy level in the target word, particularly when the masker was low-pass filtered.
Conclusions: A large mismatch in EHF level between target and masker speech improved speech recognition only when the target contained EHFs, particularly with a two-talker masker. These data support the hypothesis that EHFs convey phonetic cues to improve speech-in-speech recognition, but the data provide little evidence that EHFs convey segregation cues. Another possibility is that a high level of EHFs marks a given speech signal as important, thereby seizing the listener's attention, however, our data suggest this effect would be small. EHF cues provide a greater benefit for masked speech recognition with a two-talker masker vs. one-talker masker, perhaps due to an increase in informational masking associated with a two-talker masker.

SA218. Adaptation to Ecological Noises in Speech Recognition

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Category: Speech Perception
Background: The recognition of isolated words in noise improves as words are delayed from the noise onset. This improvement, referred to as 'adaptation to noise', can result in an increase in the proportion of recognized speech tokens of up to 30%. Despite its potential relevance for everyday listening, it is uncertain if and to what extent adaptation occurs for realistic, 'ecological' noises. The aim of the present study is to investigate this.
Methods: For 22 normal-hearing listeners (mean age = 26.4 years; SD = 5.9 years), we measured speech-reception thresholds (SRTs) (signal-to-noise ratio at 50% correct disyllabic word recognition) for natural or tone-vocoded words delayed 50 or 800 ms from the noise onset, referred to as 'early' and 'late' conditions, respectively. The vocoding was intended to preserve envelope but not natural temporal-fine-structure speech cues. Adaptation was estimated as the SRT improvement in the late relative to the early condition. Six 'ecological' noises from public repositories were selected based on their similarity with speech in terms of their long-term spectrum and level fluctuations.
Results: For natural words, adaptation was small overall and similar across the different noises (~0.5 dB). For vocoded words, adaptation was greater for the angle-grinder noise (1.9 dB), the international female fluctuating masker (IFFM) (1.6 dB), or the hair-dryer noise modulated by the IFFM envelope (1.3 dB), than for the shopping-center (1.1 dB), hair-dryer (0.9 dB), or steady-state speech-shaped (0.9 dB) noises. Incidentally, the three noises that produced greater adaptation had different long-term spectra across them, but their modulation spectra were closer to that of speech than the modulation spectra of the three noises that produced less adaptation, which were stationary in level.
Conclusions: The similarity between the modulation spectra of the noise and speech is more important for adaptation to noise to occur than the similarity between the long-term spectra of the noise and speech, at least when speech recognition relies on envelope cues. [Work supported by the Spanish Ministry of Science and Innovation (grant PID2019-108985GB-I00), and the European Regional Development Fund.]

SA219. Listening Effort in Multilinguals

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Category: Speech Perception
Background: Understanding speech in adverse listening conditions, such as background noise, may pose challenges for listeners. This is especially the case for multilinguals who can speak more than one language. Specifically, the literature shows that multilinguals experience speech perception difficulties in noisy conditions, especially in their L2, despite performing well under quiet conditions. In this study, we aimed to understand the effort exerted by multilinguals in quiet and in noise. Listening effort is defined as the cognitive resources required to perform a listening task and is known to be involved even when perceptual performance is not affected. Thus, studying how effort is implicated in multilinguals' performance may uncover challenges in speech processing that may not be revealed if just perceptual performance is examined.
Methods: Twenty-eight undergraduate students participated in the study: 18 Arabic-Hebrew-English multilinguals and 10 Hebrew-English bilinguals. Arabic-Hebrew-English participants were native Arabic

speakers, partially immersed in a Hebrew-speaking environment, who learned English in a formal setting. Hebrew-English participants were native speakers of Hebrew who learned English as a second language. During the study, listening effort, using both subjective ratings and pupillometry, was assessed in a perceptual task that involved listening to words and sentences presented in quiet and in noise. Arabic-Hebrew-English students were examined in Arabic, Hebrew, and English, and Hebrew-English participants were tested in Hebrew and English.
Results: Pupil data and subjective ratings indicated that listening effort was increased in participants' non-dominant language/s relative to the more dominant one/s. This was observed even in the quiet condition, where perceptual performance was previously shown to be comparable across languages. Specifically, among Arabic-Hebrew-English participants the pupil dilation was greater when listening to English stimuli than Hebrew ones and in Hebrew compared to Arabic. Also, among those participants, subjective ratings of effort were the highest in English and the lowest in Arabic. Among Hebrew- English bilinguals the same pattern of results was observed, with greater pupil dilation and higher subjective ratings of effort in English than in Hebrew. In addition, the findings corroborated that effort was modulated by differences in listening conditions and stimulus complexity. Specifically, increased effort was observed during processing of noisy stimuli compared to those presented in quiet, and during processing of sentences compared to words in isolation.
Conclusions: Multilinguals exerted greater listening effort in less dominant languages, even in quiet, suggesting that assessing listening effort offers the possibility to reveal differences even when those do not emerge in perceptual performance. Further, with multilingualism becoming the global norm, increased listening effort in these populations should be considered in educational settings and workplaces, where these individuals regularly deal with adverse conditions and learn in their non-dominant language.

SA220. Effect of Voice Training on Voice Cue Perception and Listening Effort in Vocoder Degraded Speech

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Category: Speech Perception
Background: Normal hearing listeners can effectively use fundamental frequency (F0) and vocal-tract length (VTL) voice cues to segregate the target speech from the masker speech in multiple-talker situations. However, when speech signal is degraded, such as in cochlear implants or vocoder-listening, sensitivity to F0 and VTL is reduced and listening becomes more effortful, likely contributing to difficulties in understanding speech. Previous studies show that listening to familiar voices, either personally familiar or due to exposure in an implicit or explicit voice training, can improve speech intelligibility in normal hearing. We investigated how voice training affects voice cue sensitivity and listening effort for non-vocoded and vocoded speech.
Methods: As an implicit short-term voice training, normal-hearing participants listened to an audio book and answered content related questions for 30 minutes. Subsequently, voice sensitivity (via just-noticeable-differences, JNDs, for F0+VTL) and listening effort (via pupillometry) were measured with the trained voice or an untrained voice as a reference, in both non-vocoded and vocoder-degraded versions. The JNDs were measured with an adaptive 3 alternative forced choice odd-one-out task, with fixed or variable CV triplets across three choice items. Pupillometry data were quantified in peak pupil dilation and latency, but also by Generalized Additive Mixed Models.
Results: F0+VTL JNDs were significantly larger for vocoded than non-vocoded conditions, and with variable items than fixed items. Contrary to our expectations, based on previous work on voice familiarity and training effects on speech intelligibility, voice training did not have a significant effect on voice cue sensitivity. Over the time course of pupil dilation response, analyzed with Generalized Additive Mixed Models, pupil dilations were significantly larger during voice discrimination while listening to untrained, vocoded speech than listening to trained, vocoded speech. No such effect was observed with non-vocoded speech.
Conclusions: These findings imply that the short voice exposure by listening to a story, as implemented in this first study within this line of research, seems not sufficient to improve voice cue sensitivity. Perhaps more intense training, using personally familiar voices, or using the same linguistic materials and tasks in training and testing, could lead to such improvement. Alternatively, voice discrimination tasks where small

F0+VTL differences are measured might not be sufficiently sensitive to reflect such improvement and voice training benefits could be better observed for speech intelligibility. On the other hand, this specific voice training seemed to provide an improvement in listening effort. Even in the absence of a clear benefit in JNDs, voice discrimination among vocoded voices was observed to be less effortful with short-term voice training. In a follow-up study, the effect of explicit and implicit voice training on speech-on-speech perception and listening effort is being investigated with normal-hearing listeners, and the preliminary data will be presented.

SA221. Brainstem Evoked Auditory Potentials in Tinnitus: A Best-Evidence Synthesis and Meta-Analysis

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Category: Tinnitus

Background: Accumulating evidence suggests a role of the brainstem in tinnitus generation and modulation. Several studies in chronic tinnitus patients have reported latency and amplitude changes of the different peaks of the auditory brainstem response, possibly reflecting neural changes or altered activity. The aim of the systematic review was to assess if alterations within the brainstem of chronic tinnitus patients are reflected in short- and middle-latency auditory evoked potentials (AEPs

Methods: A systematic review was performed and reported according to the PRISMA guidelines. Studies evaluating short- and middle-latency AEPs in tinnitus patients and controls were included. Two independent reviewers conducted the study selection, data extraction, and risk of bias assessment. Meta-analysis was performed using a multivariate meta-analytic model.

Results: Twenty-seven cross-sectional studies were included. Multivariate meta-analysis revealed that in tinnitus patients with normal hearing, significantly longer latencies of auditory brainstem response (ABR) waves I (SMD = 0.66 ms, $p < 0.001$), III (SMD = 0.43 ms $p < 0.001$), and V (SMD = 0.47 ms, $p < 0.01$) are present. The results regarding possible changes in middle-latency responses (MLRs) and frequency-following responses (FFRs) were inconclusive.

Conclusions: The discovered changes in short-latency AEPs reflect alterations at brainstem level in tinnitus patients. More specifically, the prolonged ABR latencies could possibly be explained by high frequency sensorineural hearing loss, or other modulating factors such as cochlear synaptopathy or somatosensory tinnitus generators. The question whether middle-latency AEP changes, representing subcortical level of the auditory pathway, are present in tinnitus still remains unanswered. Future studies should identify and correctly deal with confounding factors, such as age, gender and the presence of somatosensory tinnitus components.

SA222. Visual Cross-Modal Re-Organization in Young Adults With Normal Hearing and Minimal Tinnitus

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Category: Tinnitus

Background: Cross-modal reorganization occurs when the central resources of an intact sensory modality recruit the resources of a deprived sensory modality for compensatory purposes. In particular, visual cross-modal re-organization has been demonstrated to occur in acquired, post-lingual hearing loss, as adults with high-frequency hearing loss demonstrate activation of auditory cortex while passively observing a visual stimulus (Campbell and Sharma, 2014, 2020). However, these studies did not exclude participants with tinnitus, a pathology in which phantom sound is perceived in the absence of an external stimulus, and which is highly comorbid with hearing loss (Eggermont and Roberts, 2015). Research has shown that individuals with normal hearing may also suffer from tinnitus (Campbell et al., 2019). Because these individuals do not present with auditory deficits known to drive re-organization, it is unclear whether this neurophysiologic phenomenon may contribute to tinnitus in this population.

Methods: 16 participants with normal hearing and minimal tinnitus (TINN) (Grade 1 on the Tinnitus Handicap Inventory [THI; Newman et al., 1996]). 32 participants with normal hearing and no tinnitus (NTINN).

Participants viewed a high contrast sinusoidal concentric grating that morphed into a radially modulated grating or circle-star pattern. Visual evoked potentials (VEPs) were analyzed in an occipital region of

interest (OROI), left temporal region of interest (LTROI), and right temporal region of interest (RTROI). VEP amplitude and latency were recorded for P1, N1, and P2 peaks in each ROI.
VEP components were compared between TINN and NTINN groups. The latency of the VEP N1 peak latency in the RTROI was correlated with THI scores in the tinnitus group.
Results: P2 latency was significantly increased in the TINN group at the LTROI. P2 latency approached significance as it increased in the TINN group in the RTROI.
The VEP N1 component has been shown to be a biomarker of visual cross-modal re-organization in several studies (Buckley and Tobey, 2011; Campbell and Sharma, 2013, 2016). Specifically, decreased N1 latency in the right temporal region has been correlated with auditory behavior (Campbell and Sharma, 2016).
Conclusions: Two main results: 1) P2 latency was increased in the TINN group in both temporal ROIs, and 2) Decreased N1 latency in the RTROI was significantly correlated with increased tinnitus severity. The second finding is indicative of visual cross-modal re-organization in the right hemisphere to be related to tinnitus severity, even in the presence of normal hearing. It appears that this re-organization may serve a compensatory function in tinnitus, specifically in the cognitive domain.

SA223. Characterizing Complex Tinnitus Sounds Using Reverse Correlation: A Feasibility Study
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Category: Tinnitus
Background: Tinnitus affects an estimated 25 million people in the U.S. and can have a major impact on quality of life. Clinical practice guidelines for tinnitus management recommend sound therapy and cognitive behavioral therapy, both of which involve targeted exposure to external sounds. Critically, treatment outcomes have been shown to improve when the sounds used in treatment more closely match the internal tinnitus experience of the patient – e.g., the constituent frequencies of the psychoacoustic tinnitus spectrum (PTS). Current methods for characterizing tinnitus percepts (e.g., pitch matching) are most appropriate for patients with a pure-tone or narrow-band PTS. However, for many patients, evidence suggests that the PTS has a more intricate, non-tonal structure that is not easily characterized using existing methods. Here, a proof-of-concept study is described for characterizing the PTS using Reverse Correlation, a method widely used in psychophysics for unconstrained characterization of complex internal percepts.
Methods: Three (n=3) normal hearing subjects each participated in two (2) Reverse Correlation experiments, in which they performed 2000 trials (20 blocks x 100 trials/block). In each trial, subjects listened to a target tinnitus-like sound followed by a random noise stimulus. Subjects were asked to decide (i.e., “yes” or “no”) whether the target sound was present in the stimulus. Target sounds comprised examples from the American Tinnitus Association, labeled as “roaring” (experiment 1) and “buzzing” (experiment 2), chosen for their broad-band frequency spectra. Stimuli were constructed by randomly assigning power levels (-20dB or 0dB) to each of 100 Mel-spaced frequency bins between 0.1 to 13 kHz. The PTS was estimated using both conventional linear regression (LR) and a novel compressive sensing (CS) approach. Pearson's correlation coefficient (r) was used to assess estimation quality by comparing PTS estimates to their corresponding target sounds. One-sample, right-tailed t-tests were performed on the mean Fisher-transformed r values across subjects to assess significant differences from zero.
Results: For experiment 1 ("roaring"), r values using LR estimation were 0.57, 0.45, 0.62 for each subject respectively (0.54±0.09; $t(2)=8.74$, $p<0.01$), while r values using CS estimation were 0.64, 0.64, 0.72 (0.67±0.04; $t(2)=16.22$, $p<0.01$). For experiment 2 ("buzzing"), r values using LR estimation were 0.22, 0.58, 0.42 (0.41±0.18; $t(2)=3.51$, $p<0.05$), while r values using CS estimation were: 0.32, 0.73, 0.67 (0.57±0.22; $t(2)=3.78$, $p<0.05$). All r values were significant within-subjects ($p << 0.01$).
Conclusions: Reverse Correlation may allow accurate characterization of non-tonal tinnitus-like sounds. Further, results indicate that a CS approach can improve characterization accuracy over conventional approaches without requiring more trials. This strategy has broad clinical applications and may form the basis for a behavioral assay to characterize a wider variety of tinnitus percepts than currently possible. Future work will focus on validating this approach in patients suffering from tinnitus.

SA224. Medial Olivocochlear Neurons May Protect Against Tinnitus

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Category: Tinnitus
Background: Tinnitus is the often-debilitating perception of sound in the absence of a physical stimulus, which affects approximately 15% of adults in the United States. Medial olivocochlear (MOC) neurons, located in the ventral nucleus of the trapezoid body are cholinergic efferent neurons that synapse on cochlear outer hair cells (OHCs) and protect against noise damage by altering OHC electromotility. Studies using distortion product otoacoustic emissions have suggested that humans suffering from tinnitus have heightened MOC activity compared to humans without tinnitus. Animal models of tinnitus have shown conflicting results with decreases or no change in MOC activity. The objective of this project is to examine MOC activity in guinea pigs, with and without behavioral evidence of tinnitus, after noise exposure compared to control animals by examining choline acetyltransferase (ChAT) intensities in the region of OHCs.
Methods: Guinea pigs were exposed to unilateral narrow band noise at 102 dB SPL for 2 hours to induce temporary threshold shifts and tinnitus. Gap prepulse inhibition of the acoustic startle (GPIAS) was used to determine the tinnitus status of the animals pre- and post-noise exposure twice a week for 14 weeks. Non-exposed animals served as controls. Animals were then euthanized, and their cochleae removed and fixed in 2% PFA, dissected, and prepared for whole mount immunofluorescence using goat anti Chat, mouse anti CtBP2, mouse anti GluA2 antibodies, and phalloidin. Frequencey-relevant areas were determined by a cyctocochleogram, followed by imaging on a Lecia SP8 confocal microscope to collect Z-stack images for analysis.
Results: ChAT intensity beneath the OHCs was significantly increased in noise-exposed animals that did not develop tinnitus compared to those that developed tinnitus and the non-exposed controls. The increases were observed primarily in the contralateral ears for OHC rows 1 and 2, as well as row 1 of the ipsilateral cochleae. The effects were only seen in the apical half of the cochlea.
Conclusions: The results of this study suggest that animals that do not develop tinnitus after noise exposure have significantly increased MOC activity compared to noise exposed animals that develop tinnitus and sham-exposed animals. These bilateral increases suggest a compensatory increase in MOCs in response to noise exposure that may protect against the development of tinnitus, in contradiction to human DPOAE studies that suggest increased MOC activity in tinnitus subjects. These results suggest that further research is warranted into how MOC projections might contribute to tinnitus resilience.

SA225. Drug Discovery for Noise-Induced Tinnitus Based on the SBAD Model

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Category: Tinnitus
Background: Subjective tinnitus, a perceptual disorder of auditory phantoms, is the number one disability among Veterans, affecting at least one in every 10 American adults. Although hearing loss is not present in every patient with tinnitus, tinnitus is highly associated with hearing loss. For example, veterans have higher rates of tinnitus than the public due to increased noise levels they encounter while in service, including gunfire, machinery, aircrafts, and engines. There are currently no FDA approved drugs for tinnitus, partly due to a lack of dependable animal detection models. Previously, we developed and validated a behavioral-based tinnitus detection paradigm for animals called sound-based avoidance detection (SBAD). SBAD is a dual paradigm that uses negative reinforcement (electrical shocks) to infer tinnitus (silent trial) while monitoring potential confounding variables including alertness, motivation, motor functioning, and memory (sound trial). Using this validated method, we screened several classes of drugs for their effects again tinnitus.
Methods: We trained mice in a shuttle box with operant conditioning. Unilateral noise exposure (4-25 kHz, narrow band noise, 116 dB) was used to induce tinnitus in mice upon successful training. Tests in the shuttle box were performed at 1 to 4 months for tinnitus detection.
Results: Tinnitus-positive and control mice were treated with saline and various calcium channel blockers and underwent SBAD re-testing to determine their effectiveness in diminishing tinnitus behavior. Our findings indicate that tetrandrine (TET), a drug approved in China for silicosis, hypertension, and

inflammation, is effective in treating noise-induced tinnitus. However, TET has multiple molecular targets, and it is difficult to identify the specific targets that engage in tinnitus suppression. Subsequently, we found that one class of FDA-approved drugs targeting a specific calcium channel type produced similar therapeutic effects as TET in our mouse model with dose-dependent efficacy.
Conclusions: Thus, our pharmacological approach implicated this calcium signal pathway underlying noise-induced tinnitus. We are now applying both single cell RNA sequencing and single molecule fluorescence in situ hybridization to independently validate our findings and explore possible new molecular targets involved in tinnitus.

SA226. Basal Ganglia Contribution to Auditory Phantom Perception? A Volumetric Subcortical Study of Tinnitus in an Elderly Chilean MRI Cohort

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Category: Tinnitus
Background: Tinnitus is the persistent phantom perception of sound with no external auditory stimuli present. Its prevalence in the population reaches up to 14%, it increases with age and is commonly accompanied by hearing loss and cognitive decline. Although tinnitus has traditionally been considered a peripheric inner-ear problem, an increasing body of evidence from animal and human studies has shown that the central nervous system's neuroplasticity is key to understanding its pathophysiology. Interestingly, some neuroimaging studies have reported structural cortical differences beyond auditory areas, consistent with the "gating hypothesis" of tinnitus, which proposes that the frontostriatal network acts as a gatekeeper of sensory inputs. While structural changes in frontal areas support this hypothesis, a characterization of gray matter differences in subcortical areas -including the auditory pathway and basal ganglia- is still lacking.
Methods: We address this by analyzing brain volume on an MRI dataset from the Chilean ANDES cohort of cognitively healthy elderly subjects (N=106). The ANDES (Auditory and Dementia study) project is a prospective cohort of non-demented Chilean. We use MAGNETOM Skyra 3-Tesla MRI Scanner. Air conduction pure tone audiometric (PTA) hearing thresholds were evaluated, and frequencies at 0.5, 1, 2, and 4 kHz were calculated for each subject in both ears. To determine the structural gray matter changes, we preprocessed the data with both FreeSurfer and SPM and measured gray matter volume from Desikan-Killiany parcellation and a subcortical mask for auditory pathway regions. Auditory brainstem responses waveforms were averaged with alternating clicks presented at supra-threshold levels (2000 repetitions, 80 dB nHL, bandpass 0.1–3 kHz, stimulus rate 21.1 Hz, EP25, Eclipse, Interacoustics®). The amplitudes of waves I and V were measured from peak to trough. The Tinnitus group was defined by a subjective report obtained in a personal interview of the participants done by an otorhinolaryngologist expert.
Results: We found no significant differences between age, hearing loss, and auditory brainstem responses between groups. Interestingly, we found a significant volumetric increase in the tinnitus group's superior frontal gyrus and caudate, putamen, and pallidum areas but not in auditory pathway areas. Consistent with the gating hypothesis, these volumetric changes were correlated across subjects beyond global intracranial volume, suggesting a common frontostriatal network. Mixed model analysis revealed that a significant interaction between age and hearing loss was found in bilateral pallidum only for the tinnitus group, suggesting that older and more hearing impaired subjects had gradually bigger pallidum
Conclusions: Overall, our results show a broader picture of tinnitus pathophysiology, suggesting a frontostriatal network that putatively impairs sensory gatekeeping mechanisms that could underlie the phenomenology of auditory phantom perception.
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SA227. Temporal Integration of Multisensory Stimuli in Migraine

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Category: Vestibular: Basic Research and Clinical

Background: Multisensory cues generated by a single event arrive at the brain asynchronously due to variable delays in transmission, encoding, and processing. The brain must accommodate for these asynchronies in order to form a maximally useful impression of the surrounding world. The time offset over which multiple sensory inputs are interpreted as "synchronous" is known as the temporal binding window (TBW). Using flash-beep combinations of stimuli, abnormally prolonged TBW has been observed in patients with autism, dyslexia, and schizophrenia, suggesting these conditions may reduce the ability to accurately synthesize incoming information from multiple sensory modalities.
Migraine is a condition that typically involves heightened sensitivity to various sensory stimuli. For example, patients with migraine often demonstrate dramatic responses to visual and auditory stimuli, and recent work has shown better-than-average sensitivity to some vestibular stimuli. Patients with migraine also tend to have difficulty processing multisensory stimuli. For these reasons, we hypothesized that patients with migraine might have abnormal (widened) TBWs, leading to sensory confusion and potentially offering an explanation for their particular sensitivity to multimodal sensory stimuli.
Methods: In this preliminary study, we compared the TBWs of patients with migraine to normal controls using visual + vestibular stimuli (10 ms light flash and 1 Hz raised cosine yaw rotation about the earth-vertical axis) and visual + auditory stimuli (10 ms light flash and 10 ms 1000 Hz tone burst) . We also compared the vestibular sensitivity of our subjects to their TBW involving vestibular stimuli, a relationship that previously has proven robust among patients with vestibular hypofunction.
Results: Data were collected from 9 people, among whom three met criteria for classic migraine. None of these patients met criteria for vestibular migraine or persistent postural-perceptual dizziness. We found a strong correlation between the TBWs involving visual + vestibular stimuli and visual + auditory stimuli ($r=0.98$, $p<0.001$). Subjects with migraine had higher TBW than normal subjects (mean (SD); 287 ms (187) and 147 ms (132), respectively, for the visual + vestibular TBW). As opposed to previous work demonstrating that vestibular thresholds to roll+pitch motion were improved in migraine patients, we found that their thresholds to our yaw rotations were higher than average. Unlike in patients with more dramatic vestibular loss, we did not find convincing evidence that our patients' sensitivity to rotations was related to their TBW ($r=0.65$, $p=0.08$).
Conclusions: We conclude that the individual TBW "set points" are wider in patients with migraine than in those without, offering a possible explanation for their difficulty managing multisensory input and manifesting high levels of motion sickness. The TBW can be reduced with training, offering a possibly remedy for motion sickness and other migraine-related symptoms.

SA228. Sensitized Metrics for Detecting Aminoglycoside-Induced Vestibular Loss in Patients With Cystic Fibrosis: A Preliminary Study

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Category: Vestibular: Basic Research and Clinical
Background: Intravenous aminoglycosides (IV-AGs) are routinely used for management of Pseudomonas aeruginosa infections in persons with cystic fibrosis (CF). Known adverse events from these treatments may include ototoxicity, leading to tinnitus, hearing and/or vestibular loss. Identification and management of vestibular loss in CF is clinically limited. The aim of this preliminary study is to compare evidence of vestibular loss using conventional and novel metrics in patients with CF and a history of balance complaints and IV-AG therapies.
Methods: Five participants with CF (33-43 yr.; 4 females) exposed to IV-AGs were enrolled. Conventional vestibular testing included video head impulse test (vHIT) assessment of horizontal canal function. Novel metrics included custom vestibular psychophysical (VPP) testing and balance assessment using central sensorimotor integration (CSMI) tests. VPP quantified a subject's ability to perceive and detect the direction of low-amplitude rotations about an earth-vertical yaw-axis with a motion trajectory. This was defined by a single-cycle 1-Hz raised-cosine stimulus velocity whose amplitude was adaptively adjusted on sequential trials following a Parameter Estimation by Sequential Test (PEST) paradigm of 100 trials performed in the dark. VPP test results were analyzed to estimate the vestibular threshold of motion detection. The CSMI test quantified sensory contributions, motor activation, and time delay properties of the balance control system based on body sway responses to 12 continuous 20-s duration, 2° (peak-to-peak) amplitude pseudorandom

cycles of tilts of the stance surface and/or a visual surround. Two CSMI tests were performed: 1) surface-tilt with eyes closed (SS/EC) and 2) combined surface-tilt and visual-tilt with eyes open (SS+VS/EO). Both test conditions allow for the estimate of the vestibular contribution to standing balance.
Results: Four subjects had normal mean vHIT (Mean=0.95, SD=0.036) results except for the most symptomatic patient who showed reduced mean vHIT gain (0.66). In contrast to vHIT results, VPP findings showed elevated thresholds in four subjects (Mean=4.85 deg/s, SD=4.17; control subject mean=1.11 deg/s), and one within normal limits (0.92 deg/s). For CSMI, the values for SS/EC and SS+VS/EO are 49% and 45%, respectively. The mean values across the CF subjects was slightly less than published control data at 45% and 41%, respectively. However, the mean time delay of 174 ms was nearly 2 SD above the mean for controls (Mean+2SD=176 ms). The motor activation 'stiffness' factor tended to be lower than controls with the stiffness factor indicating the magnitude of corrective joint torque generated per unit of sensory-derived body sway. The combination of reduced vestibular contribution and reduced stiffness lead to subjects being particularly sensitive to balance disturbances.
Conclusions: Preliminary results support the notion that VPP and CSMI methods can identify changes in motion detection and balance control that may be indicative of vestibular damage that is not identified using conventional vHIT.

SA229. Clustering of Patients Presenting Dizziness and Vertigo: A Retrospective Study From 872 Patients

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Category: Vestibular: Basic Research an Clinical
Background: Dizziness and vertigo affect ~15% to 20% of adults yearly. In clinical practice, description of the symptoms relies on anamnesis. The interview performs by the clinician allowed to specify the nature of dizziness and vertigo (rotation sensation, instability, duration of crisis, triggered by head movement, related to deafness, tinnitus, photophobia or headache). Neurovascular risk factors (overweight, hypertension, etc.) and neurological problems (sequelae of stroke, Parkinson, multiple sclerosis) are also questioned. Like the cochlea, vestibule responds to different stimulation frequencies related to the head movements. Frequency spectrum analysis of the vestibular system are based on various tests such as the caloric irrigation for the evaluation of low frequencies, the rotatory chair movements (or the kinetic test) for the evaluation of medium frequencies and the video head impulse test (vHIT) for the evaluation of high frequencies. Here, we compare a knowledge-driven analysis from the clinician expertise and data-driven analysis resulting from machine learning investigations based on auditory and vestibular data.
Methods: In this work, we performed retrospective study from 872 patients consulting for dizziness or vertigo in an ENT practice. The data were anonymized by the clinician, centralized by the data analyst, and stored on secure data server. Patients from 8 to 100 years old (72 ± 15 years old) and both sex (65% females) were included in the study. The medical history of the patients collected during clinical interview was expressed as categorical values in a data base for analysis. For all the patients, pure tone audiogram was measured from 250 Hz to 8 kHz for both ears. Vestibular disorder was evaluated by using calorimetry, rotative and vHIT tests. Ocular functions were monitored using eye tracking in pursuit and saccade tasks. Data processing was carried out using Matlab.
Results: Different patient populations were described according to their age, sex, characteristics of the crisis, risk factors and etiology. Then, various principal component analysis was performed from i) the pure tone audiograms of the patients (2 ears, 7 frequencies, 14 dimensions), ii) the low- to high frequencies vestibular testing (calorimetry, rotation, VHIT, 16 dimensions) and iii) both the tonal audiograms and vestibular testing (30 dimensions). For each analysis, 10 clusters were identified (ascending hierarchical clustering, Euclidean distance, 100 iterations). This allowed us to identify dizziness and vestibular profiles that have been compared to knowledge-driven clusters. The clustering also showed difference in the sex repartition according to the audiograms and vestibular profiles.
Conclusions: Results obtained from this study showed that the asymmetry of hearing and vestibular deficits is an important marker to differentiate profiles of dizziness and vertigo. This clustering also provides potential feasibility in proposing diagnostic profiles to a clinician according to audiometric and vestibular loss measurements.

SA230. Changes in Calyx-Only Afferent Terminals Follow VsEP Impairment and Recovery After Moderate Noise Exposure

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Category: Vestibular: Basic Research and Clinical

Background: 120dB SPL noise causes persistent vestibular hypofunction characterized by decreases in vestibular short-latency evoked potential (VsEP) responses that have not recovered 28 days after noise exposure. Rats with VsEP deficits also have a reduction in calyx-only afferent terminal labeling in the striolar region of the saccule. We recently showed that 110dB SPL noise exposure caused a transient VsEP deficit that recovered within 7 days after noise exposure. The present study examined 110dB SPL noise-induced changes in calyx-only afferent terminal number and complexity that follow the time course of VsEP loss and recovery and compared extent of calyx loss in rats that were exposed to noise that caused permanent versus transient changes in VsEP responses.

Methods: Long-Evans rats were exposed to 110dB SPL, 120dB SPL, or sham noise conditions. In 110dB SPL noise-exposed rats, ears were collected during VsEP loss (1-day post-noise) or after recovery (7-days post-noise). In 120dB SPL noise-exposed rats, recovery was not observed, and ears were collected 28-days post-noise. All vestibular sensory epithelia were immunostained for calretinin and beta-3 tubulin to visualize calyx-only and total calyx terminals, respectively. Immunostained vestibular end organs were imaged with a Leica Stellaris confocal microscope and calyces were grouped by complexity (1, 2, 3, 4+ calyces per afferent) in 100x200 µm striolar regions of interest. Counts by group were totaled for each rat to get a total count for the region of interest.

Results: A reduction in calretinin-labeled calyx-only terminals in the otolith organs was observed 1-day after 110dB SPL noise and 28-days after 120dB noise. 7-days after 110dB noise exposure, the number of calyx-only terminals were similar to sham, and significantly greater than 1-day post-noise counts, suggesting recovery. There was no significant change in beta-3 tubulin immunolabeling of total calyces for any time point or group. This suggests that the total number of calyces does not change after noise exposure; however, it is unclear whether some calyces are lost and surviving calyces become more complex. A trend towards a higher number of very complex (4+) calyces was observed 7-days after 110-dB SPL in the saccule and significant shifts towards more complex calyces and fewer single calyces were observed in the utricle in 110dB SPL noise exposed rats. This suggests a loss of single calyces and increasing complexity of surviving calyces after recovery from noise exposure.

Conclusions: The time course of vestibular dysfunction and recovery after noise, is mirrored by reduced calretinin immunolabeling of calyx-only afferent terminals 1- but not 7-days after 110-dB SPL noise exposure, and 28-days after 120dB SPL noise exposure. Additionally, the shift towards increasing complexity of calyces 7-days after 110dB SPL noise exposure is a possible mechanism underlying recovery of vestibular function after noise insults.

SA231. New Stimulation Protocol for Aging Vestibular Afferent

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Category: Vestibular: Basic Research and Clinical

Background: Bilateral vestibular deficiency (BVD) causes chronic dizziness, blurry vision during daily activities and the vestibular prosthesis is a potential treatment for this disorder. However, the vestibulo-ocular reflex (VOR) gain restored by vestibular prosthesis (VP) in human clinical trial is lower than what we expected, and a lot of space remains for the VP performance improvement. We hypothesize that vestibular afferent physiology differs substantially in senior vs. young adult subjects. We further hypothesize that a stimulation protocol adapted to senior vestibular afferent physiology (e.g. afferent firing rate vs. head velocity) could improve the prosthetic-evoked VOR gain in comparison to the current stimulation protocol. We present a new stimulation protocol with new operating curves plus manipulation of ramped slops and intrapulse intervals developed in our laboratory.

Methods: New pulse rate verse head velocity operating curve was selected based on the physiological measurements in senior animals and computational simulations and integrated into the new stimulation

protocol. The performance of the new protocol is tested in a chinchillas model with BVD and computational simulations in our recent electrophysiology models of VIII nerve.
Results: Improvement of optimized stimulation was identified through animal experiments and computational simulations. Simulation of electrically evoked compound action potentials (eCAP) and vestibulo-ocular reflex (eVOR) reveal the potential of computational modeling for stimulation protocol selection. The vestibular afferent units recruiting rate induced by the new stimulation protocol increased in senior subjects in comparison with the young groups.
Conclusions: A novel protocol to improve vestibular stimulation protocol adopted physiological characters in senior subjects was created to improve electrical stimulation performance in senior population. Serial computational models were developed in this study for future design of stimulation for seniors.

SA232. Psychometric Models for Estimation of Asymmetric Vestibular Perceptual Thresholds
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Category: Vestibular: Basic Research and Clinical
Background: Vestibular perceptual thresholds refer to the smallest motion intensity detected or discriminated by a participant. Thresholds for yaw rotation are commonly measured by a psychophysical task using a motion platform such as a rotary chair. Participants' binary responses (left or right) to stimulus trials are used to fit a simple sigmoidal psychometric function, and then the threshold can be estimated based on pre-determined criteria. Previous studies reported that psychophysical methods could detect directional asymmetries of vestibular thresholds, indicating that psychometric methods may provide additional information to conventional vestibular function measures that assess vestibular asymmetries. The goal of this preliminary study was to further explore the relative merits of alternate psychometric models that quantify perceptual motion asymmetries.
Methods: Eight adults (34 to 59 years; 4 females) provided example data with subjects having diverse backgrounds ranging from presumed normal vestibular function to having a history of exposure to ototoxic medications. Motion stimuli were vertical-axis yaw rotations with a single-cycle 1-Hz raised-cosine velocity waveform. Subjects identified self-motion directions (left or right) to stimuli with a range of amplitudes, and their responses were fitted to psychometric functions that included symmetric left/right slopes (Model 1), asymmetric left/right slopes (Model 2), and asymmetric left/right slopes with a 'dead zone' between the two slopes (Model 3) with the dead zone defining a range of amplitudes over which a subject is essentially unable to determine motion direction. All models include a 'bias' term that could shift the psychometric curves right or left. The vestibular thresholds were estimated at 25% and 75% points of the curves for the left and right directions, respectively, and corrected by the bias value. An asymmetry index (AI) was calculated to quantify the degree of directional asymmetry. The Bayesian information criterion (BIC) was used to judge which model fits could adequately account for the data while limiting the complexity of the model.
Results: The BIC indicated that data from only 3 (1 normal and 2 pathologic) of the 8 subjects were adequately accounted for by the Model 1 symmetric psychophysical function. Among the other 5 subjects, Model 2 provided an adequate fit for 3 subjects (1 normal and 2 pathologic), with AI magnitudes ranging from 38% to 82%, and Model 3 provided an adequate fit for the remaining 2 subjects (1 normal and 1 pathologic) with AI magnitudes of 11% and 17%.
Conclusions: There is evidence for the existence of vestibular-related asymmetries in the perception of the motion direction and there may be evidence for a perceptual 'dead zone'. However, variability in experimental data may influence the reliability of model selection and the resulting measures of response asymmetry. These considerations may affect the clinical utility of vestibular psychophysical testing.

SA233. KCNQ2/3 Regulates Efferent Mediated Slow Excitation of Vestibular Afferents in Mammals
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Category: Vestibular: Basic Research and Clinical
Background: Primary vestibular afferents transmit information from hair cells about head position and movement to the CNS, which is critical for maintaining balance, gaze stability and spatial navigation. The CNS, in turn, modulates hair cells and afferents via the efferent vestibular system (EVS) and its activation of several cholinergic signaling mechanisms. Electrical stimulation of EVS neurons gives rise to three

kinetically- and mechanistically-distinct afferent responses including a slow excitation, a fast excitation, and a fast inhibition. EVS-mediated slow excitation of vestibular afferents takes seconds to peak and tens of seconds to return to baseline. It is attributed to odd-numbered muscarinic acetylcholine receptors (mAChRs) on the afferent whose activation leads to the closure of a potassium conductance and increased afferent discharge.
Likely effector candidates include low-threshold, voltage-gated potassium channels belonging to the KCNQ (Kv7.X) family, which are involved in neuronal excitability across the nervous system and are subject to mAChR modulation. Specifically, KCNQ2/3 heteromeric channels may be the molecular correlates for the M-current, a potassium current that is blocked following the activation of odd-numbered mAChRs. To this end, multiple members of the KCNQ channel family, including KCNQ2 and KCNQ3, are localized to several microdomains within vestibular afferent endings, where they influence afferent excitability and could be targeted by EVS neurons. Additionally, the relative expression of KCNQ subunits appears to vary across the sensory epithelia and among different afferent types. However, it is unclear which KCNQ channel subunits are targeted by mAChR activation and whether that also varies among different afferent classes.
Methods: In both sexes of anesthetized 6-14wk C57BL/6J mice, glass microelectrodes were used to obtain single-unit spike recordings from regularly- and irregularly-firing vestibular afferents from cranial nerve VIII, while electrically stimulating EVS neurons in brainstem. To explore the role of specific KCNQ subunits, we characterized EVS-mediated slow excitation in vestibular afferents using various KCNQ subunit-selective openers and blockers.
Results: Consistent with KCNQ channels, EVS-mediated excitation is blocked and enhanced by the non-selective KCNQ channel blocker XE991 and opener retigabine, respectively. Using KCNQ subunit-selective drugs, we observed that a KCNQ2 blocker blocks the slow response in irregular afferents, while a KCNQ2/3 opener enhances slow responses in regular afferents. The KCNQ2 blockers did not appear to affect resting afferent discharge rates, while KCNQ2/3 or KCNQ2/4 openers decreased afferent excitability.
Conclusions: We here show pharmacological evidence that KCNQ2/3 subunits are likely targeted by mAChR activation in mammalian vestibular afferents. These data together suggest that KCNQ channels play a role in slow response and discharge rate of vestibular afferents, which can be modulated by EVS in mammals.

SA234. Roles of Tmc1 and Tmc2 Channels in Vestibular Function

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Category: Vestibular: Basic Research and Clinical
Background: Transmembrane channel-like genes, Tmc1 and Tmc2, are expressed in auditory and vestibular hair cells and have been shown to form mechanosensory transduction channels. Tmc1 is expressed in mature vestibular and auditory hair cells, whereas Tmc2 is transiently expressed, suggesting they may play different roles in inner ear function. While their roles in auditory function have been well established, important questions regarding their roles in vestibular function remain to be addressed. The goal of this study was to gain insight into their roles of Tmc1 and Tmc2 in vestibular function by measuring the rotational and translational vestibulo-ocular reflexes (rVOR and tVOR) and recording single vestibular afferent activities in Tmc1 KO mice and Tmc2 KO mice.
Methods: Mice (2-3 months) lacking Tmc1 (Tmc1-/-;Tmc2+/+, n=10) or Tmc2 (Tmc1+/+;Tmc2-/-, n=6) and C57BL/6 mice (WT, n=23) were used in the study. VOR responses to sinusoidal head rotation (0.2~4Hz) (rVORs) and translation (0.2-2Hz) (tVORs) were recorded using an infrared eye tracking system. Single unit recording of the vestibular afferents was conducted under ketamine anesthesia. Vestibular afferent spontaneous firing rates, regularity and sensitivity to head rotation and translation were analyzed.
Results: While Tmc1 KO mice and Tmc2 mice exhibited VOR responses to head rotation and translation, their rVOR and tVOR responses exhibited distinct differences as compared to the WT mice. Tmc1 KO mice exhibited decreases in both rVOR and tVOR gains, but the decrease in tVOR gains was much larger. Tmc2 mice, however, exhibited nearly the same tVOR gains as the WT mice, but significantly lower rVOR gains than the WT mice. Tmc2 mice exhibited larger phase leads in both the rVOR and tVOR. Vestibular afferents were recorded from WT mice, Tmc1 KO mice and Tmc2 KO mice. Compared to the WT mice, mice lacking Tmc1 or Tmc2 exhibited lower spontaneous firing rate (WT mice: 72.8+2.2 spike/s, n=279; Tmc1 KO:

53.8+2.8 spike/s, n=183; Tmc2 KO: 56.8+3.5 spike/s, n=128), lower sensitivities to head rotation (WT mice: 0.178+0.014 spike/s/d/s, N=100; Tmc1 KO: 0.078+0.01 spike/s/d/s, n=40; Tmc2 KO: too few samples) and nasal-occipital translation (WT mice: 81.4+6.9 spike/s/g, n=87; Tmc1 KO: 54.2+6.3 spike/s/g, n=75; Tmc2 KO: 39.4+5.6 spike/s, n=46).
Conclusions: Although preliminary, these results supported the hypothesis that Tmc1 and Tmc2 channels play important and distinct roles in vestibular function. While lack of Tmc1 resulted in a larger deficit in the otolith function, lack of Tmc2 resulted a larger deficit in the canal function.

SA235. Implication of AMPA Receptor Expression in the Vestibular Nucleus and Hippocampus for Hypergravity-Induced Motion Sickness

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Category: Vestibular: Basic Research and Clinical
Background: Humans without vestibular function never suffer from motion sickness, indicating that the vestibular system is essential for the development of motion sickness. Motion sickness occurs as a result of a mismatch or conflict between the information arising from the vestibular system, the visual and proprioceptive inputs. Due to spatial disorientation reported in space, spatial memory and navigation performances could be more impaired by altered gravity. Hippocampus, a key organ for spatial memory could influenced by gravitational change. In this study, we studied the expression of AMPA receptors in the vestibular nucleus and hippocampus during the hypergravity stimulation to determine the key molecules that contribute to development and adaptation to altered gravity.
Methods: we use our gravity system, Inha G-simulator which is a centrifuge device for animal. With 4G hypergravity stimulation was exposed for 24hrs, 1week, 2weeks and 4weeks with SD male rats (aged 7~8weeks, weighing 250-300g). We checked the kaolin intake and the vestibular function with animal rotator (VOR responses). And we did western blotting and immunohistochemistry analysis to quantify the protein expression of AMPA receptors in vestibular nuclei and hippocampus.
Results: Kaolin consumption was increased after load of hypergravity, in comparison with normal control group. There was a pattern to increasing kaolin intake by hypergravity load day by day. Kaolin intake was correlated with degree of gravitational stimulus. They showed significant reduction on VOR gain compared to the control group after hypergravity exposure. Decreased VOR gains were recovered to normal range on 3~4days after stopping the hypergravity stimulation. The expressions of AMPA receptor (GluA1-GluA4) were increased significantly compared to control group in hippocampus after 4G 4weeks stimulation of hypergravity. Also 4weeks exposure group showed higher expression than 24hrs and 2weeks exposure group significantly.
Conclusions: By observing the data, hypergravity stimulation affect the kaolin intake and it was correlated with the degree of gravitational stimuli and exposure time. And also, by modulating of AMPA receptors expression, hypergravity stimulation might affect the vestibular function and spatial memory in hippocampus

SA236. Assessment for the Utricular Function Using Centered and Eccentric Rotation

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Category: Vestibular: Basic Research and Clinical
Background: Vestibular evoked myogenic potential (VEMP) is now established as a reliable examination for the otolith organs. However, since the organs are essentially linear accelerometers, it is not a physiological response. Despite centered rotation (CR) causes angular acceleration, eccentric rotation (ER) causes centrifugal acceleration additionally that stimuli otolith organ especially utricle. There are some reports to assess the utricular function by means of comparison between vestibulo-ocular reflex (VOR) in CR and that in ER. The aim of this study was to confirm the consistency of the two assessments for utricular function.
Methods: The utricular disorder group used 8 subjects with unilateral absence of ocular VEMP and normal results of video Head Impulse Test. They aged 45 to 58. The control group consisted of 6 healthy adults ranged in age from 29 to 48. The VOR gains for CR and ER were measured and the difference was obtained in both groups. Their heads were placed on the axis of the rotation during CR and shifted 30 cm forward

from the axis during ER. Sinusoidal rotation with a maximum angular velocity of 50 degrees/sec was employed at 0.1, 0.2, and 0.5 Hz in each trial.
Results: Gain differences at 0.1, 0.2, and 0.5 Hz were 0.06, 0.06, 0.16, respectively in the control group. Those were 0.07, 0.07, 0.08 respectively in the utricles disorder group. An increase in the gain difference at 0.5 Hz was seen in the control group but not in the utricle disorder group.
Conclusions: The results of this study suggest that utricular function can be evaluated from the difference in VOR gain between ER and CR at 0.5 Hz. Higher gain at higher frequencies in the ER depends on the utricular function. That can be explained as follows; while the tangential acceleration which stimulates the otolith organs especially utricle increases in the higher rotational frequency, the centrifugal acceleration which stimulates the otolith organs especially saccule is unchanged because of maximum angular velocity was fixed at each frequency. The limitation of this study is a small number of cases series. Study with a larger number is required.

SA237. Group Vestibular Rehabilitation Program: A Cost-Effective Outpatient Management Option for Dizzy Patients

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Category: Vestibular: Basic Research and Clinical
Background: This study was performed to evaluate the effectiveness of our novel group vestibular rehabilitation therapy (G-VRT) and to analyze the factors affecting outcomes.
Methods: Medical records of 64 patients with chronic dizziness who received G-VRT in a tertiary medical center between December 2019 and July 2020 were analyzed retrospectively. G-VRT program consisted of a 1-hour physical therapy session by an otologist in a small group setting, followed up with home exercises and monthly outpatient visits. Dizziness Handicap Inventory, visual analog scale, functional level scales, and video head impulse test (v-HIT) as well as the compliance to the program were evaluated.
Results: All scores of dizziness questionnaires were significantly improved after G-VRT ($P<.001$). The overall VOR gain calculated by v-HIT increased compared to the initial scores, whereas the average PR scores of all three semicircular canals significantly decreased from the initial scores, indicating enhanced vestibular compensation. ($P<.05$). Enrolled patients showed high compliance to the program.
Conclusions: G-VRT program is a cost-effective and efficient way to provide relief for chronic dizzy patients. Further case-control studies in a larger group as well as comparative studies with generic or customized vestibular exercise will be needed to validate the clinical value of G-VRT.

SA238. Loss of Sestrin 2 (SESN2) Induces an Early Onset of Age-Related Vestibular Dysfunction in Mice

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Category: Vestibular: Basic Research and Clinical
Background: The function of the vestibular system declines with age. Oxidative stress is thought to contribute to the aging process. Sestrin 2 (Sesn2) is a stress-inducible and age-related protein. It acts as an anti-aging agent mainly by its antioxidant function as well as by regulation of adenosine monophosphate-activated protein kinase and mammalian target of rapamycin complex 1 signaling. It has been reported that SESN2 plays an important role in the protection of auditory hair cells against gentamicin and age-related hearing loss. In a previous study, we showed that loss of SESN2 potentiates noise-induced vestibular deficits in mice. In the present study, we examine the role of SESN2 in age-related vestibular dysfunction by measuring the vestibulo-ocular reflex (VOR) responses and vestibular afferent activities in SESN2 KO mice.

Methods: SESN2 KO mice (male and female) aged 4 and 12 months, and age-matched wild-type mice (C57BL/6J) were used in the study. The expression of SESN2 in the vestibular epithelium was determined by immunohistochemistry with cryo-sectioned samples. VOR responses to sinusoidal head rotation (0.2~4Hz) (rVORs) and translation (0.2~2Hz) (tVORs) were recorded using an infrared eye tracking system. Single unit recordings of the vestibular afferents were conducted under ketamine anesthesia. Vestibular afferent spontaneous firing rates, regularity and sensitivity to head rotation and translation were analyzed. We analyzed vestibular afferents for spontaneous firing rates, regularity, and sensitivity to head rotation and translation.
Results: Similar to the cochlear end organs, we confirmed SESN2 was expressed in the hair cells and supporting cells of the cristae and maculae in WT mice. At 4 months of age, SESN2 KO mice exhibited similar VOR responses to WT mice. At 12 months of age, while WT mice did not exhibit significant changes in the VOR, SESN2 KO mice exhibited significant decreases in rVOR gains and increases in phase leads. Interestingly, we found that the female SESN2 KO mice showed larger decreases of rVOR gains than the male SESN2 KO mice. As for the tVOR, a gain decrease was only observed at 2Hz in the 12-month-old SESN2 KO mice. Single unit recordings were made from a total of 643 vestibular afferents in WT mice and SESN2 KO mice at 12 months of age. While the loss of SESN2 exhibited little effect on afferent spontaneous firing rates, it significantly reduced afferent sensitivities to head rotation or translation.
Conclusions: These results support the hypothesis that SESN2 plays an important role in the age-related decline of vestibular dysfunction. Supported by NIHR01DC018919 and NIHR01AG073151.

SA239. Inflammation in the Vestibular Sensory Organs of Mice Following Neonatal Infection With Murine Cytomegalovirus

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Category: Vestibular: Basic Research and Clinical
Background: In humans, infection with cytomegalovirus during the first trimester of gestation can result in hearing loss and vestibular dysfunction. The underlying mechanisms are not known, but CMV appears to disrupt the normal development of the cochlea and vestibular organs. To better understand the nature of this pathology, we have characterized inflammation and sensory cell morphology in the vestibular organs of mice after neonatal inoculation with CMV.
Methods: Studies employ the murine cytomegalovirus (MCMV) which models many of the pathologies present in human CMV infection. Mice received a single IP injection of MCMV within 12 hr of birth (P0) and were allowed to survive for 7, 14 or 24 days. Mice were then euthanized, the temporal bones were removed and fixed, and utricles and horizonal cristae were processed for immunohistochemistry as wholemounts or frozen sections. Hair cells were identified with an antibody against myosin VIIa, neurons were labeled with the TUJ1 antibody (□-III tubulin) and anti-neurofilament, and inflammatory cells were labeled for CD45. Confocal microscopy was used to image all specimens and the density of leukocytes, hair cells and calyx nerve terminals was quantified.
Results: A massive inflammatory response was observed in the vestibular organs at 7 and 14 days after MCMV inoculation. Large numbers of leukocytes occupied the stromal and connective tissues of the vestibular periphery, and leukocytes also entered the sensory epithelia of the utricle and horizonal crista. Labeled inflammatory cells made frequent contacts with hair cells and neurons, and also formed phagocytic processes that engulfed hair cells and calyx nerve terminals. Such inflammation was only observed in the ears of MCMV-infected mice. Consistent with our prior observations (e.g., Kaur et al., Front Cellular Neurosci 2014), no leukocytes were present in the vestibular sensory epithelia of sham-treated or untreated mice, while more moderate numbers of inflammatory cells were present in the stromal tissues. The number of inflammatory cells within the sensory epithelium was still elevated at P24, although their numbers had diminished and no phagocytosis was noted. Quantification of hair cell density at P14 revealed a slight reduction in hair cell numbers in both the striolar and extrastriolar regions of the utricle.
Conclusions: Cytomegalovirus infection causes damage to the developing inner ear, but it is not clear whether such damage is caused by a direct action of the virus or by resulting inflammation. Our data indicate that MCMV causes a robust inflammatory response in the developing vestibular organs of mice, which may cause subsequent cellular pathology.

SA240. Vestibular Function is Associated With White Matter Structural Integrity Change in Healthy Older Adults

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Category: Vestibular: Basic Research and Clinical

Background: The vestibular system transmits relative head motion signals from the peripheral organs to the central nervous system (CNS). Previous studies analyzed diffusion tensor imaging (DTI) results to show a positive correlation between clinical vestibular loss and cerebral white matter fractional anisotropy (FA). However, little is known about the relationship between subclinical vestibular function and white matter changes in the central vestibular pathways. This study explores whether subclinical vestibular function predicts the microstructural integrity of white matter in the central vestibular pathways.

Methods: 888 subjects (54.4% female) from Baltimore Longitudinal Study of Aging were included in the analysis. Diffusion-weighted MRI scans were acquired on 3T Philips Achieva scanners in Baltimore, Maryland. Functions of the saccule, utricle, and horizontal semi-circular canal were assessed using the cervical vestibular evoked myogenic potential (cVEMP), ocular VEMP (oVEMP), and video head-impulse test (vHIT), respectively. Continuous and categorical (any present response vs. bilaterally absent response) vestibular variables were used. White matter tract integrity was assessed using axial diffusivity (AD), radial diffusivity (RD), mean diffusivity (MD), and FA of selected white matter regions. Multiple log-linear regression was used to assess the relationship between white matter tract integrity and vestibular end-organ function, correcting for age, sex, intracranial volume, and scanner protocol. Permutation testing and bootstrapping were used to evaluate the statistical significance of such correlations.

Results: Higher bilaterally averaged VOR gain was significantly correlated with poorer white matter integrity of the left and right internal capsule, the left superior and posterior corona radiata, and the left sagittal stratum/thalamic radiation, while it was correlated with higher white matter integrity of the genu and the splenium of corpus callosum, the left inferior fronto-occipital fasciculus, the left uncinate fasciculus, and the right sagittal stratum (p-values<0.05). Higher oVEMP amplitude was correlated with poorer white matter integrity of the right internal capsule and the right sagittal stratum, while it was correlated with higher white matter integrity of the left cerebral peduncle and the right internal capsule (p-values<0.042). Higher cVEMP amplitude was correlated with higher white matter integrity of the left superior longitudinal fasciculus (p-value≈0.015). A VOR gain higher than or equal to 0.8 was correlated with higher white matter integrity of the left internal capsule (p-value≈0.018). Any present oVEMP response was correlated with poorer white matter integrity of the right cerebral peduncle (p-value≈0.042).

Conclusions: Our study suggests that age-related variation in vestibular function is related to broad changes in white matter tract integrity, notably involving the sagittal striatum which carries fibers to the visual cortex and also to non-visual sensory association cortices. Future studies are needed that further dissect the fibers within these white matter bundles to understand the relationship between vestibular loss and the integrity of specific fiber tracts.

SA241. Prevalence of New Hearing Loss in the Acute Vestibular Syndrome and Its Diagnostic Value

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Category: Vestibular: Basic Research and Clinical

Background: Hearing loss is a common symptom in acute vestibular syndrome (AVS) patients and can be of central or peripheral origin. However, prevalence of hearing loss and the diagnostic value of an audiogram to differentiate between peripheral and central causes in these patents is not yet known. Therefore, we quantified new objective hearing loss by audiogram in AVS patients to answer these questions.

Methods: We performed a cross-sectional prospective study in AVS patients presenting to our Emergency department (ED) from February 2015 – November 2020. All patients received an MRI, Head-impulse test, Nystagmus test and Test of skew (HINTS), caloric testing and an audiogram. Definite diagnosis for central cause was made by MRI and/or clear clinical findings.

Results: We assessed 71 AVS patients, of which 7 had a stroke, 6 had a minor stroke and 58 an acute unilateral vestibulopathy (AUVP). 12.7 % of AVS patients had objective hearing loss. HINTS had an accuracy of 80 % to diagnose stroke whereas HINTS with audiogram (HINTS+) 76 %. The concordance between objective and subjective hearing loss was moderate (Cohens Kappa 0.53) with 55% of the patients not realizing it.
Conclusions: We found that almost one eighth of the AVS patients have a new hearing loss and only the half of them realise it. HINTS+ proved to be less accurate to diagnose a central cause than HINTS. Therefore, audiograms have no diagnostic value in ED but are necessary to objectify a new hearing loss that was underestimated during acute vertigo.

SA242. Detection and Quantification of Calcitonin Gene-Related Peptide (CGRP) in Human Plasma Using a Modified Enzyme-Linked Immunosorbent Assay

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Category: Vestibular: Basic Research and Clinical
Background: Calcitonin gene-related peptide (CGRP) is a vasoactive neuropeptide that plays a putative role in migraine headache, in which CGRP released by activated trigeminal fibers induces sterile neurogenic inflammation and arterial vasodilation. Our laboratory is interested in investigating CGRP as a biomarker for vestibular migraine, a poorly understood but common clinical problem.
The presence of CGRP in the peripheral vasculature has spurred investigation to detect and quantify this neuropeptide in human plasma using proteomic assays such as enzyme-linked immunoassay (ELISA). However, its half-life of only 6.9 minutes and variability of assay protocols have yielded inconsistent data in the literature. Prior studies have not included critical controls, including spike-and-recovery and linearity-of-dilution trials. Here we present an ELISA protocol for purification and quantification of CGRP in human plasma validated with such controls.
Methods: Human blood was collected from healthy controls, and plasma was separated by centrifugation. Per manufacturer's protocol (Bertin Bioreagent), peptide extraction was done with a polar sorbent. Dried extracts were suspended in EIA buffer and analyzed on ELISA plates. To create positive controls, known amounts of CGRP were "spiked" into plasma, and "recovery" was quantified after extraction by ELISA. Linearity-of-dilution tests were done to determine if standard curves obtained with EIA buffer remained valid for plasma at concentrations of interest.
Results: The manufacturer's protocol (Bertin Bioreagent) did not yield acceptable recovery (80-100%) until additional steps were added, for example, to block non-specific binding. Following these modifications, we found that plasma from healthy controls contained minimal amounts of CGRP. Plasma samples spiked to 200, 100, and 50 pg/ml gave favorable yields of 107%, 103%, and 110%, respectively. Linearity of dilution was demonstrated among these spiked samples.
Conclusions: To our knowledge, this is the first study to validate an experimental assay for detecting and quantifying CGRP in human plasma. This protocol may be valuable not only to extend investigations of the role of CGRP in neuro-otological manifestations of migraine but potentially to other neurological, psychiatric, musculoskeletal, and cardiovascular diseases.

SUNDAY, FEBRUARY 12, 2023

POSTER SESSION 2

SU1. Effects of Age on Within-Channel and Across-Channel Temporal Processing and Relationship to Speech Perception in Noise

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Category: Aging
Background: Age-related changes in the auditory system can lead to difficulty perceiving speech in noise. Temporal processing plays a vital role in the perception of SIN, evidenced in prior behavioral studies. There

are two components to temporal processing: (i) within-channel, influenced by the physiology of both the peripheral and central auditory systems, and (ii) across-channel processing (e.g., of temporal coherence), primarily involving the central auditory system. Animal studies suggest that both peripheral (loss of hair cells, cochlear synaptopathy) and central changes (down-regulation of inhibition) occur in normal aging. Here, we sought to characterize the relative contributions of within-channel and across-channel temporal processing mechanisms to speech perception in noise (SPIN) in individuals with near-normal hearing sensitivity as a function of age.
Methods: Data collection on a battery of behavioral and electrophysiological measures of temporal processing is ongoing in human subjects spanning a wide age range (18-70 years) with near-normal hearing sensitivity (pulsed-tone average of thresholds for the frequency range 500 - 6000 Hz < 25 dBHL). Within-channel assays consist of behavioral gap detection thresholds (GDT) and electroencephalographic (EEG) responses elicited by gaps embedded in 4000 Hz tones with octave-band noise. Across-channel assays target temporal-coherence processing through (1) a behavioral measure of comodulation masking release (CMR) for detecting 4 kHz tones embedded in modulated narrowband-noise masker and flanked by two well-separated bands of noise that were modulated coherently or incoherently with the on-band masker; (2) an EEG-based measure using a novel twenty-tone stimulus where the co-modulation statistics are parametrically varied while keeping within-channel statistics constant. Finally, SPIN performance was measured using two tasks placing differential emphasis on peripheral masking (word identification in a multi-talker babble) and cognitive load (matrix sentence test with staggered presentation of words from different streams), respectively.
Results: Preliminary results show a trend towards increased (poorer) behavioral GDTs and reduced CMR with age. Cortical responses elicited by gaps and temporal-coherence changes were less robust with increasing age. SPIN performance also degraded with age, as anticipated
Conclusions: Comparison of within-channel and across-channel temporal processing assays to SPIN outcomes will give us insight into the relative contributions of age-related peripheral and central auditory changes to everyday hearing.

SU2. The Influence of Progressing Synaptopathy for Distinguishability of Speech-Related Stimuli in the Aging Rat

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Category: Aging
Background: Mammalian inner hair cells connects to the brain via a multitude of exclusive spiral ganglion neurons within the auditory nerve. Over the lifetime a large number of these connections are lost even without traumatic events (cochlear synaptopathy). In view of this, loss the number of cochlear hair cells – inner and outer hair cells – remain unaffected and audiometric hearing thresholds (audiograms) as well as cochlear amplification by outer hair cells (otoacoustic emissions) seem normal in the clinical testing. However, dynamic range coding for sound levels and temporal resolution range are most likely to be impaired in the situation of synaptopathy, which is why it is expected that speech understanding will be also disturbed.
Methods: We examined the hearing performance of three groups of laboratory rats of different ages, young (1-4 months, average ca. 2.2 months), middle aged (10-19 months, average ca. 13 months), and old (20-30 months, average ca. 25 months). We used auditory brainstem responses to analyze hearing thresholds and activation of the auditory nerve (ABR wave I) and the ascending auditory pathway (ABR wave II, and IV) by quantifying response amplitudes and latencies for a variety of frequency and level specific auditory stimuli (click, noise-burst, and pure-tone pips). Outer hair cell function was tested by otoacoustic emission

(DPOAE) threshold, amplitude and growth. Testing auditory steady-state responses (ASSR) and fast adaptation of DPOAEs (MOC reflex) the time resolution of the ascending auditory responses and efferents were tested, respectively. On a subgroup of rats of either age, we presented speech-related short syllable stimuli with contrasts for steady-state vowels (/i/–/y/; /o/–/u/) and consonants (/di/–/bi/; /du/–/bu/) to test the discrimination of low and high frequency contrasts for onset and steady state auditory stimuli.
Results: The time course of the hearing performance in the aged rat matched well with the observations, which have long been described for several rodent animal models and point out that synaptopathy and auditory fiber loss may take place in a dynamic manner long before the loss of hair cells. The changes in audiometric thresholds with age are subtle. However, systematic changes in temporal resolution, dynamic coding range for temporally modulated auditory stimuli, and the central brain responses to complex speech-like sounds alter in the aging rat in a specific way.
Conclusions: We present data that illustrate the interrelationships between peripheral and central auditory processing as they may be relevant for cognitive tasks required in speech perception. We will test the relevance of our findings for human speech intelligibility and the diagnostic potential of a method that uses speech-like stimuli for evoked responses in future trials on the behavioral animal model.
This work was supported by the Deutsche Forschungsgemeinschaft DFG KN 316/13-1, DFG RU 713/6-1, NEURON JTC 2020, BMBF 01EW2102 CoSySpeech.

SU3. Increased Forward Masking Effects Reflect Age-Related Auditory Temporal Processing Deficits
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Category: Aging
Background: Aging has profound effects on sensory systems, many of which are critical to communication. Indeed, older adults report problems in hearing despite having normal audiometric thresholds (ONH) and it is unclear how changes in the brain due to aging differ from younger normal-hearing listeners (YNH). The literature suggests that there are age-related reductions in neural inhibition of central auditory processing and strong evidence for age-related temporal processing deficits. However, there is limited understanding of how these deficits interact with level effects, particularly at peripheral stages of the auditory system. Therefore, we wanted to integrate these aspects to investigate whether aging has consequential effects on brainstem processing, providing insight on how ONH listeners can experience hearing problems as a form of "hidden hearing loss". We hypothesized that the auditory brainstem responses (ABR) Wave V amplitudes will be decreased with increased latencies in ONH participants across conditions. Furthermore, we predicted that ONH participants will demonstrate later masking release at a higher masker-to-target intervals (MTIs), validating the effects of aging on temporal processing.
Methods: We will record ABRs from 15 YNH and 15 ONH participants in response to a single-pulse target and masker. The target stimulus at 90 dB peSPL was presented with broadband masker stimuli at both 65 and 80 dB SPL. Additionally, we presented the target stimulus alone to establish baseline responses. To test for temporal processing deficits and the effect of forward masking, we presented the masker stimuli at different MTIs at 5, 100, and 200-ms monaurally through the right ear. At least 1500 artifact-free responses were recorded via a two-channel recording.
Results: Preliminary data demonstrated that ONH participants displayed lower Wave V amplitudes and increased latencies compared to YNH participants. For YNH participants, we found a significant decrease in amplitude and increase in latency at the 5-ms conditions in comparison to the target only condition, demonstrating the presence of forward masking. We determined that masking release had occurred for conditions at which latencies and amplitudes were equivalent to those obtained to the target alone. The YNH participants demonstrated masking release at the 100-ms interval while ONH participants only showed masking release at the 200-ms interval.
Conclusions: Our results help elucidate the fundamental effects of aging on temporal processing in the central auditory system. Despite having near-normal audiometric thresholds, ONH listeners can experience hearing difficulties in real-world situations outside of the clinical environment. Thus, our study gains insight into further understanding this "hidden hearing loss" phenomenon and future experiments should focus on extending stimuli to more complex time-varying signals such as speech. Delineating these effects of aging will pave the way for better clinical diagnosis of hearing disorders and personalized treatments for different forms of age-related hearing loss.

SU4. Individual Variability in Early Markers of Sensorineural Hearing Loss With Age and Ototoxicity

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Category: Aging

Background: Recent studies found that envelope-following-responses (EFRs) are a promising marker of age-related or ototoxic induced cochlear synaptopathy (CS) in animals. The differential character of EFR markers of CS is important because humans may have an unknown mixture of sensorineural hearing damage (e.g. inner or outer hair cell loss, CS). This is in contrast with measurements on animals where the type of hearing injury can be better controlled. Next to that, it is not yet clear whether CS is the underlying cause of speech intelligibility declines with age. Furthermore, thus far developed EEG-based non-invasive diagnostic tools of sensorineural hearing loss (SNHL), do not explicitly quantify the extend to which CS contributes to inner hair cell (IHC) loss. Here, we study declines in early markers of hearing damage in an aging population and in patients receiving cis-and carboplatin chemotherapy. We hypothesize a faster age-decline in patients who have already developed SNHL or are receiving chemotherapy.

Methods: A total of 108 Flemish subjects participated in this study and were divided into two groups: (i) a control group with normal audiograms (i.e. air conduction thresholds at 4kHz never exceeded 20 dB HL) (n=89, 18-65 years), (ii) older adults with a sensorineural hearing loss (SNHL) (n=19, 45-75 years). We adopted a test battery that comprised audiograms measured at standard and extended high frequencies (EHF), envelope following response (EFR) to a rectangularly amplitude modulated (RAM) pure-tone with a carrier frequency of 4kHz and speech intelligibility scores (Flemish Matrix test). We calculated the declining rate of each metric with the increasing age. Currently, we are measuring adults who receive cis- or carboplatin chemotherapy (n=10, 38-75) with the same test battery.

Results: Within the normal hearing group, the increasing age accounted for 23.6% of the variation in EFR magnitude, with adjusted R2 = 22.7%; a medium size effect according to Cohen (1988). Ageing statistically significantly predicted the EFR magnitude, $F(1, 87) = 26.91$, $p < .0005$; (mean PTA 4.79 ± 4.21 standard deviation; SD). Hearing impaired group: (mean PTA 19.81 ± 10.35 SD).

Conclusions: The evidence from our large aging cohort supports that CS due to age, plays an important functional role in the RAM-EFR magnitude reduction. We found that the EFR reductions observed in the older subjects are consistent with age-related CS, but that it remains challenging to parse out the role of IHC loss. The implications of added SNHL and chemotherapy to the RAM-EFR-with-age curve will be presented.

Acknowledgements: This work was supported by the European Research Council (ERC) under the Horizon 2020 Research and Innovation Program (grant agreement No 678120 RobSpear and No 899858 CochSyn).

SU5. Reduced Mitochondria Cytoplasmic Occupancy in the Stria Vascularis of Aging Mouse Cochlea

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Category: Aging

Background: Age-related hearing loss (ARHL) is a global concern affecting the communication and livelihood of older adults. Previous studies suggest that mitochondrial dysfunction contributes to aging pathophysiological alterations including hearing loss. Here, we characterize ultrastructural changes in mitochondria within sensory and non-sensory cell types of mouse cochlea. Mitochondria, characterized as cellular power plants, catalyze the phosphorylation of ADP to ATP to supply energy needed for various biophysical cochlear processes such as maintaining fluid ion homeostasis and generating the endocochlear potential. Ultrastructural analysis using Imaris cell imaging software provides a unique opportunity into quantitatively evaluating mitochondrial structural alterations due to aging. This study addresses the hypothesis that energy consuming cochlear cells of the stria vascularis undergo a rapid reduction in mitochondrial cytoplasmic occupancy due to aging.

Methods: Transmission electron microscopy was used to visualize ultrastructural changes of mitochondria in the stria vascularis, hair cells, supporting cells, and spiral ganglion neurons of young adult (2-4 months), middle age (12-15 months), and aged CBA/CaJ mice (>24 months). Auditory brainstem response

measurements were conducted to evaluate hearing sensitivity. Imaris machine learning segmentation was used to quantify mitochondria cytoplasmic occupancy.
Results: Quantitative ultrastructural analysis using Imaris showed higher mitochondria cytoplasmic occupancy within primary processes of strial marginal cells compared to other cochlear cell types examined within young adult CBA/CaJ mice. Primary processes of strial marginal cells forming indigitations with strial intermediate cells revealed elongated mitochondria occupying ~75% of cytoplasmic area. Middle-age and aged mice showed a reduction in mitochondria occupancy within marginal cell processes with less defined cristae and more abundant mitophagosomes. Mitochondria were rounder and more organized along the outer hair cell border with nearly complete mitochondria loss in aged mice. Mitochondria of supporting cells revealed only ~10% of cytoplasmic occupancy with a rounded shape and random distribution. Lastly, mitochondria of type I spiral ganglion neurons showed moderate mitochondria cytoplasmic occupancy with mitochondria of varying shapes occupying ~50% of cytoplasmic area of the soma of young adult mice. A reduction in mitochondria occupancy was observed in type 1 spiral ganglion neurons of aged mice.
Conclusions: The application of Imaris machine learning segmentation has allowed quantification of mitochondria structure in multiple cochlear cell types of an aging mouse model. The study emphasizes the importance of mitochondria in energy consuming cochlear cell types within the stria vascularis. Specifically, mitochondria in strial marginal cells of young adult mice occupies nearly the full length of marginal cell processes, which clearly diminishes in aging mice. Further studies should investigate the functional role of mitochondria within marginal cells to determine the impact in ARHL since marginal cells are crucial in generating the endocochlear potential.

SU6. How Do MEG Signatures of Spatial Attention Vary Among Older Adults?
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Category: Aging
Background: People often face the challenge of understanding speech when other speakers are present. Listeners with normal hearing can deploy preparatory spatial attention to improve intelligibility in spatialised settings, although children who have hearing loss from a young age deploy preparatory spatial attention to a lesser extent than children with normal hearing. It is currently unclear how older adults, who have a lifetime of preserved hearing—but commonly experience age-related declines in their hearing—deploy spatial attention. Differences in spatial attention could help to explain why older adults vary so widely in their ability to understand speech when competing speech is present. Here, we investigated how age and audiometric thresholds relate to preparatory spatial attention among older adults.
Methods: We recruited adults aged 55-80 years. We measured their audiometric thresholds and tested their ability to understand a target phrase in the presence of two competing phrases, which were spoken by different talkers and presented from different locations. The target talker was cued visually by an arrow (left or right), which was presented 100 or 2000 ms before the talkers started speaking—thus providing a short or longer interval for participants to prepare spatial attention. While participants completed this task, we recorded concomitant MEG responses and pupil dilation.
Results: Preliminary results (26 participants) reveal wide variability in MEG signatures of preparatory attention among participants. Some participants evoke a large preparatory response throughout most of the preparatory interval, others evoke a shorter preparatory response for part of the preparatory interval, whereas others evoke little or no preparatory response. In this sample, greater magnitude preparatory responses (which occur before acoustic stimulation) were associated with higher (i.e., worse) audiometric thresholds (and did not correlate with age).
Conclusions: Preliminary results suggest that preparatory spatial attention varies widely among older adults, and (at least partially) relates to age-related hearing loss. Possibly, older adults attempt to compensate for declines in hearing by deploying spatial attention to a greater extent. This contrasts with our previous findings in children with hearing loss, who seem to deploy spatial attention to a lesser extent than children with normal hearing. This underscores that hearing loss may have different effects on auditory cognition depending on when in life hearing loss occurs. The final analyses will confirm these preliminary findings in a larger sample, examine the nature of these MEG responses in more detail, and examine whether these effects are reflected in pupil dilation.

SU7. Altered Neural Encoding of Vowels in Noise in Gerbils With Age-Related Hearing Loss Does Not Affect Perceptual Vowel Discrimination

Amarins Heeringa*[1], Carolin Jüchter[2], Rainer Beutelmann[2], Georg Klump[3], Christine Koeppl[1]
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Category: Aging

Background: Understanding speech in a noisy environment, as opposed to speech in quiet, becomes increasingly more difficult with increasing age. Using the quiet-aged gerbil as an animal model, we here study the effects of aging on speech-in-noise processing. Specifically, our aim is to study perceptual vowel discrimination and the encoding of these vowels by single auditory nerve fibers, to elucidate some of the underlying mechanisms of the age-related deficit in speech-in-noise perception.

Methods: To determine perceptual vowel discrimination, young-adult (n = 9) and quiet-aged (n = 10) Mongolian gerbils were trained to discriminate a deviant vowel in a sequence of vowel standards. The German vowels /e/, /i/, and /a:/, naturally spoken in a consonant-vowel-consonant logatome with /b/ as flanking consonant, were presented against an ICRA-1 noise masker with speech-like spectral properties at 5 dB SNR. The logatome had a fixed level of 65 dB SPL. Response delays and hit rates were measured to dissociate easy vs difficult discriminations.

In a different set of young-adult (n = 7) and quiet-aged (n = 5) gerbils, we recorded responses from single auditory-nerve fibers while presenting the same speech stimuli as in the behavioral experiment. Neural response dissimilarity between vowel responses was determined using delta-CI, a spike timing-based discrimination metric. Various neural representation schemes were used to study auditory-nerve vowel encoding.

Results: For both young-adult and quiet-aged gerbils, the behavioral discrimination between /e/ and /i/ was more difficult to make than /e/ vs /a:/ or /i/ vs /a:/, based on longer response delays and lower hit rates. No interaction effect of age and vowel comparison on response delays and hit rates was found, suggesting that perceptual vowel discrimination in noise was not affected qualitatively by aging.

Spike timing-based discrimination in fibers of young-adult gerbils agreed with the perceptual vowel discrimination, showing lower delta-CIs for the /e/ vs /i/ comparison. In quiet-aged gerbils, neural discrimination was better in the /a:/ vs /i/ comparison than in the other two, thus disagreeing with perceptual discrimination. Representation schemes, based on the spectrum of the inter-spike-interval histogram, revealed stronger encoding of both the fundamental and the lower formant frequencies in fibers of quiet-aged gerbils. Elevated thresholds in combination with a fixed stimulus level, i.e. lower sensation levels of the stimuli for old individuals, can explain these findings.

Conclusions: In this study, we showed that neural encoding of naturally-spoken vowels in noise was altered in gerbils with age-related hearing loss, predicting enhanced discrimination in the auditory nerve. However, this did not affect perceptual vowel discrimination. Ongoing research in our lab will now focus on perceptual discrimination and encoding of consonants, as well as studying neural encoding of these stimuli at higher auditory processing stages.

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SU8. Oxidative Stress (OS) is Independent of Age-Related Autophagy Pathway in Cochlea

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Category: Aging

Background: Although the mechanisms of age-related hearing loss (ARHL) are not completely understood, it is likely that overlapping and interacting pathways are associated with aging in the inner ear. Oxidative stress is implicated in the aging cochlea, and autophagy is a metabolic turnover cellular process, where cellular components are processed for degradation. Autophagy plays a key role in the high energy metabolic cochlea. In the present study, we test the hypothesis that age-related oxidative stress and inflammation are linked to autophagy, mitochondrial DNA mutation(s) and apoptosis, as part of ARHL.

Methods: Young (n=6, 3mon) and aged (n=6, 30 mon) CBA/CaJ mice served as the auditory animal model. Auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) were recorded to measure hearing changes. In vitro cell treatments using HEI-OC1 cells were conducted with different

experimental paradigms: dose-dependent and time-course. Western blot, immunohistochemistry and imaging with confocal laser scanning microscopy were used for the analysis of biomarkers of oxidative stress and autophagy, along with qRT-PCR and ligation mediated -PCR (LM-PCR) to detect gene expression changes and DNA fragmentations (apoptosis).
Results: We mimicked oxidative stress in vitro by applying H2O2 in HEI-OC1 cells and our results indicated that there was no significant difference of LC3II and p62 protein expressions under the stimulation with H2O2 vs non-treatment, and no differences in the expression of mCherry-GFP-LC3 observed using confocal microscopy. Inflammation as indicated by increases in NFkB, Akt, and IL-1B, and cell death by DNA fragmentations were upregulated in response to H2O2 in the HEI-OC1 cell line. In aged cochlea of CBA/CaJ mice, one missense mutation, with the frequency varying from 0.08% to 66.23% was observed. In total, 7 mutations were identified in vivo, and all of them did not show homoplasmy. The missense mutations found in aged cochlea of CBA/CaJ mice were identical to the mutations induced by H2O2 in the HEI-OC1 cell line. ABR threshold shifts up to 30 dB occurred in treated mice with bafilomycin (autophagy inhibitor) compared to non-treated CBA/CaJ mice. In addition, DPOAE amplitudes decreased, and thresholds increased in treated mice, revealing that outer hair cell function was damaged due to blocking the autophagy pathway in the cochlea. However, prestin protein expression was not affected by H2O2 treatment. Emulating age-related autophagy blocking in the cochlea by stimulating HEI-OC1 cells with chloroquine and 3BDO did not induce an inflammatory response, apoptosis inducing factor (AIF) nuclear translocation or cell death signaling, which appeared under the presence of H2O2 or in the aged cochlea samples of CBA/CaJ mice.
Conclusions: It appears that there is minimal overlapping of mechanisms of oxidative stress and autophagy in the pathogenesis of ARHL, but both are important contributors for inner ear aging processes.

SU9. Neural Signatures of Predictive Coding During Auditory Temporal Coherence Detection in Cochlear Implant Users

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Category: Auditory Cortex and Thalamus: Human Studies
Background: Listeners with cochlear implants (CI) face difficulties in detecting auditory objects especially when presented in noisy backgrounds, which are not only explained by peripheral factors (e.g., hearing loss). In CI users, we found that performance on a stochastic figure-ground (SFG) task, which relies on auditory grouping/regularity detection mechanisms, was correlated with speech-in-noise performance. This indicates the involvement of these mechanisms in mediating speech-in-noise perception. However, the auditory cognitive mechanisms by which the brain predicts the emergence of regularity, and their contribution to speech-in-noise perception, are not fully understood. Therefore, this study aimed to investigate the neural mechanism(s) of predicting auditory object emergence in a group of CI users.
Methods: 10 bilateral CI subjects with at least one-year experience using a CI, as well as 10 healthy control subjects (HC) were recruited for this study. Participants performed the SFG task, wherein they were required to detect the emergence of temporally regular tone pips in a cloud of tone pips presented at random times and frequencies. Detection of the auditory object was indicated with a button press. High-density EEG (64-channels) was recorded as the subjects performed the task. Planned analyses include measuring beta and gamma power in regions of interest as derived from the literature (superior temporal gyrus, supramarginal gyrus, intraparietal sulcus, anterior cingulate cortex), as potential indices of auditory object prediction. To investigate the relationship between task performance and neural indices we will correlate beta/gamma power with SFG task accuracy.
Results: EEG data were processed for cochlear implant artifact using ICA decomposition, and the resultant data showed evoked potentials at the stimulus change period from ground to figure + ground.
Conclusions: These results demonstrate that a neural signature of auditory figure detection can be recorded in an experienced bilateral cochlear implant population. The same time periods will be analyzed for induced power changes as potential indices of predictive coding.

SU10. Selective Cortical Responses to Harmonic Sounds

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Category: Auditory Cortex and Thalamus: Human Studies
Background: Sounds like speech and music are generally harmonic, with frequency components that are integer multiples of a common fundamental frequency (f0). Information in harmonic sounds is believed to be extracted in two ways: via individual frequency components, and via estimation of the fundamental frequency. The first mechanism is applicable irrespective of whether sounds are harmonic; the second is specific to harmonic sounds. The brain basis of these two mechanisms remains unknown. While previous work has shown higher neural responses to harmonic sounds compared to noise, it is not clear whether this result was specific to harmonic sounds, or whether it would also be driven by sounds that have sparse spectra but are not harmonic.
Methods: Participants in an fMRI experiment listened to melodies composed of harmonic or inharmonic notes. Inharmonic notes contained frequency components that were jittered in frequency to be inconsistent with a single f0. Melodies were presented in silence and in background noise. Listeners also heard noise "melodies" in which notes were spectrally matched to harmonic tones. We first identified pitch-sensitive cortical voxels as those that responded more to harmonic tones than to spectrally matched noise. We then measured the response of these voxels to each of the conditions, using independent data.
Results: Voxel responses were similar for harmonic and inharmonic melodies when tones were presented in quiet. However, responses were higher to harmonic melodies when tones were presented in noise, a manipulation known to cause listeners to rely more on representations of f0. This result was specific to pitch-sensitive voxels; it was not observed more broadly in sound-sensitive cortical voxels.
Conclusions: The results suggest that mechanisms selective to harmonic sounds are present in the auditory cortex and are active when sounds occur in noise. However, these mechanisms appear to be co-located with mechanisms that respond equally to harmonic and inharmonic sounds, and which are engaged when sounds are presented in quiet.

SU11. Tracking Structural Changes in Sound Sequences: A Comparative EEG Study Across Human and Non-Human Primates

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Background: Sensitivity to regular acoustic patterns is a pillar of perception and communication. Previous human EEG studies have characterized a neurophysiological signal associated with the implicit detection of structural changes within rapidly unfolding tone sequences. This consists of a sustained activity increase, triggered by the onset of a regular pattern (i.e. a sequence made of a repeated set of tones, REG). Notably, when a REG sequence is interrupted by a random arrangement of tones (RAN sequence) the sustained response drops and settles at a lower level. Hence, such sustained activity has been hypothesised to reflect the building-up and/or abandoning-of a predictive model of the structure underlying a given sequence. So far, this hypothesis has only been tested in humans. Here we ask whether the sustained activity reflects the functioning of a phylogenetically conserved process, possibly aimed to identify (and respond to) behaviourally-relevant events.
Methods: We recorded EEG from 2 macaques (macaca mulatta), as well as 20 human participants, passively listening to sound sequences (50-ms tone-pips drawn from a pool of 20 log-spaced values; range 444-4000 Hz). We compared EEG responses to (i) regularly repeating patterns of 5 tones (REG), (ii) sequences containing a transition from one regular to a different regular pattern (REG1-REG2) and (iii) sequences containing a transition from a regular to a random pattern (REG-RAN).
Results: We report that the sustained response to rapid tone sequences can be extracted from the EEG of macaques. Ongoing analyses are assessing whether non-human primates track structural changes similarly to how humans do.
Conclusions: Introducing new analytical approaches in the field of non-human primate neuroimaging, this study might shed light upon the origins of the neural processes involved in tracking structural changes in sound sequences.

SU12. Many But Not All Deep Neural Network Audio Models Predict Auditory Cortex Responses and Exhibit Hierarchical Layer-Region Correspondence

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Background: Deep neural networks are commonly used as models of the visual system, but are less explored in audition. Prior work provided examples of audio-trained neural networks that produced good predictions of auditory cortical fMRI responses and exhibited correspondence between model stages and brain regions, but left it unclear whether these results generalize to other neural network models.
Methods: We evaluated brain-model correspondence for nine publicly available audio neural networks that varied in both their architecture (spanning convolutional neural networks, recurrent neural networks, and transformers) and training task (ranging from automatic speech recognition and speech enhancement to audio captioning and audio source separation). To supplement these external models and systematically explore the effect of training task, we trained ten models ourselves: two architectures trained separately on each of four tasks as well as on three of the tasks simultaneously. The primary brain-model similarity evaluation metric was the variance explained by linear regression mappings fitted from model features to brain responses. To ensure that the conclusions were robust to the choice of evaluation metric, we repeated key analyses using representational similarity metrics. We also used two different fMRI data sets to assess the reproducibility and robustness of the results (Norman-Haignere et al., 2015, n=8; Boebinger et al., 2021, n=20) with data from a total of 28 unique participants.
Results: First, most deep neural network models produced better predictions of brain responses than a baseline spectrotemporal filter-bank model of the auditory cortex. This result was dependent on task-optimization of the neural networks; models whose weights were permuted, destroying the structure learned during model training, performed below the baseline model. Second, most models exhibited a systematic correspondence with the hierarchical organization of the auditory cortex, with earlier model stages best matching primary auditory cortex and later stages best matching non-primary cortex. These findings generalized across fMRI datasets and evaluation metrics. Third, not all models produced good brain predictions and hierarchical stage-region correspondence, suggesting that certain architectures and training tasks yield models that match the brain better than others. Finally, we investigated how the task a model is trained on influences its match to brain. We observed task-specific prediction improvements for specific brain responses, e.g., with speech tasks producing the best predictions of speech-selective brain responses. The best overall predictions were produced by models trained on multiple tasks.
Conclusions: The results indicate that many deep neural networks replicate aspects of auditory cortical computation, and indicate the important role of training tasks in obtaining models that yield accurate brain predictions.

SU13. Distracting Synchrony: Multiplexed Measures of Temporal Processing and Auditory Distraction
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Category: Auditory Cortex and Thalamus: Human Studies
Background: Behavioral evidence shows that not all noise is equal. Participants perform better on speech-in-noise tasks when masked by white noise as opposed to multi-talker babble, and simple tone stimuli are released from masking when the masker differs in a task-relevant feature. These effects are more profound in individuals with clinical difficulty hearing in noisy environments. Poor performance in the more difficult noise conditions could reflect degraded target representations, exaggerated noise representations, or some combination of the two. To disambiguate these possibilities, we utilize a new psychophysics and EEG paradigm to jointly measure auditory nervous system synchronization to both target and distracting noise stimuli across a wide range of stimulus timescales.
Methods: We developed a stimulus consisting of concatenated amplitude modulated (AM) tones that produces temporal features along four nested timescales, ranging from stimulus temporal fine structure (~500 Hz) to embedded context (~0.5 Hz), and everything in between. These stimulus features are captured in the EEG recorded during presentation, including the frequency following response (FFR), envelope following response (EFR), and envelope change following response (ECFR). Participants report trial-by-trial whether target stimuli were presented in random arrangements or repeating patterns both in a psychophysical task and during the EEG recording session.

In conjunction with this target stimulus, a distractor stimulus of AM tone sequences was designed with temporal features that fall between those of the target stimulus. These temporal features include a carrier change following rate and distractor-EFR. The distractor utilizes perceptually salient features, rather than broadband masking, to challenge accurate perceptual classification of target context.
Results: Behavioral analyses show a significant drop-off in performance in the presence of the distractor. Analysis of scalp EEG from young adult participants demonstrated that FFRs, EFRs, and ECFRs all decreased in amplitude when the target stimuli were accompanied by the distractor compared to the target alone. The decrease was largest for the longer timescale ECFRs and much smaller for the FFR. Separate analyses for distractor temporal features produced robust synchronization peaks when the distractor was presented with the target. Distractor synchronization measures are compared to behavioral performance in the psychophysical task and in traditional speech-in-noise tasks.
Conclusions: Using carefully constructed stimuli, we are able to simultaneously capture synchronization to both attended target and unattended distracting sounds across multiple timescales. In normal hearing subjects, these measures show promise as an objective measurement for difficulty hearing in noise and temporal processing deficits

SU14. Induced Alpha and Beta Electroencephalographic Rhythms Covary With Single-Trial Speech Intelligibility in Competition

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Category: Auditory Cortex and Thalamus: Human Studies
Background: Understanding speech in competing sounds is a challenging task that is critical for successful communication in everyday environments. Neurophysiological studies across species suggest that processing of sensory information, including speech, may be subserved by intrinsic brain oscillations. Specifically, brain rhythms in different canonical frequency bands may mediate the perceptual processes of both auditory grouping and selective attention, as well as more linguistic aspects of speech processing such as predictive coding. While prior work has explored how evoked (i.e., phase-locked) and induced neural oscillations relate to selective processing of a target speech source in an acoustic mixture, we know of no prior studies that have directly tested, at the single-trial level, whether the overall magnitude of induced brain oscillations in different canonical bands relate to simultaneously measured trial-wise speech intelligibility in the presence of competing sounds.
Methods: Here, we recorded human electroencephalography (EEG) while simultaneously measuring the intelligibility of target speech sentences presented amidst two different competing sounds: multi-talker babble or speech-shaped noise.
Results: Our results show that both parieto-occipital alpha (7–15 Hz; thought to modulate attentional focus) and frontal beta (13–30 Hz; associated with speech-motor predictive coding) induced oscillations significantly covary with trial-wise percent-correct scores for speech in competition; importantly, alpha and beta power provide significant independent contributions to predicting single-trial behavioral outcomes. Moreover, we observed large individual differences in the across-trial distribution of the overall magnitude of alpha and beta power as well as in the alpha-to-beta power ratio. This individual variability in neural patterns raises the possibility that listeners employed different task strategies: some may have relied heavily on focusing attention, while others may have depended on predictive coding.
Conclusions: These results can inform models of speech processing and help in the design of noninvasive measures to index different neural mechanisms and processes that together support complex listening.
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SU15. Encoding of Temporal Coherence in Mouse Auditory Cortex During Non-Stationary Listening Effort

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Category: Auditory Cortex and Thalamus: Structure and Function

Background: Temporal coherence (TC) between frequency channels in the sound stream is one of the key perceptual attributes that aids in segregating a target from background. TC-aided stream segregation is particularly important during attentive and effortful listening and is hypothesized to be encoded in the auditory cortex (ACX).
Methods: To understand the neural encoding of TC, we performed two-photon imaging and electrophysiology in ACX of mice while they performed a non-stationary listening effort task. Mice detect TC in an ongoing random tone cloud to get sugar water as reward. This task design requires sustained attention to segregate TC from tone cloud, and we manipulated this attentional intensity by changing reward size in different blocks of trials (see De Gee et al, 2022 for task details).
Results: We find that neurons robustly respond to tone clouds by increasing their firing rate, but in response to TC emergence they modulate their firing rate in several distinct ways. Both in imaging and electrophysiology data, a small proportion of neurons increased their firing rate, however a substantial proportion either did not respond or reduced their firing rate in response to TC. The response profile of neurons that got suppressed was significantly faster compared to the neurons that got enhanced in response to TC.
Conclusions: In ongoing work, we are further characterizing the response properties of these two groups neurons during different phases of the task, to understand the encoding of TC in relation to listening effort.

SU16. Extratelencephalic Neurons Encode Learned Stimulus Categories and Behavioral Choice

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Category: Auditory Cortex and Thalamus: Structure and Function

Background: Auditory-guided behavior is ubiquitous in everyday life, whenever auditory information is used to guide our decisions and actions. Nestled amongst several populations, extratelencephalic (ET) neurons reside in the deep layers of auditory cortex (ACtx) and provide a primary means of routing auditory information to diverse, sub-cortical targets associated with decision-making, action, and reward.
Methods: To investigate the role of ET neurons in auditory-guided behavior, we developed a head-fixed choice task, where mice categorized the rate of sinusoidal amplitude-modulated (sAM) noise bursts as either high or low to receive a water reward. We first established ACtx necessity using bilateral optogenetic inhibition (with GtACR2), then used two-photon calcium imaging alongside selective GCaMP8s expression to monitor the activity of ET (N=3 mice, n=~180 neurons/day/animal) and layer (L)2/3 intratelencephalic (IT) (N=3 mice, n=~450 neurons/day/animal) populations.
Results: Clustering analyses of ET and L2/3 IT populations revealed heterogenous response motifs that correlated with various stimulus and task variables. One such motif, primarily present in ET neurons, corresponded to “categorical” firing patterns (i.e., neurons that responded best to low or high sAM rates). This categorical selectivity was not present early in training, and longitudinal recording revealed that ET neurons shifted their response profiles dynamically across learning to reflect these discrete perceptual categories. Our stimulus set included a sAM rate at the category boundary, rewarded probabilistically, allowing us to investigate stimulus-independent choice. Using statistical approaches to visualize high-dimensional neural activity we found that ET population activity, in response to this boundary stimulus, reflected behavioral choice, regardless of reward outcome. Further quantification using neural decoding analyses confirmed that behavioral choice could be robustly predicted from ET activity. Both choice and categorical selectivity were notably lessened in the L2/3 IT population, hinting at a unique ET role.
Conclusions: Critically, ET categorical selectivity was only evident during active behavioral engagement and disappeared during passive presentation of identical stimuli. This suggests that learned categorical selectivity is shaped via top-down inputs that act as a flexible, task-dependent filter, a hypothesis that we are actively pursuing. These results suggest that the ACtx ET projection system selectively propagates behaviorally-relevant signals brain-wide and is critical for auditory-guided behavior.

SU17. The Effects of Early Postnatal Noise Exposure on the Development of Perineuronal Nets in Rat Inferior Colliculus and Auditory Cortex

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Category: Auditory Cortex and Thalamus: Structure and Function
Background: In the rat auditory system, a period of increased vulnerability to external stimuli (critical period, CP) starts with the onset of hearing (PD 12) and ends around three weeks later. It is expected that the closure of the CP is paralleled with the maturation of perineuronal nets (PNNs), lattice-like extracellular matrix structures that appear around the soma and proximal dendrites of mainly parvalbumin-expressing inhibitory neurons. Exposure to loud sound during the CP can significantly affect the morphology and electrophysiology of neurons in the rat central auditory system. Whether the exposure can also change the pattern of PNN maturation remains unknown.
Methods: min. The changes of PNNs was evaluated in the brain sections of inferior colliculus (IC) and auditory cortex (AC), stained for Wisteria floribunda agglutinin in the exposed rats aging from PD 14 to PD 106, and compared with non-exposed controls. For analysis, we further divided IC into the central nucleus (CIC), dorsal cortex (DIC), and external cortex (EIC), and auditory cortex into the superficial (I-IV) and deep layers (V-VI).
Results: In all three structures of the IC (CIC, DIC, EIC), PNNs were visible already at PD 14 in a greater number in exposed than control animals. The number of PNNs continued to increase during the development, but the difference between exposed and control groups remained to be present across all IC structures. In the AC, no PNNs were discernible until PD 21, when they were again more expressed in the exposed animals, particularly in the deep layers. The difference remained significant in PD 28, but has diminished during further development. Therefore, it seems that the accelerated development of PNNs in exposed animals might be at least partly a general phenomenon.
Conclusions: To summarize, we confirmed that PNNs develop in the AC later than in subcortical auditory structures. Second, we found that the development of PNNs appeared to be more accelerated in the noise-exposed animals than in the non-exposed controls. These results suggest that noise exposure may lead to premature closing of the CP window, thus limiting the plasticity of early postnatal development in the central auditory system. This study was supported by the Operational Programme Research, Development and Education in the framework of the project "Centre of Reconstructive Neuroscience", registration number CZ.02.1.01/0.0./0.0/15_003/0000419, and by the project INTERACTION LTAIN19201.

SU18. Excitatory-Inhibitory Circuit Modifications in the Emergence of Hyperexcitability after Acoustic Trauma

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Category: Auditory Cortex and Thalamus: Structure and Function
Background: Cortical hyperactivity is a cardinal signature of peripheral deafferentation. In the adult auditory cortex (ACtx), noise exposure causing high-frequency cochlear sensorineural hearing loss induces cortical hyper-responsivity and perceptual hypersensitivity to spared mid-frequency tones (McGill et al., eLife 2022). Here, we use high-density columnar single unit recordings combined with multi-channel optogenetics to address the precise relationship between excess central gain, disinhibition, and altered encoding of long-range intracortical inputs.
Methods: We used an intersectional virus strategy in adult mice to express channelrhodopsin in parvalbumin+ (PV) GABAergic neurons in the right ACtx and a somatically restricted, red-shifted opsin in the left ACtx, allowing us to independently measure from single neurons in the right primary ACtx (A1): 1) auditory responsiveness, 2) PV-mediated spike suppression, 3) firing rate changes evoked by excitatory callosal inputs from the left ACtx. Translaminar recordings were performed in awake head-fixed mice up to 3 days after acoustic trauma (16-32 kHz noise at 103 dB SPL for 2 hours) or sham exposure.
Results: Regular spiking (RS) units in deafferented high-frequency A1 map regions exhibited substantially increased responses to spared mid-frequency tones following acoustic trauma (n=389 units in 6 mice) compared to sham exposure (n=361 units in 5 mice). Although fast-spiking (FS) unit excitability (reflected by the direct activation of PV neurons) did not change after acoustic trauma, PV-mediated inhibition onto the RS units was strikingly decreased in the exposed group. Excess gain and reduced inhibition were observed in all layers but were most prominent overall in layer 6. While bottom-up sound-evoked responses were increased, long-range intracortical excitatory inputs were not, suggesting that acoustic trauma changes

the balance of thalamocortical and intracortical inputs in addition to its expected effect on the balance of excitation and inhibition.
Conclusions: The sudden and steeply sloping loss of afferent cochlear input caused by acoustic trauma triggers a disinhibition-compensatory mechanism in which RS units become less sensitive to feedforward inhibition from PV neurons. The direct stimulation of callosal-excitatory inputs did not show hyperresponsiveness as to sound input, suggesting differential changes between thalamocortical excitatory inputs versus cortico-cortical excitatory inputs. Our ongoing studies explore how excess gain in deep layers of the A1 column are reflected both in thalamocortical inputs as well as outputs to the basolateral amygdala.

SU19. Neuronal Integration of Acoustic Signals in an Insect Auditory System

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Category: Auditory Cortex and Thalamus: Structure and Function
Background: Mechanoreceptors in hearing organs translate sound-induced mechanical responses into neuronal signals, which are processed and forwarded to the brain along a chain of neurons in the auditory pathway. In bushcrickets, axons of the sensory cells in the hearing organ, that is located in the front leg ears, project to the prothoracic ganglion, where the first-integration step takes place. There, a relatively small number of local and projecting interneurons process incoming signals to facilitate frequency spectrum analysis, temporal pattern recognition and directional orientation. During this first-integration step of neuronal signals, inhibition is well described in bushcrickets. However, information about advantage in processing during hearing about what is lost and what is gained is still missing.
Methods: Here, we systematically characterize in the bushcricket species Mecopoda elongata the neuronal processing at the level of sensory cells, the prothoracic ganglion as well as in ascending neurons projecting to the brain. We collected data about the temporal pattern of spiking along this auditory pathway and the impact of inhibitory interneurons. To this end, we measured extracellularly spiking of sensory neurons in the leg, the activity of the prothoracic ganglion using multielectrode arrays and the response of the neck connectives with hook electrodes during tonal and natural song stimulation. To gain access to the role of inhibition, we eliminated sensory input from the contralateral ear.
Results: Our experiments reveal the timing sequence of spikes along the auditory pathway in bushcrickets from the sensory neuron to the neck, which connects to the brain. Specifically, from the ear to the neck about 14 ms are needed and spike numbers decrease. Furthermore, we show the spatio-temporal pattern of excitation within the prothoracic ganglion and in particular the impact of inhibitory interneurons projecting from the contralateral side. Interestingly, we found long-lasting increases in local field potential amplitudes, that point to amplification processes on the synaptic level.
Conclusions: This may play a role during communication of M. elongata males with females.

SU20. Neural Correlates of Auditory Distance Perception and of Auditory Distance Cues

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Category: Auditory Cortex and Thalamus: Structure and Function
Background: Perceiving the sound source distance is of key value in many everyday activities. The psychoacoustics of distance perception and its neuronal correlates are poorly understood. Previous studies identified planum temporale (PT) and superior temporal gyrus (STG) as auditory cortical areas important for intensity-independent auditory distance processing based on the direct-to-reverberant energy ratio (DRR) and the interaural level difference (ILD) cues. However, it is not clear whether the area represents the distance percept per se and/or one of the intensity independent acoustic cues ILD and/or DRR. In a previous study [Doreswamy et al. (2021) ARO Abstract #M7], we conducted behavioral and neuroimaging experiments in a virtual reverberant environment. Here, we perform advanced computational analyses to identify the cortical areas encoding the distance cues and distance percepts.
Methods: The auditory distance stimuli were simulated using a single set of non-individualized binaural room impulse responses (BRIR) measured on a listener that did not participate in this study. The auditory stimuli were broadband noise bursts varying in distance (15–100 cm) on the left-hand side along the interaural axis while the ILD/DRR cue availability was manipulated such that the cues varied with distance either congruently or incongruently. The behavioral experiment involved a distance discrimination task for

various stimulus pairs. The discrimination performance was used to confirm that distance perception with congruent cues is better than with incongruent cues. The imaging experiment was a sparse-sampling adaptation fMRI in which the stimuli were random sequences of noise bursts presented from various distances either with congruent or with incongruent cues. Univariate and split-half correlation multivariate pattern analysis (MVPA) were performed on the previously identified ROIs in volume space and compared to the previous surface-based results.
Results: Behavioral results showed that subjects performed better when cues varied with distance congruently. There were no significant effects in fMRI univariate contrast between congruent vs incongruent stimuli, while significant contrasts were observed between stimuli containing DRR and ILD-only stimuli. On the other hand, MVPA found a significant difference between congruent and incongruent conditions. All these effects were observed in the right hemisphere, contralateral to the stimulus azimuth.
Conclusions: These results suggest that the auditory cortex ROI encompassing the PT and STG encodes both the auditory distance cues and the distance percepts. And, while the cue encoding is non-distributed, detectable by univariate analysis, the percepts are encoded in a distributed network detectable only by multivariate analysis. However, the extent to which the encoding of cues and percepts is overlapping cannot be determined by the current analysis.
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SU21. Designing Novel Psychophysical and Electrophysiological Markers of Cochlear Synaptopathy Based on Temporal Fine Structure Coding Fidelity

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Category: Auditory Nerve
Background: At present, it is estimated that over 10% of individuals with clinically-normal audiograms have significant difficulty understanding speech-in-noise (SPiN). Cochlear synaptopathy (CS) – the loss of synapses connecting the cochlea to the auditory nerve (AN), caused by aging or noise exposure – is thought to be an important factor contributing to this problem. Most recent studies addressed CS through its impact on temporal envelope coding, thus missing a component of sounds that is pivotal to SPiN coding, namely their temporal fine structure (TFS). In the present work, we investigated whether complex tones resembling speech vowels could be used to design novel, complementary tools to assess CS through its impact on TFS-coding fidelity.
Methods: To test this idea, we designed a battery of psychoacoustical detection thresholds and electrophysiological frequency-following responses (FFRs) measurements based on spectrally-complex signals carrying well-defined TFS cues. All measurements were conducted in younger normal-hearing individuals (yNH group, n=18) as well as older individuals with normal hearing (oNH, n=16) or mild-to-moderate hearing loss (oHI, n=14). SPiN scores were also measured: SRTs were obtained with Matrix tests using steady-state, speech-shaped noise presented under several frequency-filtering conditions.
Results: We will present and discuss all the results obtained from this large data-set. One important result was that the strength of FFRs was strongly reduced in older compared to younger listeners, and negatively correlated to age. In contrast, we found no differences in FFR strength between older listeners with or without sensorineural hearing-loss. These data suggest that cochlear damage per se does not impact the neural coding of TFS cues, but that CS is an important factor underlying the impact of age on TFS-coding capacities. However, we observed no relationships between these neural measurements and SRTs, nor between psychoacoustical detection thresholds of spectral envelope shapes and SRTs in either group, even when speech and noise material were filtered in the low-frequencies so as to maximally engage the use of TFS cues.
Conclusions: Taken together, these results suggest that FFRs to spectrally-shaped complex signals could provide a novel perspective to monitor CS through its impact on TFS-coding. Yet, these joint psychophysical and electrophysiological results call for further work to design a psychoacoustical test that would provide a robust behavioral proxy of TFS-coding fidelity for such spectrally-shaped sounds. Overall, this project paves the way toward novel non-invasive biological markers of CS based on the neural encoding of spectral cues of sounds.

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SU22. Evaluating the Predictive Potential of the Polarity Effect on Auditory Nerve Health Using the Electrically Evoked Compound Action Potential in Guinea Pigs

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Category: Auditory Nerve

Background: Cochlear implants (CIs) directly stimulate the spiral ganglion cells (SGCs) of the auditory nerve, which in the absence of hair cells degenerate over time. Several computational models predict that anodic currents stimulate the SGC cell body and central axon, whereas cathodic currents stimulate the peripheral processes (PPs) (e.g. Resnick et al., 2018, Hear. Res. 361). The difference in excitation efficacy between the two polarities is known as the polarity effect, which is hypothesized to be an estimate for SGC degeneration – in particular the extent of degeneration of PPs prior to that of the cell bodies. For an intact auditory nerve, a preference for cathodic stimulation is expected, while with substantial PP degeneration anodic stimulation would be more effective. Since this theoretical polarity effect has not been indisputably experimentally demonstrated, we here compare electrically evoked compound action potentials (eCAPs) for separate pulse polarities to SGC and PP histology in order to establish whether the polarity effect is in fact a predictor of auditory nerve health.
Methods: eCAPs were recorded in 51 chronically or acutely implanted guinea pigs using a PULSAR implant (MED-EL, Innsbruck, Austria) with biphasic current pulses with alternating polarity. The polarity effect on various eCAP outcomes, such as amplitude, slope, threshold, and latency was measured and compared between normal-hearing and ototoxically deafened guinea pigs, and related to quantified histology of SGCs and PPs. Artifact-only recordings were performed post-mortem immediately after euthanasia in order to obtain artifact-free eCAPs for the individual pulse polarities (i.e., anodic-first and cathodic-first).
Results: The applied artifact reduction method for individual polarities adequately reduced the stimulus artifact. The cathodic-first stimulus had higher excitation efficacy: in both normal-hearing and deafened guinea pigs the eCAPs evoked by cathodic-first stimulation had higher amplitudes than those evoked by anodic-first stimulation. For all animals and conditions, the eCAP latency was substantially shorter for cathodic-first stimuli, suggesting that the cathodic phase was the excitatory one in both pulse configurations. In all normal-hearing animals eCAPs could be evoked with anodic-first pulses (with roughly half the amplitude of cathodic-first-evoked eCAPs), while in deafened animals with approximately 50% SGC survival anodic-first pulses regularly failed to evoke eCAPs. None of the examined eCAP polarity effects correlated with SGC survival across animals.
Conclusions: The observation of an overall preference for cathodic-leading stimuli in agreement with the existing literature in animals. In contrast to what is suggested in the literature on the polarity effect, we did not observe a shift in excitation preference from cathodic to anodic currents after deafening. Whether or not the polarity may still be informative of neural health will be based on the ratio of PP and SGC survival.

SU23. Optimization of Recording Parameters for Enhanced Visualization of Early Auditory Evoked Potentials

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Category: Auditory Nerve

Background: Early auditory evoked potentials (AEPs) – such as electrocochleography (ECochG) and the auditory brainstem response (ABR) – have been widely used to examine the cochlear synapse in humans, which correspond to the compound action potential (cAP) in ECochG and wave I in the ABR. If cochlear synaptopathy (CS) occurs in humans, it is paramount to optimize our methods of recording the cAP/wave I component in order to utilize it as a proxy for CS.
This study focused on electrode placement, or montage. Theoretical principles suggest that recordings are optimized when electrodes are placed in the same plane as the direction of neural propagation. We tested this by recording early AEPs in horizontal and vertical montages. In doing so, we aim to provide optimized methods for recording the cAP/wave I component.
Methods: In 35 normal hearing young adults, ECochGs to a 100-µs broadband click (90 dB nHL, alternating polarity) were collected in vertical and horizontal montages, each using a gold-foil tiptrode in the

right ear canal as the reference electrode and stimulus transducer. Snap electrodes placed on high center forehead (Fz) and contralateral mastoid (M1) served as active and ground electrodes (vertical active: Fz, horizontal active: M1). For each montage, two repeatable traces were collected and added to produce a single waveform for analysis. Peak latency and peak-to-trough amplitude of cAP/wave I and wave II were compared between montages, as well as earlier ECochG components (SP and cAP amplitudes re: baseline, SP/AP ratio).
Results: Vertical montage recordings produced significantly larger cAP/wave I peak-to-trough amplitudes compared to the horizontal montage, resulting in an average increase of 73.9%. Wave II behaved opposite, showing a 54.5% decrease in amplitude in the vertical montage. Latency differences prove important for understanding these montage effects on cAP/wave I amplitude. Vertical montage recordings resulted in slightly earlier cAP/wave I latencies and significantly later wave II latencies. This delayed onset of wave II in the vertical montage allowed more time for the cAP/wave I trough to reach its maximum, rather than being cut short as it was in the horizontal montage. Vertical montage recordings therefore represent a more accurate measure of cAP/wave I amplitude. Lastly, earlier ECochG components remained consistent across vertical and horizontal recording montages.
Conclusions: An optimized measure of cAP/wave I amplitude is vital as it continues to be used as a proxy of CS. Our results indicate that electrode montage plays an important role in the outcomes of cAP/wave I visualization and measurement. ECochGs collected in a vertical montage produce a more accurate and sensitive measure of cAP/wave I characteristics compared to a horizontal montage. As CS continues to be explored in humans, we conclude that using a vertical electrode montage over a horizontal montage is ideal.

SU24. Evidence-Based Recommendations for Determining Sample Sizes for Experiments Assessing Auditory Nerve Function Using Electrocochleography

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Category: Auditory Nerve
Background: The physiology of the cochlea and auditory nerve can be assessed with electrocochleography (ECochG), a technique that involves measurement with an electrode placed near, on, or within the cochlea. Some studies and applications have centered on measuring the amplitudes of the auditory nerve compound action potential (AP) and summating potential (SP). Despite the common use of ECochG, the variability of repeated measurements from individuals or groups of participants is not well understood. Previous studies reported excellent reliability using the intraclass correlation coefficient, though information for determining sample sizes and powering ECochG-based studies is not available. Indeed, the variability of ECochG measurements may contribute to the difficulty of translational research efforts aimed to identify cochlear synaptopathy in humans. Here we use a Bayesian model to provide guidance on determining sample sizes for powering ECochG-based studies.
Methods: ECochG measurements were made from younger adults (N=36) with normal hearing using a tympanic membrane electrode. We constructed a Bayesian model to predict minimum detectable differences (Dmin) for AP and SP amplitudes from this dataset, where each participant contributed multiple repeated ECochG recordings for a total of N=128 measurements. The model was used to simulate the Dmin for hypothetical experiments that assessed disease or treatment effects using repeated- (RM) and independent-measurement (IM) designs. The model predicted Dmin for a given number of participants and repeated measurements.
Results: AP and SP amplitudes were positively correlated. AP amplitudes spanned a range of approximately 18 dB, and SP amplitudes spanned a range of approximately 21 dB. Repeated measurements from individual participants fell over a range of approximately +/- 6 dB, relative to the participant's mean AP and SP values. Increasing the number of repeated measurements or participants (or both) exponentially decreased Dmin in dB. When the number of repeated measurements increased from one to eight, Dmin for AP amplitudes decreased from 7.1 dB to 2.5 dB for one participant per group, and from 1.3 dB to 0.4 dB for 32 participants per group. Dmin for AP amplitudes was approximately 1.2 times lower than Dmin for SP amplitudes. Dmin for RM experiments was approximately 3.6 times lower than for IM experiments.
Conclusions: With a suitable number of participants and repeated measurements, ECochG can be used to reliably detect small amplitude differences resulting from disease or treatment. Our findings provide evidence-based recommendations for the design and sample size determination of experiments using ECochG amplitude measurements. Accounting for the variability of ECochG measurements should result in

more consistent findings in the clinical and basic assessments of hearing and hearing loss, either hidden or overt.

SU25. Auditory Brainstem Implant-Evoked Responses of Auditory Cortical Neurons in a Chronic Mouse Model

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Category: Auditory Prostheses
Background: The auditory brainstem implant (ABI) is the only alternative to cochlear implants (CI) for hearing rehabilitation in patients with Neurofibromatosis type 2 (NF2). The ABI stimulates second order auditory neurons of the cochlear nucleus (CN) using a multichannel surface array. Unlike the CI, ABI performance remains modest, and most users struggle to understand speech, especially in the absence of lip reading, but still show improvement over time with consistent device use. To improve the design and implementation of the ABI, it is critical to understand how it activates neurons within the central auditory system. We therefore developed a novel mouse model of the ABI that enables a chronic study of how the ABI activates neurons within the primary auditory cortex (A1) using two-photon calcium imaging in awake mice.
Methods: Mice were implanted with a conformable two or three-channel ABI surface array on dorsal CN following suboccipital craniotomy and received a cranial window over A1 for two-photon imaging. Transgenic mice expressing the Ca2+ reporter GCamP6s in Thy1+ cells allowed us to record the activity of L2/3 pyramidal neurons. Acoustic stimuli of broadband noise bursts or ABI stimuli of balanced, biphasic pulse-trains or amplitude-modulated pulse trains were delivered. To characterize neuronal responses to the ABI, we modified the pulse amplitude, pulse rate, pulse duration, modulation rate and modulation depth.
Results: As described previously by electrophysiological studies using the CI, we observed three populations of responsive neurons in A1: neurons that responded selectively to the acoustic stimulus, selectively to the ABI, or to both stimulus types (n = 800 cells from 3 mice). ABI responsive neurons exhibited selectivity for distinct features of the stimulation including pulse rate and modulation depth. Ongoing analyses will determine if neurons tuned to ABI stimulation features are spatially organized within A1.
Conclusions: The characterization of cortical responses to features of the ABI stimulation will help to identify stimulation strategies to optimize the activation of cortical neurons. Further development of this ABI mouse model will permit future studies on the specific neuronal cell types recruited by ABI stimulation paradigms and the effects of learning to use the ABI on specific cortical circuits.

SU26. Design of a Hybrid Opto-Electrical Cochlear Implant

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Category: Auditory Prostheses
Background: Deafness and hearing loss are widespread in the world. At-present, about 20% of the global population live with debilitating hearing loss. Assistive technologies, such as hearing aids, middle ear, and cochlear implants (CIs) can help people requiring hearing rehabilitation.
While CIs are widely used, their technology based on electrical stimulation exhibits low spatial precision due to the current spreading in the cochlea, affecting sound perception. CIs performance could be enhanced with optical stimulation, particularly infrared neurostimulation (INS). Infrared light can be tightly focused in small areas of the cochlea, enabling the activation of more discrete, independent neural structures and without prior genetic incorporation of light sensitive molecules (opsins).
To incorporate INS into a CI, several features must be considered, such as number of electrical and optical channels, energy requirements, size of the CI, novel coding strategies and light delivery systems. In the present work, we describe a prototype of a hybrid opto-electrical CI (oeCI).
Methods: The hybrid oeCI includes five building blocks: A) an electrical driver with fully integrated 24 channels. Each channel has two current stimulators, providing biphasic-current pulses (milliamps range) and charge-recovery circuits; B) an optical driver with fully integrated 16 channels. These channels modulate

light sources between different current levels defined by input voltages. The inputs are controlled with 16 digital-analog converters (DACs), one DAC per optical output; C) an ultra-low power circuit module containing a 32-bit microcontroller with an integrated low power radio communication supporting Bluetooth and including onboard antenna; D) a digital MEMs microphone; E) a medical grade lithium-ion battery that can be recharged in three hours and with hundreds of cycles.
Circuits were designed in a 4-layer PCB with a dimension of 3x3 cm2 using the software Altium Design 2019 (Altium, USA). The first prototypes were built by OSH Park (Chicago, Illinois, USA). The top surface has the electrical driver and the bottom, the optical driver and DACs. The inner layers were chosen as ground planes (GND). The MEMS microphone and the microcontroller were attached to the top surface.
To deliver light along the cochlea, we are exploring two strategies. First, inserting light sources arrays into scala tympani of the cochlea. Second, delivering light using bundles of polymeric waveguides.
Results: The device testing is ongoing. The entire oeCI was designed to run for more than a day of continuous stimulation. The battery chosen can provide a long life span of the device (years).
Conclusions: To characterize the hybrid electrodes and determine the most suitable approach (light sources arrays or waveguides), insertion force measurements are ongoing in cadaveric human cochleae as well as in a matrix printed model of the human scala tympani. Furthermore, propagation losses and coupling efficiency are ongoing in the waveguide to determine its performance.

SU27. Evaluating Midbrain Responses to Optogenetic Stimulation of the Auditory Pathway by a Novel Large Conductance, Red-Shifted Channelrhodopsin Variant

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Category: Auditory Prostheses
Background: Contemporary electrical cochlear implants (eCI) directly stimulate spiral ganglion neurons (SGNs) using electric current to partially restore auditory function in patients diagnosed with profound sensorineural hearing loss or deafness. However, patients report poor speech understanding in noise, which can be explained by the limited number of independent stimulation channels. Multiunit recordings from the inferior colliculus (IC) of anaesthetized animals further demonstrated the limited spatial resolution from eCI stimulation, which could be fundamentally improved by spatially more selective optogenetic cochlear implants (oCI). Here, towards clinical translation, we engineered and characterized novel variants of the channelrhodopsin ChRmine to increase the light sensitivity and thereby optimize the power oCI budget.
Methods: Mongolian gerbils were injected at postnatal day six (n=12) with PHP.S_hSyn-ChRmine-mutant. At the age of 86±17 days, animals underwent surgery under anesthesia to access the cochlea where an optical fiber (200 µm, Thorlabs) was placed through the round window targeting towards the modiolus. First, optically evoked auditory brainstem responses (oABR) were assessed and intensity thresholds measured. Only animals showing ABR to cochlear optical stimulation underwent a contralateral craniotomy where a 32 electrode linear silicon shank was inserted along the tonotopic axis of the IC for characterizing peri-stimulus time histograms (PSTH), spike probability, following rate and vector strength. Physiology was followed by immunohistochemical estimation of the density and transduction of SGNs.
Results: All animals show optically evoked ABRs, a median threshold to d' of 1 of 0.68 mW (n=7, for 1 ms stimuli at 10 Hz) and a d' of 2 at 1.095 mW. PSTH collected show an average tOn of 2.7ms and an average tOff of 33.9ms. The fraction of responsive units dropped below 50% when using stimulation rates over 150Hz. For all animals (n=12) confocal imaging of extracted cochleae show robust expression from base to apex.
Conclusions: We successfully established IC recordings using Mongolian gerbils expressing a mutated version of ChRmine. These animals show robust expression of the opsin throughout the SGNs and optical stimulation of the cochleae elicited robust IC activity at low light intensities. The ChRmine-mutant is an interesting candidate for chronic optogenetic cochlear implant experiments involving the use of low power optical emitters.

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SU29. In Vitro and in Vivo Characterization of Improved Channelrhodopsin ChRmine Variants for Optogenetic Activation of the Auditory Pathway

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Category: Auditory Prostheses
Background: Electrical cochlear (eCI) implants provide partial hearing restoration to individuals with severe to profound sensorineural hearing loss by direct electrical stimulation of spiral ganglion neurons (SGNs). To overcome wide-spread electrical neural excitation associated with eCIs, stimulation of SGNs using optogenetics presents an attractive solution to address this limitation. For translating optogenetic hearing restoration, it is critical to select a channelrhodopsin (ChR) with large photocurrents, red-shifted activation, low desensitization, and ideally fast kinetics. ChRmine is a bacteriorhodopsin-like cation ChR (BCCR) which mediates very large peak photocurrents when compared to algal ChRs but displays rapid desensitization. Here we evaluate the optogenetic utility of ChRmine mutants we engineered for less desensitization.
Methods: In vitro characterization of electrophysiological properties of ChRmine mutants was performed by whole cell patch-clamp recordings of transfected neuroma glioblastoma (NG) cells expressing one of three ChRmine-mutants or the wild-type (WT) upon excitation with light at a wavelength of $\lambda = 532$ nm. For in vivo characterization, adeno-associated-virus (AAV) carrying transgene WT or mutant ChRmine under the human synapsin promotor were injected into the round window of neonatal C57Bl6/J mice (postnatal day 6). Six to ten weeks after injections, a laser-coupled fiber (594 nm) was inserted into the round window to measure optically evoked auditory brainstem responses (oABRs). Subsequently, the cochleae were extracted for immunohistological analysis by lightsheet microscopy to evaluate the number of transduced cells as well as membrane expression profiles.
Results: From whole cell-recording, photocurrents of the 4 ChRmine variants peaked between 514 to 518 nm. All ChRmine mutants demonstrated less desensitization than the WT as well as large stationary photocurrent densities and high light sensitivity. Eleven and eight mice were injected with the ChRmine mutant #3 and WT, respectively. oABRs were elicited in all animals, with amplitudes in the range of 8 to 15 μV, and mean thresholds of 0.79 +/- 0.4 mW (mutant) and 5.7 +/- 6.4 mW (WT), during optical stimulations of 1 ms at 10 and 20 Hz (please note that 594 nm is not optimal for ChRmine. The oABR amplitude dropped with stimulations rates over 150 Hz. Preliminary histological data show robust expression of ChRmine WT and mutant #3 in SGNs throughout all turns of the cochlea.
Conclusions: The ChRmine variants exhibit large photocurrents and comparatively slow kinetics, which promises robust neuronal photoactivation at moderate ChR expression levels and at low light intensities. ChRmine mutant #3 hence sets a lower bound of the power requirement for optogenetic hearing restoration with a theoretically estimated thresholds at optimal wavelength of 0.1 mW. Future work will need to speed up its deactivation kinetics and to further characterize the utility of BCCR variants for optogenetic stimulation of the auditory pathway.

SU30. Investigating Current Spread in Cochlear Implant Users With the Panoramic ECAP Method

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Category: Auditory Prostheses
Background: The Panoramic ECAP (PECAP) Method uses Electrically-Evoked Compound Action-Potentials (ECAPs) to estimate the variation in current spread and neural health along the length of the electrode array in individual cochlear implant (CI) users. PECAP provides a platform for detailed assessment of the effects of various stimulation types on the spread of electrical current within the cochlea. Some CI devices can provide so-called focused electrical stimulation by partially returning the electric current to adjacent, intra-cochlear electrodes (partial tripolar (pTP) stimulation). Another method attempts to achieve the opposite: to provide blurred stimulation by stimulating multiple electrodes simultaneously. We applied these manipulations to evaluate their effects on the spread of current when compared to monopolar mode. We further investigated the effect of cochlear region and array type on current spread.
Methods: ECAPs were recorded using the forward-masking artefact-cancellation technique from 12 Advanced Bionics CI users in monopolar mode for all electrodes activated in the participant's map and for every combination of masker and probe electrode. Two test electrodes were then selected, one apically and

one basally located along the CI electrode array. ECAPs were then also obtained in response to pTP and two blurred stimulation modes (with 3 and 5 electrodes stimulated simultaneously) in the two test electrodes. Stimuli for all presentation modes (monopolar, pTP, or blurred) were scaled to comfortable loudness for each participant. Data were then submitted to PECAP, and the current-spread estimate for the test electrodes was compared between the monopolar condition and the focused and blurred conditions. We concurrently investigated the effect of array type (23 straight vs 20 perimodiolar) and cochlear location on current spread using monopolar PECAP data from Cochlear CI users.
Results: PECAP analysis revealed an increase in current spread as a result of blurred stimulation applied at the apical side of the array, but not at the basal side. There was a trend of higher current spread detected when stimulating 5 electrodes simultaneously compared to 3 electrodes, but this did not reach statistical significance. PECAP also revealed an increase in current spread as a result of stimulating in pTP mode at the group level, primarily driven by large effects in 2 participants. PECAP also found that current spread was significantly broader for straight and at the apex, with a marginal interaction suggesting that the array effect may be greater at the apex.
Conclusions: The results suggest that blurred stimulation more effectively increases current spread at the apical side of the array than the basal side, and that in some cases pTP stimulation increases current spread instead of reducing it. It also provides a detailed comparison of the effect of array type on current spread across the electrode array, indicating wider current spread for straight compared to perimodiolar arrays.

SU31. Orally Administered CSF-1R Specific Kinase (c-FMS) Inhibitor PLX-5622 Suppresses Intracochlear Macrophage Infiltration Following Cochlear Implantation Without Affecting Fibrosis
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Category: Auditory Prostheses
Background: In patients with sensorineural hearing loss, non-functional sensory cells can be bypassed by a cochlear implant (CI) to restore hearing and improve quality of life. The foreign body response (FBR) following cochlear implantation (post-CI) comprises of infiltration of macrophages and other immune and non-immune cells and fibrosis. FBR post-CI is associated with negative effects on CI outcomes: increased electrode impedances, decreased battery life, delayed loss of residual acoustic hearing, or in rare instances, device failure. Following CI, the non-specific immunosuppressive drug, dexamethasone suppresses cochlear macrophage infiltration and reduces the fibrotic response. However, it is unknown whether there is a causal relationship between macrophage infiltration and fibrosis. This study aims to investigate the extent to which macrophage depletion by an orally administered CSF-1R specific kinase (c-FMS) inhibitor PLX-5622 modulates the FBR post-CI in a murine model.
Methods: 10-12-week-old CX3CR1+/GFP Thy1+/YFP mice on C57Bl6 background with normal hearing were fed chow containing 300 mg/kg PLX5622 provided by Plexxikon or control chow for the duration of the study. 7-days after starting the experimental diets, 3-channel cochlear implants were implanted in the left ear via the round window while right ear served as an unoperated control. Serial impedance, transimpedance matrix (TIM) and neural response telemetry (NRT) measurements were acquired throughout the study. Electric stimulation began 7 days post-CI until 10-, 28- or 56-days post-CI for 5 hrs/day, 5 days/week, with the threshold-level 30 CL below NRT threshold and comfort-level determined with behavioral response. Cochleae harvested at 10, 28 or 56 days post-CI were cryosectioned at 30 μm parallel to the mid-modiolar plane, labeled with antibody against α-Smooth Muscle Actin (α-SMA) to identify myofibroblasts and quantify the fibrotic response. Using IMARIS image analysis software, the outlines of scala tympani, Rosenthal canal, modiolus and lateral wall for each turn were traced manually to measure region volume. Density of DAPI+ nuclei, CX3CR1+ macrophages, Thy1+ spiral ganglion neurons (SGNs) and ratio of volume of α-SMA+ space/volume of scala tympani were calculated.
Results: Cochlear implantation in normal diet subjects caused infiltration of cells, including macrophages, into the cochlea: initially diffuse throughout the cochlea and later localizing to the the scala tympani of the basal turn by 56-days post-CI. Fibrosis was evident in the base of implanted cochlea. Mice fed with chow containing PLX5622 showed very few macrophages throughout the implanted cochleae at both early and late timepoints. However, scala tympani fibrosis was not reduced relative to normal diet subjects.
Conclusions: These data suggest that depletion of macrophages does not appreciably prevent intracochlear fibrotic responses post-CI.

SU32. Dexamethasone-Eluting Cochlear Implants Reduce Intracochlear Foreign Body Response and Electrical Impedance Following Surgery in a Murine Model

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Category: Auditory Prostheses

Background: The foreign body response (FBR) following cochlear (CI) can result in fibrosis and neo-ossification. Post-CI FBR can negatively affect the outcome of CI including raised electrode impedances, decline in battery life, further loss of acoustic hearing after initial hearing preservation, or in rare cases, device failure. This study aims at investigating the effects of dexamethasone, a potent anti-inflammatory glucocorticoid, on the intracochlear FBR and CI electrical impedance after cochlear implantation in a murine model.

Methods: 10-12-week-old CX3CR1+/GFP Thy1+/YFP mice on C57Bl6 background with normal hearing, were implanted with a 3-channel cochlear implant (dexamethasone-eluting or standard implant) in the left ear via the round window; right ear being an unoperated control. Implant functionality was tested with serial impedance and neural response telemetry (NRT) measurements. Starting on post-operative day 7 until final time-point, electrodes were stimulated for 5 hrs/day, 5 days/week with stimulation threshold-level 30 CL below NRT threshold and comfort-level determined by behavioral response. Cochleae were harvested at 10, 28 or 56 days post-operatively, 4% PFA fixed, 30μm thick cryosections parallel to the mid-modiolar plane were labeled with antibody against α-Smooth Muscle Actin (α-SMA) to label myofibroblasts and quantify the fibrotic response. The outlines of scala tympani, Rosenthal canal, modiolus and lateral wall for each turn were traced manually to measure the volume of each and to quantify nuclei and density of CX3CR1+ macrophages and spiral ganglion neurons (SGNs). The volume of α-SMA-positive fibrotic tissue was measured and the ratio of the volume of α-SMA+ tissue/volume of scala tympani calculated.

Results: Accounting for open circuits, the dexamethasone-eluting group had decreased impedance values compared to controls, most prominent at later time-points. Transimpedance matrix measures showed increased access and polarization resistance over time in the control versus dexamethasone-eluting groups. Cochlear implantation caused infiltration of cells and CX3CR1+ macrophages into the cochlea: initially generalized throughout the cochlea and becoming predominantly localized to the scala tympani of the basal region of the cochlea by 56 days post-CI. Fibrosis was observed in the scala tympani throughout the time investigated following standard CI. Cochlear implantation did not cause appreciable degeneration of SGNs. Compared to cochleae implanted with standard arrays, cochleae implanted with dexamethasone-eluting arrays showed significant reduction in the inflammatory response evident by reduced macrophage density and cellularity in scala tympani, Rosenthal's canal, the lateral wall and modiolus and reduced fibrosis in scala tympani.

Conclusions: Cochleae implanted with dexamethasone-eluting electrodes displayed a reduction in the inflammatory and fibrotic response for sustained period. These findings were associated with a decline in impedance measures, supporting the notion that the FBR increases the electrical impedance.

SU33. Access Resistance and Polarization Impedance Measurements in Cochlear Implant Patients With Rising Electrode Impedances

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Category: Auditory Prostheses

Background: Some cochlear implant users present with electrode impedances that increase while using electrical stimulation and decrease with device rest. In the present study, multi-point electrode impedance measurements were used to decode the mechanism for fluctuating or rising electrode impedances in cochlear implants. Electrode impedance measured at the end of the first phase of the biphasic pulse is known as total impedance (Tp), which is the sum of access resistance (Ra) and polarization impedance (Zp). Ra represents the resistance of the cochlear environment to the flow of current between the intracochlear stimulating electrode and the extracochlear ground electrode. In contrast, Zp represents the electrochemical electrode-electrolyte interface at the surface of the electrode. Ra can be analyzed into two additional components:

near- field (Rn) and far- field (Rf) resistance. Zp can be separated and analyzed into Warburg capacitance (Cp) and Faradaic resistance (Rp). These subcomponents were analyzed to determine the underlying mechanism for fluctuating electrode impedances in cochlear implants.
Methods: Electrode impedances were measured at 6, 12, 18, 24, and 75-µs during the presentation of cathodic leading biphasic pulses in eight Nucleus cochlear implant patients. In these patients, electrode impedances increased with electrical stimulation and decreased with device rest.
Results: An increase in electrode impedances was associated with an increase in near- field resistance of the Ra component, and both decreased Warburg capacitance and increased Faradaic resistance of the Zp component. Thus, an increase in electrode impedances with electrical stimulation can be attributed to increased resistance to the transfer of charge between the electrode surface and the cochlear electrolyte.
Conclusions: An increase in electrode impedance with the use of electrical stimulation is due to changes in the electrochemical reaction at the electrode-electrolyte interface. Further studies are needed to determine if alterations in electrical stimulation parameters can limit the increase in electrode impedances in patients with rising impedance pattern.

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SU35. Contributions of Peripheral and Cortical Neural Synchrony to the Categorization of Voice Onset Time in Cochlear Implant Recipients

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Category: Auditory Prostheses
Background: Voice onset time (VOT) is a complex acoustic feature that serves to distinguish consonants such as /b/ from /p/ (Lisker, 1986). Acuity for VOT identification is highly dependent on the synchronized neural response at the peripheral auditory system, evoked by the onset of voicing (Sinex et al., 1991). Theoretically, reduced neural synchronization in the auditory system could result in poor VOT identification acuity. Unfortunately, the contributions of peripheral and cortical neural synchrony to the perception of temporal cues to voicing in cochlear implant (CI) users have not been systematically evaluated and therefore remain unknown. To address this need, this study evaluated the relationships between peripheral and cortical neural synchrony and categorical perception of VOT stimuli in quiet and noise conditions in postlingually deafened CI users.
Methods: To date, eight subjects (eleven ears) have been recruited and tested for this study. All subjects were implanted with a Cochlear™ Nucleus® device in the test ear(s) with full electrode insertions. The stimulus was an /aba/-/apa/ vowel-consonant-vowel continuum with VOTs ranging from 0 ms to 88 ms. Speech-evoked auditory event-related potentials (ERPs) and behavioral categorization of VOT were measured both in quiet and in noise (speech-shaped noise at 10 dB signal-to-noise ratio, SNR) conditions. Neural synchrony in the auditory nerve and the auditory cortex were quantified using the phase-locking value (PLV) (Harris and Dubno, 2017; Harris et al.,2021). The psychometric function of VOT categorical perception was obtained by plotting the percentage of /aba/ response as a function of VOT. Relationships between PLVs and VOT results measured in different testing conditions were evaluated using Pearson Moment correlation tests.
Results: For results measured in the quiet condition, our preliminary results showed a significant positive correlation ($r = .61$, $p = .034$) between PLV results measured for the AN and the slope of the psychometric function. For results measured in the noise condition, there was a significant positive correlation ($r = .93$, $p = .022$) between the cortical PLV and the slope of the psychometric function. No other significant relationship was found.
Conclusions: These preliminary results suggest that VOT perception in quiet is related to peripheral neural synchrony, while VOT perception in noise is related to cortical neural synchrony.

SU36. Using Focused Cochlear Implant Stimulation Psychophysical Thresholds to Estimate Variation in Cochlea Dead Regions and Speech Understanding Outcomes in Adult Users

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Category: Auditory Prostheses

Background: There is growing evidence that areas of poor spiral ganglion cell survival, or neural dead regions (NDRs), negatively impact cochlear implant (CI) user speech perception outcomes. When activating CI electrodes within the NDRs, this variability in local neuron survival results in unintended activation of neighbouring regions with better neuron survival, effectively masking and smearing the local frequency information, leading to poorer speech perception. While the presence of a NDR at a single electrode can be confirmed using traditional psychophysical forward masking paradigms, the process is laborious for both clinicians and patients.
In contrast, this study uses a clinically friendly psychophysical detecting threshold assessment to estimate patient-specific variations in neuron survival along the CI electrode array. Our test leverages the difference in sensitivity of focused (bipolar) and broad (monopolar) stimulation modes to local variations at the electrode-neuron interface to pinpoint the location and size of patient-specific NDRs. We hypothesize that: 1) specific patterns of variation in psychoacoustic detection thresholds between electrodes can be used to reliably detect location of dead regions; and 2) a larger variation in neuron survival estimated using our threshold method is associated with poorer speech understanding outcomes.
Methods: Twelve post-lingually deaf adult CI users with at least one year of implant experience were recruited for the study. Psychophysical detection thresholds were measured at least once every four and two electrodes for monopolar and bipolar stimulation modes, respectively. The monopolar thresholds were subtracted from bipolar thresholds to estimate neural health along the cochlear implant electrode array. The presence of potential NDRs identified by thresholds was validated using a forward masking paradigm. Speech perception outcomes were assessed using Consonant-Nucleus-Consonant (CNC) words in quiet and Bamford-Kowal-Bench (BKB) sentences in babble noise.
Results: Preliminary data show that forward masking probe electrodes selected within potential NDRs identified by psychophysical thresholds resulted in a shift or widening of the psychoacoustic tuning curve peak towards the region of better neural health. This suggests that psychophysical thresholds are effective for identifying NDRs.
Variation in estimated neural health along the cochlea was negatively correlated ($p<0.05$) with both BKB and CNC scores.
Conclusions: Focused stimulation thresholds offer a clinically viable opportunity to estimate the variation in cochlea dead regions in cochlear implant users. The test identifies patient-specific neurological limitations which are associated with poorer speech understanding outcomes and enables development of targeted rehabilitation strategies for improving speech outcomes in cochlear implant users

SU37. Round Window Entry Alone Without Cochlear Implantation Induces Cochlear Inflammation
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Background: Cochlear implants are an established and highly effective intervention for sensorineural hearing loss. Implant performance is affected by the biological response to the chronic presence of a foreign body in the inner ear. The tissue response to cochlear implantation is characterized by inflammation, fibrosis, and neo-ossification. In this study, we performed cochlear surgery in transgenic mice to investigate the role of cochlear macrophages in the inflammatory response to labyrinthine entry.
Methods: CX3CR1+/DTR and wild type adult mice were treated with diphtheria toxin or saline over the period of one week (250ng/20g mouse weight, IP injected on 7, 4, 2 days before surgery). They were anesthetized and through a postauricular incision, the bulla was opened, and the round window membrane was perforated on the right side. The left ear was unoperated. Cochleas were harvested at 7, 14 and 21 days after surgery, and were fixed, decalcified, sectioned, and immunohistochemistry was performed for antibodies for CD45 (common leukocyte antigen) and iba1 (ionizing calcium binding adaptor protein 1: macrophages). Cochlear sections were imaged using confocal microscopy.
Results: Round window puncture resulted in cochlear inflammation that was notable 7 days after surgery and continued to be detected at days 14 and 21 days post operatively. Cochleas showed the most abundant inflammatory cells in the lower basal turn. Leukocytes were concentrated in the scala tympani and spiral ligament. Diphtheria toxin effectively eliminated cochlear mononuclear phagocytes in CX3CR1+/DTR mice, and the inflammatory response was markedly reduced in these animals, particularly at POD7. Diphtheria toxin mediated monocyte/macrophage depletion gradually subsided allowing inflammatory cells

to repopulate the inner ear. By 21 days postoperatively, some mice that had previously been macrophage depleted demonstrated a significant number of migrated myeloid cells in the operative ear. Leukocyte number was highly varied in animals that did not undergo CX3CR1 depletion. Some operated ears showed robust inflammatory responses, primarily in the basal turn scala tympani while others were comparable with the unoperated left ear.
Conclusions: Round window entry alone could elicit an inflammatory response in the cochlea without device insertion. Labyrinthine disruption is sufficient to elicit a significant transient inflammatory response and could contribute to cochlear scarring and fibrosis. Depletion of macrophages at the time of surgery reduced the number of migrating inflammatory cells during the first postoperative week. When DT treatment was discontinued, macrophages repopulated the cochlea. We will further evaluate how macrophage depletion and the timing of depletion affects the long-term outcome of cochlear remodeling associated with chronic implantation of a mouse cochlear implant.

SU38. Listening-Effort Costs of High Performance With Cochlear Implants

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Category: Auditory Prostheses
Background: Individuals with cochlear implants (CIs) often report effortful listening, especially in noisy environments. CIs provide limited spectral resolution, contributing to a highly degraded auditory signal. In addition, noise in the acoustic environment can further degrade the auditory signal of interest. This combination of signal degradation by the device and by noise could lead to increased reliance on top-down processes to understand speech with a CI. It was hypothesized that listening effort would be higher with poorer spectral resolution in older adults with acoustic hearing listening to CI simulations, and that listening effort would be higher during a sentence-in-babble recall task than during a sentence recall task in quiet in older adults with CIs.
Methods: Older adults with clinically normal hearing (0.25-4 kHz) attended to monaurally presented sentences in quiet and vocoded sentences over circumaural headphones. Vocoding simulated a range of spectral resolutions that could be provided by a CI. Older adult listeners with CIs also attended to sentences presented either in quiet or in continuous four-talker babble. Participants were instructed to listen to the sentence and repeat it when prompted. Sentence keyword recall accuracy was recorded and pupil dilation, an index of listening effort, was measured while the listeners heard, recalled, and reported the sentence.
Results: Increased pupil dilation was observed with poorer spectral resolution when sentence keywords were recalled accurately in fifteen older adults with acoustic hearing. In addition, preliminary data from older adult listeners with CIs showed varied patterns in the listening effort exerted during this task when sentence keywords were recalled accurately. Some listeners had similar pupil dilation over time in the quiet and babble conditions, but others showed larger pupil dilation in babble, consistent with the hypothesis, or smaller pupil dilation in babble, inconsistent with the hypothesis.
Conclusions: These results suggest that listening with reduced spectral resolution is effortful, and that the ability to exert higher effort in the presence of acoustic degradation may depend on the listener. One possible explanation is that motivation plays a role, and that listeners with CIs sometimes reserve mental effort for situations where they are more likely to succeed in understanding speech. Another possible explanation is that increased difficulty in the task is needed to reveal differences in listening effort with acoustic degradation in listeners with CIs.
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SU39. A Prospective Study of Cochlear Implantation in Single-Sided Deafness: Audiological Outcomes, Device Use, and Health Utility

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Category: Auditory Prostheses
Background: Cochlear implantation (CI) in single-sided deafness (SSD) has unique implications for patient performance and auditory processing. While studies have examined a spectrum of audiological outcomes, there lacks an understanding of how audiological outcomes are related to real-world patient quality of life,

health utility, and device use. We report outcomes from a prospective observational study of CI in SSD that evaluates patient performance across multiple dimensions, including audiological performance in quiet and in noise, quality of life, health utility, and device use.
Methods: 46 study participants with SSD and undergoing CI were enrolled prospectively. The deafened ear was implanted using either a Med-El-Flex-28 or Cochlear-Ltd-632 electrode between 2020 and 2022. For each patient, assessments were performed pre-implantation and at 3-, 6-, and 12-months post-CI activation, and consisted of CNC word and phoneme recognition in the implanted ear, AzBio (+8 dB SNR) with varying speech and noise presentations (S[0]N[0], S[SSD]N[AH], S[AH]N[SSD]), Spatial Hearing Questionnaire (SHQ) for quality of life related to sound localization, Tinnitus Handicap Inventory (THI), and the Health Utility Index Mark 3 (HUI3). Device-use data was acquired from manufacturer software. Subgroup analysis was performed based on duration of deafness. Statistical analysis was performed using R (Boston, MA).
Results: In the SSD ear, mean (range) CNC word scores pre-implant and at 3-, 6-, and 12-months post-activation were 1% (0%-10%), 26% (8%-76%), 26% (4%-68%), and 25% (8%-64%), respectively. Mean (range) CNC phoneme scores were 5% (0%-32%), 42% (17%-87%), 45% (17%-89%), 43% (28%-85%), respectively. No statistically significant changes were observed in AzBio scores in the auditory configuration S[AH]N[SSD]. However, scores in the S[0]N[0] and S[SSD]N[AH] configurations demonstrated statistically significant increases in performance by 12% and 29%, respectively, by 6 months post-activation ($p<0.01$). THI scores demonstrated a 47% reduction in self-reported tinnitus by 6 months post-activation. Mean HUI3 score was 0.57 pre-implant, lower than scores for common conditions such as anxiety (0.68), diabetes (0.77), and COPD (0.65), and comparable to the HUI3 score for stroke (0.58). Scores improved to 0.76 by 3 months post-activation. Average device wear-time was 7.8 hours/day (range, 0.5-17.6). Notably, patients with >10 years duration of deafness at implantation achieved comparable post-implantation CNC scores as those with <1 or 1-10 years duration of deafness but reported reduced SHQ scores and device wear-time ($p<0.01$).
Conclusions: Patient-reported health utility in SSD was significantly reduced and comparable to many other debilitating conditions. CI for SSD achieved expected improvements in word recognition, hearing in noise, patient-reported spatial hearing, tinnitus severity, and health utility. Longer-deafened patients reported less improvement in quality-of-life measures and reduced device wear-time despite significant improvement in CNC word scores, implicating central mechanisms of compensation, decompensation, and binaural integration in the SSD-CI population.

SU40. Robust Voice Emotion Recognition Under Cognitive Load

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Category: Auditory Prostheses
Background: Identifying voice emotion in speech is crucial for our daily communication. Many studies have shown that voice emotion perception depends on the quality of input signals. For example, when speech signals are spectrally-degraded (e.g., listening through a cochlear implant), voice emotion perception is often affected. Voice emotion perception can be improved when speech signals are exaggerated in child-directed (vs. adult-directed) speech. In everyday life, speech signals often unfold under conditions of cognitive load (e.g., a concurrent visual task) that do not directly impact speech signal quality. To date, it is less understood about the impact of cognitive load and its interaction with speech signal quality on voice emotion perception.
Methods: Stimuli were semantically-neutral sentences spoken by a female English talker in each of the five emotions (angry, happy, neutral, sad, and scared). The sentences were spoken in child-directed and adult-directed conditions. Using a five-alternative, forced-choice paradigm, adults with normal hearing performed an emotion recognition task on unprocessed and 8-channel vocoded versions of those sentences. Participants performed the recognition task alone (i.e., auditory-only condition) or concurrently with a visual work memory task of low load (remembering four same images) or high load (remembering four different images).
Results: Consistent with prior work, emotion recognition performance was better for unprocessed than vocoded speech and child-directed than adult-directed speech. The effect of the emotion category on recognition performance depended on whether sentences were child-directed or adult-directed. For child-directed speech, participants exhibited better performance for happy sentences but poorer performance for

neutral sentences. For adult-directed speech, participants exhibited better performance for neutral sentences but poorer performance for scared sentences. Finally, performance remained largely unchanged between auditory-only and dual-task conditions.
Conclusions: Voice emotion perception appears to be relatively automatic and to be dominated by input signal quality. The effort to improve voice emotion perception, e.g., in cochlear implant users, may primarily target bottom-up signal quality.

SU41. Sheep as a Large-Animal Model for Otology Research: Temporal Bone Extraction and Middle Ear Access

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Category: Auditory Prostheses
Background: Sheep are a suitable large-animal model for otology research due to similarities with human anatomy and ossicular velocity. For example, sheep have been used to study middle and inner ear implantable hearing devices, such as cochlear implants. However, a method for temporal bone extraction in sheep has not been described, and existing literature on middle ear access via the facial recess in sheep is limited. We describe a method for efficient temporal bone extraction and middle ear access via the facial recess in sheep, including relevant anatomic measurements from micro-CT scans.
Methods: Six temporal bones from the heads of 3 adult female Q fever-negative Hampshire sheep were extracted using a scalpel, oscillating saw, mallet, and chisel. Each head was skinned sufficiently to perform a craniotomy with removal of the brain and brainstem. The saw, mallet, and chisel were used to remove the anterior portion of the head. After disarticulation of the C1 vertebra, the posterior portion of the skull was cut in half in a sagittal plane. Soft tissue was removed, yielding individual temporal bones. Mastoidectomy and access to the middle ear via the facial recess was performed using an otologic drill. Micro-CT scans were obtained of each temporal bone at an 80-micron resolution. The following dimensions were measured for each specimen using the open-source software 3D-Slicer: perpendicular distance between the plane tangent to the superior aspect of the external auditory canal (EAC) and the "pseudo-tegmen" (layer of bone separating the mastoid from the soft tissue-filled space containing the sheep temporal artery, i.e., superior limit of the mastoidectomy), and narrowest and widest diameters of the EAC.
Results: Temporal bone extraction was successful in 6/6 temporal bones, and 6/6 temporal bones could be secured in a standard human temporal bone holder. Mastoidectomy and middle ear access were successful in 5/5 specimens, with the following observations: hypo-pneumatized mastoid; low-lying pseudo-tegmen; chorda tympani (sacrificed in all cases) and lateral semicircular canal difficult to identify; preservation of the incus buttress not necessary; thick facial nerve; surgical access to round window possible without sacrificing facial nerve. Mean (SD) distance between pseudo-tegmen and EAC was 2.3 mm (0.8 mm). Mean (SD) widest diameter of the EAC was 6.7 mm (0.8 mm). Mean (SD) narrowest diameter of the EAC was 4.3 mm (0.3 mm).
Conclusions: This extraction method efficiently yields individual sheep temporal bones that fit in standard human cadaveric temporal bone holders, allowing for streamlined storage and experimentation. Mastoidectomy and middle ear access via the facial recess in sheep can be performed similarly to humans with the following surgical considerations: hypo-pneumatized mastoid, low-lying pseudo-tegmen (superior limit of mastoidectomy), difficulty identifying chorda tympani and lateral semicircular canal.

SU42. Dual Region Recordings in the Sound Localization Pathway of Barn Owls to Investigate Stimulus Selection of Salient Stimuli

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Category: Binaural Hearing and Sound Localization
Background: Barn owls are specialists in sound localization. Their well-described midbrain stimulus selection network, a circuit containing a map of auditory space dedicated to localizing salient sounds, provides a unique opportunity to study the flow of information between midbrain and forebrain. This project worked towards investigating how the circuit conducts bottom-up relay for salient stimuli in environments with competing sounds. Earlier in vivo recordings in the owl's optic tectum (OT) have shown that gamma oscillations are spatially tuned to both visual and auditory information, and may play a role in stimulus

selection. However, previous recordings in deep midbrain structures, like OT, have relied on single electrodes in a single region and an open question remains regarding how oscillations contribute to information flow during stimulus selection.
Methods: Towards this end, we recorded spike responses and local field potentials in OT and one of its downstream forebrain regions simultaneously in anesthetized owls.
Results: We observe heterogeneity in tuning properties to binaural cues in the forebrain, ranging from peaked tuning curves to broad tuning to contralateral space. However, while tuning may differ between brain regions, firing rates and gamma power positively correlate both during spontaneous activity in the absence of stimuli and during presentation of stimuli, suggesting connectivity.
Conclusions: Consistent with previous reports, there is a transformation in coding scheme from midbrain to forebrain, with broader forebrain ITD tuning shape. Additionally, spiking activity and gamma power correlate between these regions with overlapping ITD tuning, suggesting feedforward convergence from OT to entopallium. Future experiments will determine the role of gamma oscillations in promoting stimulus selection during presentation of two competing sounds, and determine whether gamma power is predictive of bottom-up relay towards salient stimuli during sound orientation behavior.

SU43. Spatial Speech Understanding in Single-Sided Deafness Listeners

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Category: Binaural Hearing and Sound Localization
Background: Single-sided deafness (SSD) creates inherent disruptions in critical cues needed to for accurate localization of sound sources, and leads to reduced speech intelligibility in complex acoustic scenes. In multi-talker scenarios, listeners can reorient themselves to improve the signal-to-noise ratio when the target talker is off-side. In SSD listeners, effective head orientation can allow for better-ear listening, although this may prove ineffective if the target cannot be accurately located.
Use of a cochlear implant (CI) for SSD may result in improved speech understanding in these situations, both due to improved speech understanding in the poorer hearing ear and increased access to spatial hearing cues.
We studied orienting behavior using both spatial and speech paradigms to examine the relationships of localization and speech intelligibility as a function of signal-to-noise ratio in SSD listeners.
Methods: Three groups of adult SSD listeners were evaluated: congenital/early-onset SSD, acquired/adult-onset SSD, and adult-onset SSD with CI. Four speech streams, randomly selected from the Coordinated Response Measure (CRM) corpus were presented simultaneously to the listener through four speakers in a 360° horizontal array at +3 dB, +5 dB, and +8 dB SNRs, with the target talker always presented in the front hemifield. Each speech stream consisted of a call sign, color and number. Listeners were instructed to locate the source of the target talker and report the associated color/number. Head orientation and velocity of the head movement were recorded using a custom head tracker to obtain localization accuracy and response times. Overall percent correct identification of color/number of the target talker was computed and compared across groups.
Results: Localization of the target talker and response accuracy was found to be poorer in SSD listeners when compared to normal hearing individuals. Accuracy and reaction times were improved for signals presented to the normal hearing ear compared to those presented to the deafened ear. CI improved SSD listeners' orientation to the target talker and response accuracy. Orienting behavior suggests a better ear listening strategy. SNR negatively affected listeners performance on color/number identification. Orienting behavior had only a small effect on speech intelligibility at higher SNRs.
Conclusions: Disruption of binaural cues through SSD results in significant difficulty identifying target talkers in complex spatial listening environments. This increased difficulty in orientation to the talker results in poorer speech understanding in complex, unpredictable listening situations for SSD listeners. Listeners can leverage compensatory head movements to improve signal level at the better hearing ear. Intervention through cochlear implant for SSD resulted in improved performance in these situations, but these individuals continued to lag behind those with normal hearing.

SU44. Discrimination Training and Reweighting of Interaural Level Vs. Time Difference Cues

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Category: Binaural Hearing and Sound Localization
Background: Normal-hearing listeners weight binaural localization cues depending on the sound's frequency content. Interaural time differences (ITDs) dominate at low frequencies, and interaural level differences (ILDs) dominate at high frequencies. The contribution of each cue to an azimuthal localization percept also depends on environmental factors such as room acoustics. Furthermore, using visual feedback, ITD/ILD weighting was shown to change for stable sound conditions through lateralization training in virtual reality (VR). This study aims to induce similar reweighting using a simple left/right discrimination task without VR. And, it examines how the resulting reweighting depends on the target azimuth and spatial disparity between target components.
Methods: Participants were divided into three groups, a group trained to increase their ILD weighting (n=10), a group trained to increase the ITD weighting (n=14), and a no-training control group (n=11). All the groups completed an identical pre- and post-assessment involving a relative discrimination task without feedback, using various spatially inconsistent ITD and ILD combinations. The stimuli consisted of pairs of narrowband noises (fc = 2.8 kHz) generated such that the first noise contained ITD corresponding to one azimuth and ILD corresponding to another azimuth, while the second noise had the azimuths corresponding to ITD and ILD swapped. The subject indicated whether the perceived location of the noise moved to the left or to the right. The training groups completed three sessions of adaptive relative discrimination training, including feedback (correct/incorrect) always consistent with the ILD azimuth (ILD training) or the ITD azimuth (ITD training). After each incorrect response, the auditory stimulus was repeated with the correct answer shown on the screen.
Results: Responses followed the ILD azimuth significantly more often in the posttest than in the pretest for the ILD training group, while the effect of training was much weaker and not significant in the ITD training group, and no effect was observed in the control group. And, while the relative weight varied with target location and spatial disparity, the increased ILD weight in the ILD group was independent of these spatial factors.
Conclusions: Binaural cue reweighting can be achieved by a simple discrimination training when the goal of training is to increase the ILD weight, but not when it is to increase the ITD weight. A possible reason for this asymmetry is that the study was performed in virtual anechoic environment in which the ITD weight is already maximized as the ITDs are not distorted by reverberation in this environment.
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SU45. Effects of Ischemic Stroke on Binaural Perception
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Category: Binaural Hearing and Sound Localization
Background: Stroke-induced lesions at different locations in the brain can affect various aspects of binaural hearing, including spatial perception. Previous studies found impairments in binaural hearing resulting from lesions all along the auditory pathway from brainstem nuclei up to the auditory cortex. Currently, structural magnetic resonance imaging (MRI) is used in the clinical treatment routine of stroke patients. In combination with structural imaging, an analysis of binaural hearing enables a better understanding of hearing-related signaling pathways and of clinical disorders of binaural processing after a stroke. However, little data are currently available on binaural hearing in stroke patients, particularly for the acute phase of stroke and possible recovery effects within the first months after stroke onset. Here, we sought to address this gap in an exploratory study of patients with ischemic stroke.
Methods: We conducted psychoacoustic measurements using two tasks of binaural hearing: binaural tone-in-noise detection, and lateralization of stimuli with interaural time- or level differences. The exact location of the stroke lesion was established by previously acquired MRI. An additional general assessment included three-frequency audiometry, cognitive assessments, and depression screening. Fifty patients participated in the experiments, on average five days after their stroke onset. The same experiments were also conducted in a subgroup of these patients in the subacute and the chronic phase of stroke. Lesion locations include brainstem areas, basal ganglia, thalamus, temporal lobe, and other cortical and subcortical areas.
Results: Lateralization impairments were found in most patients with lesions within the primary auditory pathway. Lesioned areas at brainstem levels led to distortions of lateralization in both hemifields, thalamus lesions were correlated with a shift of the whole auditory space, whereas some cortical lesions

predominantly affected the lateralization of stimuli contralateral to the lesion and resulted in more variable responses. Lateralization performance was also found to be affected by lesions of the right, but not the left, basal ganglia. Lateralization also appears to have been affected by lesions in non-auditory cortical areas. Binaural tone-in-noise detection was unimpaired in most patients, regardless of the lesion site. Recovery of binaural hearing performance within the first months after stroke onset was found in several patients.
Conclusions: The study showed that binaural hearing was affected in the acute phase of stroke in many patients, even with no or only slight symptoms of stroke. The inclusion of participants with various lesion locations allowed identifying brain areas on, but also outside the primary auditory pathway, to be involved in binaural processing. Effects of binaural hearing impairment in this heterogenous data set were visible on an individual level and on a lesion group level.
Support: ERC Starting Grant No. 716800 to M.D.

SU46. Measuring and Modeling Real-World Sound Localization
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Category: Binaural Hearing and Sound Localization
Background: The ability to localize sounds in the world is critical to perceiving our environment, but is nonetheless not perfectly accurate. These performance limits remain incompletely documented and understood, in part because research on sound localization has tended to rely on synthetic stimuli (tones and noises). Measurements of everyday sound localization have the potential to reveal new insights and to provide benchmarks for models of sound localization, which ultimately must explain real-world competence.
Methods: We used a speaker array to measure human localization of a wide variety of natural sounds in a real-world setting (a classroom). We quantified overall localization accuracy as well as the accuracy with which specific sounds were localized in azimuth and elevation. Lastly, we assessed whether a stimulus-computable neural network model of sound localization could reproduce human performance.
Results: Human localization exhibited some known effects previously seen with synthetic stimuli, such as more accurate localization near the midline, and was overall fairly accurate apart from front-back confusions. However, some sounds were localized better than others. The qualitative effects seen in human localization were also evident in the model. The model also predicted human localization accuracy for individual sounds well above chance, though predictions were below the noise ceiling, indicating room for modeling improvements.
Conclusions: The results provide a benchmark for models of sound localization and a framework to enable quantitative predictions of human performance in this domain.

SU47. Localization of Free-Field Sound Sources After Stroke
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Category: Binaural Hearing and Sound Localization
Background: Spatial hearing relies an accurate encoding of sound level and temporal information in the two ears, on binaurally sensitive neurons in the brainstem encoding the respective interaural differences and on the integrity of neural computations in more central structures along the auditory pathway. While many studies have investigated the effects of peripheral deficits on auditory perception, the effects of damages to central structures remain less explored and poorly understood. At the population level, stroke represents a relatively prevalent pathology whose individual and often multimodal effects are commonly different in the left and right hemisphere. Visual neglect of the contralesional hemifield, for instance, is well studied, whereas in the auditory domain adverse effects have been anecdotally reported but often not quantified. Systematic characterization of an individual's directional hearing deficits could potentially serve as the basis for designing compensatory binaural algorithms for stroke-related spatial distortions. Previously, the effects of stroke on binaural hearing have predominantly been investigated with simplistic headphone stimuli that provide unnatural combinations of spatial cues with limited ecological validity. Such experiments may not be informative about the behavioral relevance of stroke-related spatial hearing deficits outside of the laboratory. Yet, characterization of these deficits for real sound sources is the necessary first step in the

development of individualized algorithmic interventions that could potentially ameliorate stroke-induced spatial distortions.
Methods: We investigated the localization performance of chronic-phase mild stroke survivors (N=14) in a free-field localization experiment (source identification task). Lesion locations across participants included both cortical and subcortical structures. The experiment followed a constant stimuli paradigm and included three interleaved stimulus conditions using low-frequency- (0.125-0.5kHz), high-frequency- (2-8kHz) and broadband (0.125-8kHz) stimuli, presented from azimuths spanning the frontal hemifield. All stimuli were derived from pink noise and had a duration of 200ms. We computed localization performance metrics (e.g., RMS-error, bias and variability) from the response data and compared them to results from participants who had not suffered a stroke.
Results: Localization performance varied significantly across stroke survivors. While many of them performed relatively normally, some participants showed signs of localization deficits. Notably, this is the case also for two out of four audiometrically normal subjects younger than 50 years.
Conclusions: We did not find adverse spatial distortions comparable to the strikingly apparent stroke-induced deficits reported in the visual domain. Despite this, we find it likely that localization deficits similar to those we found in young participants might be present in some of the older participants, but obscured by localization deficits associated with aging. Further analyses with more participants will elucidate the current data.
Support: ERC Starting Grant No. 716800 to M.D.

SU48. Rate Limits Based on Change Responses Evoked by Fine-Structure and Envelope Interaural-Time-Difference

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Category: Binaural Hearing and Sound Localization
Background: With increasing numbers of bilateral cochlear-implant (BiCI) users, predominantly children, there are high demands for efficient tests to support diagnostics of binaural-hearing and to assist BiCI fitting. Here a series of interaural-time-difference (ITD) change detection paradigms including both psychoacoustic and electroencephalogram (EEG) measures were used and developed as potential clinical tools. The test procedures, analysis methods, and the response morphologies were characterized with normal-hearing (NH) listeners for both temporal fine-structure and envelope ITD changes at different frequencies. Previous studies suggested that ITD sensitivity of BiCI users is limited by the stimulation rate and is similar to envelope ITD sensitivity of NH listeners. Data obtained here will assist future studies in BiCI users and design of clinical protocols.
Methods: Three adaptive psychoacoustic tasks were performed to obtain: 1) the upper-carrier-frequency-limit (f_(uplim_c)) of fine-structure ITD (ITD_FS) sensitivity for 40-Hz sinusoidal-amplitude-modulated (SAM) tones; 2) the upper-modulation-frequency-limit (f_(uplim_m)) of envelope ITD (ITD_ENV) sensitivity for high-frequency SAM tones (f_c = 4000Hz); 3) the upper-limit-of-pulse-rate (f_(uplim_pps)) of interaural-pulse-time-difference (IPTD) sensitivity for filtered-clicks (CI simulation). In three EEG experiments, cortical-auditory-evoked-potentials (CAEPs) of the stimuli onset and offset, auditory-change-complex (ACC) responses evoked by ITD changes, and the auditory-steady-state-responses (ASSRs) were recorded simultaneously for each stimulus type: 1) SAM tones with ITD_FS changes, f_m = 40Hz, and f_c = [400, 800, 1200, 1600]Hz; 2) SAM tones with ITD_ENV changes, f_c = 4000Hz, and f_m = [40, 80, 160, 312]Hz; 3) filtered-clicks with IPTD changes, f_pps = [40, 80, 160, 312]pps.
Results: 1) The behavioural f_(uplim_c), f_(uplim_m), and f_(uplim_pps) thresholds were 1393 ± 284Hz, 206 ± 99Hz, and 206 ± 97pps. 2) The ACC morphologies evoked by ITD_FS changes were similar to onset- and offset-CAEPs. However, the ACC-N1P2 amplitudes were smaller than onset-CAEPs, and the peak latencies of offset-CAEPs were the shortest while they were longest for ACC. The 40-Hz-ASSR amplitude increased gradually with increasing f_c. Wavelet analysis showed clear interaction between detectable transient CAEPs and 40-Hz-ASSRs in time-frequency domain for f_c<1600 Hz. 3) The ACC-N1P2 amplitudes evoked by envelope ITD changes (experiment 2 and 3) were either not detectable or much smaller than those evoked by ITD_FS changes. Their ASSRs were smaller than those low-frequency SAM tones with f_c≤1600Hz. However, the ASSRs of filtered-clicks were larger than for high-frequency SAM tones, and 40-Hz-ASSR>160-Hz-ASSR>80-Hz-ASSR>320-Hz-ASSR for both stimulus types.

Conclusions: These tools could be used to assess binaural hearing, especially for binaural temporal processing abilities. The differences between the ACC responses evoked by envelope ITD and fine structure ITD imply, at least to some extent, that there are different neural mechanisms involved in the perception of these two ITD cues. Studies with BiCI users are ongoing. This work was supported by Medical Research Council grant MR/S002537/1 to D.V.

SU49. Notched Noise Improves On-Frequency Envelope ITD Encoding

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Category: Binaural Hearing and Sound Localization

Background: Sensitivity of humans to interaural time differences (ITD) in the stimulus envelope improves with increasing sound pressure level and thus suggests that auditory nerve (AN) fiber temporal coding becomes more reliable. The response synchrony of AN fibers to the stimulus envelope, however, decreases with increasing level due to rate saturation (above about 30 dB SPL). A possible explanation for this apparent contradiction is that at higher stimulus levels, the auditory system compensates for the decrease in on-frequency synchrony (coded by fibers tuned to the carrier frequency) by utilizing synchrony in off-frequency channels, where the rate is not saturated. Previous discrimination experiments have assessed the role of ITD information in off-frequency channels by using spectrally-flanking notched noise to prevent off-frequency listening (Bernstein and Trahiotis, J. Acoust. Soc. Am. 5, 2008). These experiments showed that ITD thresholds increased only moderately by a factor of two and thresholds did not get worse with increasing stimulus level. It was therefore concluded that humans rely primarily on on-frequency ITD sensitivity, leaving open the question why envelope ITD sensitivity improves with level. In this study, we explored an alternative hypothesis: In the presence of notched noise, outer hair cells cause a reduced sensitivity to the on-frequency channel, decreasing its driven rate. This, in turn, restores on-frequency phase-locking at intermediate sound levels. If true, subjects would exploit off-frequency channels in the absence of notched noise but on-frequency channels in the presence of notched noise.

Methods: Single-unit AN fiber recordings were collected using glass electrodes from young-adult ketamine/xylazine anesthetized gerbils. Monaural 128-Hz sinusoidally amplitude modulated (SAM) tones at the fiber's best frequency were presented with and without notched noise at several noise levels and notch widths, to assess on-frequency envelope synchrony.

Human sensitivity to ongoing envelope ITD was measured with 128-Hz SAM tones centered at 4 kHz in addition to (1) binaurally presented notched noise, (2) monaurally presented notched noise, and (3) no notched noise.

Results: The AN recordings showed a significant increase in temporal synchrony to the envelope when notched noise was added.

In the behavioral experiment, the sensitivity was significantly improved when the notched noise was presented binaurally than for the monaurally presented equivalent. This suggests that for the monaurally presented notched noise, there was a mismatch between the two ears in the channel that best encoded the AM information.

Conclusions: Based on our results, we suggest that the notched noise causes a reduced on-frequency sensitivity, which in turn results in better temporal synchrony of AN fibers compared to the no noise condition. This supports our hypothesis that, in the absence of notched noise, envelope ITD sensitivity originates primarily from off-frequency channels, at least for SAM-tones.

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SU50. Characterization of OPN1-Expressing DCN Cell Types in Mice

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Category: Brainstem: Structure and Function

Background: Noise-induced hearing loss (NIHL) permanently impairs approximately 40 million adults. Although preventative measures can be achieved, avoiding loud noise exposure is difficult. While it is known that NIHL has the ability to damage inner ear hair cells, the downstream effects on the auditory system are still being investigated. Structural and functional changes in the axon initial segment (AIS) - a key macrodomain responsible for action potential initiation and propagation - were described in a subset of

avian cochlear nucleus neurons after hearing loss, resulting in an increase in excitability. However, whether similar changes occur in mammals is unknown. Neurons of the dorsal cochlear nucleus (DCN) are shown to change their electrical excitability after noise-induced hearing loss (NIHL), and are thought to be important in the initiation of tinnitus. While multiple mouse models can mark specific cell populations, specific labeling of some neuron types in the DCN is lacking. Here, we report a novel marker for a subset of cells in the DCN that will facilitate analysis of AIS structure after NIHL.
Methods: To help identify parent cell populations, experiments were performed in VGAT-ChR2-EYFP and VGAT-tdTtomato mice to identify inhibitory versus excitatory neurons. Tissue was collected, cyrosectioned, and immunostained with a variety of antibodies. Z-stack images were acquired using the Zeiss 780 confocal laser-scanning microscope.
Results: Examination of message and protein expression patterns in the cochlear nucleus identified osteopontin (OPN1) as a promising marker of cells (Friedland et al., 2006, Allen ISH Atlas). Immunofluorescence revealed OPN1 (RRID: AB_2783651) as a potential novel marker of pyramidal neurons, one of the principal excitatory cells of the DCN. OPN1 marked pyramidal cell bodies, proximal dendrites, and the proximal portion of the AIS, which was further identified by immuostaining for known AIS marker ankyrin-G. Neurons in the pyramidal cell layer that labeled with OPN1 but not VGAT-EYFP or tdTomato were deemed pyramidal neurons. These neurons shared similar morphology and orientation to reports in the literature. Interestingly, the pyramidal cell AIS could arise from either the soma or the proximal basal dendrite. We also observed a small subset of OPN1-expressing cartwheel cells (VGAT-tdTomato and OPN1 expressing), indicating that clear separation of the cell populations requires using both markers.
Conclusions: We have identified OPN1 as a novel marker of pyramidal cells that can facilitate their unequivocal identification and facilitate analysis of AIS changes after NIHL. The dendritic versus somatic origin of the AIS likely has implications for the balance of synaptic control of spike output from these cells. This work will give critical insights into potential mechanistic changes in patients with noise-induced hearing loss.

SU51. NF107-Cre Mouse: Expression in the Spiral Ganglion and Cochlear Nucleus

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Category: Brainstem: Structure and Function
Background: Auditory nerve fibers (ANFs) terminate in the cochlear nucleus (CN), but how the molecularly distinct classes of spiral ganglion cells (SGCs) interact with CN neurons is not well understood. We are using optogenetics in the NF107-Cre mouse line to study synaptic responses in all classes of CN neurons to clarify projection patterns and synaptic responses previously made using electrical stimulation. However, which classes of SGCs express this transgene, and the details of their central projection are not known. Here, we provide additional characterization of the expression pattern in SGCs and the CN.
Methods: NF107-Cre mice were crossed with Ai32 (ChR2-EYFP fusion protein) mice, resulting in the expression of ChR2-EYFP in a subset of SGCs. Cochleas from adult mice were examined in surface preparations and with whole-cochlea light-sheet microscopy, after being stained with antibodies to Tuj1 (SGCs), EYFP/GFP (transgene expression) and Pou4f1 (low-SR, high-threshold SGCs; Sherrill et al. 2019). The CN and brainstems were also cleared and immunostained for EYFP to examine central expression patterns and projections. Slice electrophysiology was used to examine synaptic responses to optical stimulation of the auditory nerve and nerve terminals.
Results: In the cochlea, only a subset of SGCs express the NF107 transgene product as identified by membrane-delimited EYFP. Most cells expressing ChR2-EYFP had nuclear Pou4f1 expression, although not all Pou4f1-expressing SGCs expressed ChR2-EYFP. This suggests that expression is limited to a population of low-SR high-threshold SGCs. This expression pattern was visible in all turns of the cochlea. ChR2-EYFP expression was also visible in many classes of cochlear supporting cells and in the vestibular ganglia. Centrally, the classical pattern of ANF innervation of the CN was visible, including endbulbs of Held in the anterior ventral CN, and multiple sizes of terminals in the deep layer of the DCN. Terminal density was particularly high just beneath the granule cell laminae of the VCN. A few individual CN neurons (~5 per CN) also expressed EYFP. The inferior colliculus had scattered expressing cells, and some putative descending fibers could be seen in the dorsal/intermediate acoustic stria. In slices, photostimulation

of expressing axons and terminals generated EPSCs in all classes of CN projection neurons, but very rarely in cartwheel cells.
Conclusions: This mouse line appears to preferentially drive recombination in type Pou4f1-expressing SGCs. The NF107 BAC construct contains the full coding region for collagen glycosyl transferase type 2 (Colgalt2), along with a complete upstream region. Colgalt2 is expressed specifically during development in type 1c SGCs (Petitpre et al., 2022), consistent with the overlapping expression pattern with Pou4f1 seen here. Thus, this mouse line may provide a useful platform to selectively study the central projections of type 1c ANFs, and their functional changes after hearing loss.

SU52. Activity Dependent Intrinsic Plasticity Regulates Repetitive Firing of Superior Olivary Neurons Prior to Hearing Onset

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Category: Brainstem: Structure and Function
Background: Before hearing onset, auditory neurons exhibit patterns of spontaneous firing that link tonotopically related neurons. To understand how intrinsic firing patterns of central auditory neurons are regulated in the face of developmental changes in synaptic strength and dendritic morphology, we examined intrinsic firing and other physiological properties during patch-clamp recordings in slices of the gerbil medial superior olive (MSO), a nucleus that conveys spatial cues for horizontal sound localization.
Methods: We used whole-cell current clamp electrophysiological recordings in Mongolian gerbils leading up to hearing onset in MSO (postnatal day 5-12) and slightly after in LSO (postnatal day 5-15). Horizontal slices 200 μm thick containing MSO and LSO were sectioned and then perfused with oxygen and kept at 35 °F during recording.
Results: We found that prior to hearing onset (postnatal day 12), trains of action potentials elicited with current steps exhibit both Na and Ca components: all but the first one or two spikes were eliminated by 10 μM nifedipine or 500 μM ICA123149, blockers of L-type (Cav1; n=) and persistent Na channels (Nav1.1/1.3; n=5). In response to ramp currents (rise time: 100 to 1000 ms), repetitive spiking was insensitive to excitation slope. Subsequent blockade of persistent Na current in ICA123149 suppressed repetitive firing, and the residual transient action potential required excitation to reach threshold within 100 ms (n=5). Conversion of repetitive to transient firing pattern could be induced by trains of synaptic activity during the course of in vitro experiments. Trains of excitatory inputs to MSO neurons in gerbil brainstem slices were delivered prior to hearing onset (P3-P11). Subthreshold synaptic stimulation (10 monolateral stimuli at 100 Hz, repeated 20 times at 0.5 Hz) produced striking decreases in both input resistance and resting potential (–66±1 mV to –76±1 mV, n=9, p=0.03). The decrease in repetitive firing was correlated with the magnitude of decrease in input resistance even when the influence of the altered resting potential was offset with DC current injection. These effects persisted for the duration of recordings (>30 min. post-stimulus, up to one hour). Activity-dependent changes in input resistance, resting potential and firing pattern were dependent on group I metabotropic glutamate receptors, intracellular calcium influx via NMDA receptors, calcium-induced calcium release, and activation of calcineurin.
Conclusions: Taken together, our results show that action potential initiation mechanisms in pre-hearing neurons exhibit are distinct from those of post-hearing neurons and are powerfully regulated by intrinsic plasticity. We propose that this intrinsic plasticity provides an activity-dependent way to negatively adjust firing output gain in the face of developmental increases in synaptic strength.

SU53. Effect of Synaptic Noise on Level and Temporal Coding in Lateral Superior Olive Neurons

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Category: Brainstem: Structure and Function
Background: Integration of multiple synaptic inputs is a hallmark of interneuronal information processing, and excitatory-inhibitory integration is of utter importance in lateral superior olive (LSO) neurons, enabling sound localization. About 40 weak excitatory input fibers from the ipsilateral cochlear nucleus (CN) converge on a given LSO neuron. By contrast, only ~4 strong inhibitory fibers do so from the medial nucleus of the trapezoid body (MNTB; Gjoni et al. 2018; (doi.org/10.1113/JP276012), Müller et al. 2019 (doi.org/10.1113/JP277757)). Differences in interaural sound pressure level (ILD) and temporal disparities (ITD) are integrated in the CN -> LSO <- MNTB circuit. ‘Class 3 excitability’, i.e., phasic firing in response

to step current injection, is often seen in other auditory neurons, accompanied by a sensitivity against the rate of depolarization. Adding noise to excitable systems influences the output considerably by creating slope-based stochastic resonance (Gai et al. 2010; doi.org/10.1371/journal.pcbi.1000825). Physiologically, noise arises from 1) temporal jitter of converging inputs and 2) noise in the strength of these inputs. The influence of rate-sensitivity and integration of synaptic noise in mature LSO neurons is unknown. This motivated us to combine dynamic-clamp experiments with modeling of input spiking patterns to analyze ILD and ITD integration in these important sound localization neurons.
Methods: Using in-vitro whole-cell recordings, we stimulated LSO neurons with sinus-amplitude-modulated (SAM) currents. Next, we performed dynamic-clamp experiments to analyze the effects of non-linear currents evoked by conductance injections and their contribution to the rate-sensitivity. Finally, we used computational modeling to create temporal jitter in input spiking. Each spiking pattern was convolved to time-varying conductances using a unitary synaptic conductance template. Summed excitatory and inhibitory conductances thus simulate the convergent inputs from CN and MNTB neurons. This scenario allows to analyze the effects of synaptic noise on output generation and the sensitivity of LSO neurons to virtual sound paradigms.
Results: Stimulating phasic LSO neurons with SAM currents revealed a strong sensitivity to the rate of depolarization. Current threshold was lowest at 200-500 Hz, with a logarithmic mean of ~270 Hz for P30-40 animals. Similar results were obtained at >P60, with a mean of ~240 Hz. SAM conductances increased the minimal frequency at which LSO neurons generated spikes. Adding synaptic noise (temporal jitter) to the conductance was insufficient to evoke spikes in response to low-frequency SAM stimuli.
Conclusions: Phasic LSO neurons are sensitive to the rate of depolarization, creating a band-pass filter for temporal integration. Noise in synaptic excitation is filtered by leaky LSO neurons and provides no further coding information. Spikes occur only when temporal jitter by modeled input fibers is low, emphasizing the importance of enhanced phase-locking by CN neurons. Ongoing experiments will reveal whether noise through synaptic inhibition extends the range of ILD and ITD integration.

SU54. Cochlear Synaptopathy Impairs Suprathreshold Tone-In-Noise Coding in the Cochlear Nucleus
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Category: Brainstem: Structure and Function
Background: Permanent damage to synapses between inner hair cells and high-threshold auditory nerve fibers (ANFs) produces hearing impairment in the absence of threshold shifts. Cochlear synaptopathy is a potential major health issue in humans, as many listeners with clinically normal audiograms still have difficulty hearing in noisy settings and aged human temporal bones demonstrate widespread synapse loss despite no explicit otopathology. Previous behavioural studies have shown no effect of synaptopathy on tone-in-noise detection thresholds, but this has not been confirmed by neural recordings, nor has the effect of synaptopathy been shown on suprathreshold neural responses in noise. Here, we examine the effect of synaptopathy on tone-in-noise coding on the direct recipients of ANFs – cells in the cochlear nucleus.
Methods: Guinea pigs received a unilateral sound overexposure to the left ears (7 kHz centered, half-octave noise at 102 dB SPL for 2 h), producing unilateral temporary threshold shifts. At 4 weeks post-exposure, loss of auditory nerve synapses and reduced ABR wave 1 amplitudes were observed specifically on the left side. A separate group of animals received sham noise exposures. Single unit responses were recorded from several cell types in the cochlear nucleus. Puretone stimuli (2-24kHz; 0-90 dB SPL) were used to generate receptive fields and rate-level functions in the presence of either 0-, 40- or 60-dB SPL continuous broadband noise.
Results: The synaptopathy-inducing noise exposure did not affect mean single-unit tone-in-noise thresholds, nor the lowest tone-in-noise thresholds in each animal, demonstrating equivalent tone-in-noise detection thresholds compared to sham animals. However, synaptopathy reduced neural responses in response to suprathreshold tones in the presence of background noise, particularly in small cells of the cochlear nucleus.
Conclusions: These results demonstrate that despite not altering tone-in-noise thresholds, synaptopathy results in reduced activity in the cochlear nucleus in response to suprathreshold tones in the presence of background noise. Overall, these data have implications for hearing in the presence of background noise and offer insight to designing future objective tests for cochlear synaptopathy.

SU55. Kv4.2 and Kv4.3 Channels Regulate Signal Processing of Bushy Neurons in the Cochlear Nucleus

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Category: Brainstem: Structure and Function
Background: A-type currents are voltage-gated, Ca2+-independent K+ currents with rapid activation and inactivation, and are widely expressed in the nervous system to regulate electrical properties of neurons. In the cochlear nucleus, principal bushy neurons are specialized in processing temporal fine structure of sound, which is underlied by the expression of a unique combination of voltage-gated ion channels. However, it remains unclear whether and how A-currents contribute to the signal processing of bushy neurons due to multiple contradicting reports. Several immunohistological studies revealed Kv4.2 and Kv4.3 expression in bushy neurons (Fitzakerley, Star et al. 2000, Pal, Por et al. 2005, Bortone, Mitchell et al. 2006, Rusznak, Bakondi et al. 2008), but there were also electrophysiological studies that found no A-type currents in these neurons (Rothman and Manis 2003, Cao, Shatadal et al. 2007, Fu, Zhang et al. 2021).
Methods: Combining electrophysiology with immunohistochemistry, we investigated the expression and function of Kv4.2 and Kv4.3 channels in bushy neurons using mature CBA/CAJ mice of either sex.
Results: We performed voltage clamp recording from bushy neurons and isolated an A-type current using two different Kv4-selective inhibitors, the Patx-1 and Jingzhaotoxin-X. Under current clamp mode, blocking A-currents with Jinzhaotoxin-X increased spike half-width and decreased current threshold in response to direct current step injections. No significant changes were found in the resting potential, input resistance, membrane time constant, as well as the spike amplitude. We then stimulated the auditory nerve with high-rate stimulus trains and found that the evoked spikes in bushy neurons showed increased spike latency but decreased vector strength after Jingzhaotoxin-X. Finally, we performed immunostaining experiments and confirmed the expression of Kv4.2 and Kv4.3 in bushy neurons.
Conclusions: These findings showed that bushy neurons do express Kv4.2 and Kv4.3 channels in mature mice, which likely play significant roles in promoting temporal processing in these neurons.
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SU56. Role of Synaptic Vesicle Refilling in Maintaining Reliable Neurotransmission at a Sound Localization Center

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Category: Brainstem: Structure and Function
Background: Glycinergic connections from the medial nucleus of trapezoid body (MNTB) to the lateral superior olive (LSO) are tuned for synaptic reliability and temporal precision at sustained high-frequency stimulation (60 s; > 100 Hz). These features are achieved by a high quantal content and efficient replenishment of the readily releasable pool (RRP) in the MNTB axon terminals. During sustained neurotransmission, the RRP is continuously replenished with preformed or recycled synaptic vesicles (SVs). The latter need to be refilled with neurotransmitter, a process driven by an electrochemical gradient generated by the vacuolar H+-ATPase (V-ATPase) and maintained by the Na+/H+ exchanger (NHE). The molecular bases of RRP replenishment at MNTB-LSO synapses are still poorly understood. Here, we investigated the role of SV recycling in synaptic plasticity at MNTB-LSO synapses upon inhibition of SV refilling during sustained high-frequency stimulation.
Methods: Whole-cell recordings were performed on LSO principal neurons in acute coronal slices from postnatal day 11 mice at physiological temperature. Evoked inhibitory postsynaptic currents (eIPSCs) were recorded while electrically stimulating MNTB fibers at 10-200 Hz in 60-s trains. Each train was followed by a 60-s recovery period at 1 Hz. SV refilling was blocked by pharmacologically inhibiting V-ATPase activity and NHE activity using folimycin/ bafilomycin and EIPA, respectively (1 to 5 µM; 30-60 min vs 100 µM; 10 min).
Results: Short-term depression (STD) was more pronounced upon inhibiting V-ATPase activity. At 50 Hz, controls maintained stable steady-state amplitudes at ~40 % of the baseline. After V-ATPase inhibition, eIPSC amplitudes became reduced ~4-fold higher at steady-state levels. Despite a significant decline in the RRP (~3-fold vs control), MNTB-LSO synapses considerably recovered from STD, although only partially (30-50 % of baseline level at 10-200 Hz). In controls, fractional recovery was ~100 %, whereas it was significantly smaller upon V-ATPase inhibition (70 %). MNTB-LSO synapses were also stimulated for 4-min|100-Hz and 3-min|1-Hz upon V-ATPase blockade. Still, recovery from STD was not completely abolished, reaching ~30 % of the baseline. A complete collapse of neurotransmission was achieved only by

inhibiting both V-ATPase and NHE activity pharmacologically. The dual inhibition of SV refilling led to a further 6-fold decrease of eIPSCs amplitude at steady-state levels after sustained high-frequency stimulation. Moreover, recovery from synaptic depression was ~3-fold reduced compared to merely blocking the V-ATPase.
Conclusions: Neurotransmission at MNTB-LSO inputs did not fully collapse upon V-ATPase inhibition, even after increasing inhibitor concentration or prolonging the timing of stimulation. Only the simultaneous inhibition of both V-ATPase and NHE led to a substantial decrease in synaptic performance, suggesting a minimal contribution of preformed SVs in maintaining robust and reliable neurotransmission at high frequencies. We conclude that the remarkable performance of MNTB-LSO inputs during sustained high-frequency stimulation depends mostly on RRP replenishment through recycled SVs.

SU57. The Lateral Paragigantocellular Nucleus is a Source of Widespread Cholinergic Projections to the Subcortical Auditory System

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Category: Brainstem: Structure and Function
Background: Acetylcholine affects the entire auditory system by modulating temporal processing, setting neuronal excitability and supporting plasticity. In the subcortical auditory system, the main sources of cholinergic input are thought to be the pontomesencephalic tegmentum and superior olivary complex. Cholinergic neurons in the lateral paragigantocellular nucleus (LPGi), a nucleus in the reticular formation located caudal to the superior olivary complex, project to the cochlear nucleus and the inferior colliculus, and thus constitute a third source of cholinergic input to some regions. However, the extent of cholinergic projections from the LPGi is unknown.
Methods: We used adult, normal-hearing, transgenic ChATCre mice of either sex (C57/CBACaJ mixed background). In a first experiment, we injected AAV into the LPGi leading to Cre-dependent fluorescent protein expression in cholinergic neurons. We then identified anterogradely labeled axons in nuclei throughout the subcortical auditory system, from cochlear nucleus to medial geniculate nucleus.
In a second experiment, we confirmed the anterograde results with retrograde tracers and immunochemistry. We injected fluorescent retrograde tracers in selected target nuclei and used antibodies against choline acetyltransferase (ChAT) or vesicular acetylcholine transporter (VAChT) to identify cholinergic neurons.
Results: Following AAV deposit in LPGi, cholinergic axons and boutons were present in the cochlear nucleus, superior olivary complex, nuclei of the lateral lemniscus, inferior colliculus, intercollicular tegmentum, nucleus of the brachium of the inferior colliculus and the medial geniculate nucleus. The axons were present bilaterally in all identified nuclei, with a prominent ipsilateral bias. In nuclei with multiple subdivisions, cholinergic LPGi axons were present in most or all subdivisions, though densities of axons sometimes differed between subdivisions.
Deposit of retrograde tracers in select nuclei, including the inferior colliculus and nuclei of the lateral lemniscus, labeled VAChT+ or ChAT+ neurons in the LPGi. Consistent with our anterograde study, retrogradely labeled immunopositive cells were present bilaterally with an ipsilateral bias.
Conclusions: Cholinergic neurons of the LPGi project to nearly all areas of the subcortical auditory system, from the cochlear nucleus to the thalamus. These projections are similar to the widespread cholinergic projections of the pontomesencephalic tegmentum and are more extensive than those from the superior olivary complex. Comparisons with other studies suggest that the cholinergic projections from different sources (i.e., LPGi, pontomesencephalic tegmentum, superior olivary complex) overlap in many target areas, possibly converging on the same target cells. Presumably, the projections from different sources serve different functions. The LPGi receives ascending auditory input from the cochlear nucleus and descending input from the midbrain and cortex, so it could contribute to bottom-up and top-down modulation. Further insight into LPGi functions will require more information on the inputs to LPGi neurons and the conditions under which they are active.
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SU58. In Vitro Evidence for a New Category of Ventral Cochlear Nucleus Projection Neuron

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Category: Brainstem: Structure and Function

Background: We recently provided evidence from in vivo recording and labeling experiments (Lu et al., ARO 2020) that, in gerbils, cells formerly described as "octopus" can be divided into 2 cell types based on anatomical and physiological features. We now refer to these cells as octopus and squid cells. One critical distinguishing anatomical feature is the axonal course out of the cochlear nucleus (CN), octopus cell axons via the intermediate acoustic stria (IAS) and squid cell axons via the trapezoid body (TB). We have recorded from and labeled cells in brain slices of both mouse and gerbil PVCN and compared their intrinsic and synaptic features and cell anatomy. Confirming our in vivo data, we find that PVCN cells with axons heading to or toward the IAS show distinct differences in membrane and synaptic features when compared to those in the PVCN with axons heading to or toward the TB.
Methods: 240 micron thick coronal or sagittal slices of 17-22 day old mice or 16-19 day old gerbil cochlear nuclei that included the PVCN were made. Patch electrodes containing neurobiotin were used to record from cells. A stimulating electrode was inserted into fascicles of the auditory nerve (AN) near the recorded cell and single shocks or shock trains applied.
Results: In current clamp, current pulses to generate IV curves from the two sets of cells revealed several differences. In both species, a hyperpolarization induced sag (a measure of IH) was greater, a depolarization induced drift in membrane potential and the spike height were larger, spike threshold lower and input resistance higher in squid cells. Both received excitatory AN inputs, but AN shocks also generated large IPSPs in squid (but not octopus) cells with a slightly longer latency that often had a lower shock threshold than the direct AN excitatory inputs. The glycinergic antagonist, strychnine, blocked these inhibitory inputs. Anatomically, the dendritic trees could have a similar appearance but we have not quantified dendritic parameters yet. The octopus cell axons would head dorsally to or toward the IAS occasionally giving off a collateral. On some occasions they would arise from the soma and head ventrally for 20-50 microns but would then abruptly turn and head dorsally. Squid cells axons would head ventrally and rostrally to or toward the TB with an occasional collateral.
Conclusions: An In vitro evaluation confirms our conclusion from in vivo experiments that there are two cell types, octopus and squid with significant differences in membrane and synaptic features. The axons of these cells take two different courses out of the CN presumably to different targets. These synaptic, membrane and projection differences would strongly suggest that their functions in auditory processing are likely to differ.

SU59. A Heterogeneous Neuron Population Might Underlie Frequency Integration in the Intermediate Nucleus of the Lateral Lemniscus

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Category: Brainstem: Structure and Function
Background: In the auditory brainstem pathways sound frequencies are mainly represented tonotopically. Yet frequency and cross-frequency integration is considered to generate crucial information about the acoustic environment. Neurons in the intermediate nucleus of the lateral lemniscus (INLL) have been suggested to be involved in cross-frequency integration, while their biophysical, synaptic and morphological features remain largely uncharacterized so far.
Methods: We use in vitro whole-cell recordings to characterise INLL neurons, their synaptic inputs and assess their morphology as well as in vivo single unit recordings to detect frequency integration.
Results: One main finding is that the population of INLL neurons generates a large continuum of membrane time constants (τ) ranging from sub-milliseconds to more than 100 ms. This continuum of integration times can be explained by differences in input resistance. According to the differences in τ the subthreshold parameters, the action potential generation as well as the firing behaviour are adapted. Thereby the population of INLL neurons ranges continually from onset to adapting to sustained and spontaneously firing neurons. The biophysical differences between neurons are not regional specific. Moreover, the dendritic structure and cell soma size do not correlate with τ. Thus, cell location and morphology are no determinants of the biophysical behaviour of INLL neurons. The time course of EPSCs and to some extend IPSCs are correlated with τ. Paired pulse analysis shows that EPSCs tend to facilitate increasingly in neurons with slow τ, while IPSCs tend to increasingly depress. Thus, PSC time courses are linked to the integration time of INLL neurons. Dynamic clamp recordings simulating EPSG dynamics show that integration time depends on an interplay between EPSC time course and τ. Finally, our in vivo recordings indicate that about a third of INLL neurons show a strong modulation in their tuning curve by a two-tone paradigm. By presenting the

best frequency paired with a second tone of a different frequency, the tuning curve changes including best frequency. This finding indicates that INLL neurons do not have a static best frequency they respond to and that frequency integration exists at this level.
Conclusions: Taken together our data shows that the INLL is composed of a heterogeneous neuronal population that is not bound to a strict anatomically defined tonotopy. This population rather provides a substrate of continuous temporal integration times serving to process sounds in the frequency domain.

SU60. Comparing Methods for Deriving the Auditory Brainstem Response to Continuous Speech in Human Listeners

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Category: Brainstem: Structure and Function
Background: Our lab has been developing encoding model techniques to derive the Auditory Brainstem Response (ABR) from continuous natural speech. The model can be considered as a linear system, where a nonlinearly processed stimulus is taken as the input (i.e., regressor), the electroencephalogram (EEG) data as the output, and the ABR as the impulse response deconvolved from the EEG and the regressor. We have explored several regressors over the past years aiming to obtain robust and clinically meaningful ABRs, and found each regressor has its own advantages and limitations. In this study, we quantitatively analyzed and compared three regressors, hoping to help guide decisions on what approach to choose when deriving ABRs from natural speech and other natural sounds.
Methods: The stimuli in this study include: 1) natural speech from an audiobook, and 2) that same audiobook, re-synthesized as "peaky" speech by aligning the phase of the harmonics at glottal pulses and making the speech impulse-like, but maintaining the intelligibility.
Three regressors were studied: 1) the half-wave rectified stimulus waveform (HWR; Maddox and Lee, 2018), 2) glottal pulse train corresponding to the "peaky" speech stimulus (Polonenko and Maddox, 2021), and 3) the auditory nerve modeled firing rate (ANM), generated from a computational auditory periphery model.
From 22 subjects, we deconvolved ABRs from the two stimulus classes using the three regressors. We analyzed their ABR waveforms' signal-to-noise ratio (SNR), their spectral coherence between the predicted EEG and the real EEG, and their efficiency in acquisition time required for waveforms to reach a threshold SNR of 0 dB.
Results: For natural speech stimuli, the ANM regressor derived canonical ABR waveforms including wave I, III and V, while the HWR only showed a broad wave V (glottal pulses are not an appropriate regressor for unprocessed speech). The ANM also outperforms the HWR in SNR and spectral coherence. All subjects reached threshold SNR within 6 minutes for the ANM, in contrast to > 20 minutes for HWR.
For peaky speech stimuli, both the ANM and glottal pulse regressors provided canonical ABR waveforms including early ABR components that HWR was not able to show. The ANM slightly outperforms the glottal pulses in SNR and spectral coherence. All subjects reached decent SNR within 7.5 min for ANM, but only 5 minutes needed for glottal pulses.
Conclusions: Both the ANM and glottal pulse regressor provided comparable high-quality ABRs, quick acquisition, and substantially outperformed the HWR regressor. The glottal pulse regressor has the disadvantage of being applicable only to re-synthesized peaky speech, but the advantage of providing ABRs in meaningful physical units (i.e., microvolts). The ANM regressor has the advantage of being applicable to both natural and peaky speech, and in principle other natural sounds such as music.

SU61. A Novel Method for Maximizing Efficiency and Reducing Test-Time for the Auditory Brainstem Response Test

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Category: Clinical Otolaryngology and Pathology
Background: Since early identification and characterization of hearing loss is important, only objective estimates of frequency-specific hearing thresholds need to be obtained. In particular, audiologists may schedule an auditory brainstem response (ABR) test – a test that can require up to an hour or two, and necessitate a separate appointment, causing delays in diagnosis. Additionally, time taken to record frequency-specific ABRs usually exceeds the time made available by patient sedation/cooperation. Previous

attempts of reducing test-time have not been popular in clinical settings due to expensive hardware and software requirements. In the current study, we examined the potential of an ABR acquisition paradigm that can significantly shorten testing-time without sacrificing accuracy. This simple and intuitive technique does not require additional equipment. We used a chained-stimuli approach which interleaves several discrete stimuli between the two ears in a fashion that maximizes acquisition efficiency while minimizing response adaptation by testing several frequencies at the same time.
Methods: Thirty normal hearing young adults participated in the study (male=10; age range=18-35years). The ABR-eliciting stimuli was a train of either tone-bursts or narrow-band-iChirps (4000Hz, 2000Hz, 1000Hz, and 500Hz) sequentially placed (from high to low frequency to avoid neural adaptation and upward spread of excitation) and presented in an interleaved manner to each ear. The stimuli train was jittered (5%) in time to avoid any neural entrainment to periodicity. ABR responses were recorded simultaneously from two inverting sites- C7 and linked mastoids. Responses were recorded at stimulus levels of 40, 30, and 20 dBnHL.
Results: Both the tone-burst and narrow-band-iChirp stimuli elicited replicable ABR waveforms at all stimulus levels tested. Two-way interactions between intensity and stimulus revealed that the response amplitude was significantly larger for the narrow-band-iChirp stimulus, compared to the tone-burst stimulus, especially at threshold ($F = 4.74$, $p < .01$). By design, chirp stimuli can produce greater neural synchrony (and therefore larger response amplitude) since it removes the desynchronizing effects of different traveling wave delays associated with a click stimulus. Due to this enhanced neural synchrony, responses can be identified even at lower stimulus levels compared to responses elicited by tone-burst stimuli. The C7 electrode site resulted in better SNRs, waveform morphology, and thus larger response amplitudes than the linked mastoid site ($F = 54.11$, $p < .001$). C7 electrode site yielded responses less contaminated by post-auricular muscle artifacts. Thus, we were able to record frequency-specific ABRs by interleaving the stimulus, without compromising the responses.
Conclusions: In effect, our method and stimuli put together creates a recording paradigm/protocol that can yield binaural ABR thresholds in closer to 22 minutes, which is 1.8 times quicker compared to the conventional threshold determination using the ABR clinically. This novel recording paradigm is thus a quick and effective clinical tool in threshold determination.

SU62. Creation of a Virtual Human Temporal Bone Laboratory Using an Open Digital Repository
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Background: Temporal bone laboratories are unique source of material for auditory and vestibular research. Unfortunately, there are only few otopathology laboratories in the world. The high concentration of necessary knowledge and resources to process and study temporal bones in few facilities has hampered progression of hearing and balance research. The long-term commitment of the laboratory is to enhance the utility of human temporal bones for modern auditory/vestibular research in cooperation with the NTBB Hearing and Balance Registry, and to make the information available to the greater scientific community. Therefore, through an open-source digital repository called “Elevator”, a cloud-based digital source, we aimed to create a virtual collection of digital slides of our entire human temporal bone archives.
Methods: The University of Minnesota Otopathology laboratory has more than 2,200 human temporal bones representing a variety of otologic diseases. The collection also has temporal bone sections from 19 animal species. These temporal bones were amassed through decades of procurement and processing. The temporal bones had been previously fixed in 10% formalin, decalcified with EDTA, embedded in celloidin, and horizontally sectioned using a sliding microtome at the thickness of 20um. Each tenth section was stained with Hematoxylin and Eosin and mounted on glass slides. The stained slides are currently being scanned using a high-resolution tissue scanner at a 20x magnification. The slides were uploaded to a cloud-based database (Amazon Web Services; Amazon, Bellevue, Washington). The digitized slides were then categorized at “Elevator” in designated folders under multiple categories that can be organized and accessed remotely.
Results: A virtual collection of 2,200 human temporal bones is being created in “Elevator”. The resolution of the scanned slides is suitable for cellular level analysis including cochlear and vestibular hair cells and ganglion neurons. Specimens can be archived according to tags and categories, such as age, sex, otological

disease or disorder, and others. All data is de-identified prior to digitization. In Elevator, it is possible to digitally manipulate the specimens and add comments, notations, and drawings, without altering the source file. We also plan to link the (de-identified) medical records to allow connecting potential symptoms and diseases with the histopathological findings. The digital archive can be directly linked with "Canvas", a platform for virtual courses in which we plan to establish virtual courses on otopathology. As such, we will be able to access and demonstrate otopathology in the digital specimens at real time.
Conclusions: Elevator, an open digital library affords housing of digital human temporal bone sections and organizes collections in folders that can be categorized with flags based on donor history and pathology, facilitating data search and access. Elevator's public-facing website is accessible to external researchers, constituting a critical resource for data dissemination, remote human otopathology research and training.

SU63. Update on the SPI-3005-501 Clinical Trial in Tobramycin Receiving Cystic Fibrosis Patients: Stop Ototoxicity

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Category: Clinical Otolaryngology and Pathology
Background: SPI-3005-501 is a Phase 1/2 clinical trial of ebselen (SPI-1005) to prevent and treat tobramycin induced ototoxicity. We previously reported that the incidence of tobramycin induced ototoxicity in the Phase 1b (observational) study was higher than expected using pure tone audiometry (PTA) and the sensitive range for ototoxicity (SRO) (93%), distortion product otoacoustic emissions (DPOAEs) between 4-8 kHz (80%), words-in-noise (WIN) (40%), with less significant change in the Tinnitus Functional Index (TFI) and Vertigo Symptom Scale (VSS), two validated patient reported outcomes (PRO). These changes were observed 2 and 4 weeks after the last infusion of tobramycin (10 mg/kg/d) that ranged from 11-21 days. In addition, concomitant oral SPI-1005 at 600 mg BID did not alter the pharmacokinetics of IV tobramycin. Here, we report the interim analysis of the ongoing Phase 2b interventional study that has achieved approximately 50% of the target enrollment to determine if ebselen treatment reduces the incidence of aminoglycoside ototoxicity.
Methods: 37 of 80 patients have been enrolled on standard treatment for acute pulmonary exacerbation including IV tobramycin (≥10 days). Patients were screened and then randomized (1:1:1:1) to placebo or one of three oral doses of SPI-1005 (200, 400, or 600 mg BID) and treated for 21 days, beginning 2 days after the start of IV tobramycin infusion. Changes in PTA from baseline were calculated at 2 and 4-weeks after the end of tobramycin treatment and ASHA cochleotoxic criteria were applied. Changes in all other measures were also calculated from baseline. The rates of change were then compared between the completed observational and ongoing interventional studies to determine if the ongoing Phase 2b study should continue or be halted for lack of efficacy.
Results: Both the observational and interventional enrollees have a similar baseline incidence of hearing loss (39% vs 39%), mean age (31 vs 29 years), genotypes, concomitant meds, and days of IV tobramycin (14.8 vs 14.2), respectively. Similar levels of ototoxic change using PTA and the SRO were found at 2 weeks post-tobramycin in the observational (89%) and interventional (83%) studies. However, at 4 weeks, the rates of ototoxicity diverged between the observational (93%) and interventional (60%). At 4 weeks, the change in WIN score also diverged from 40% to 26%, respectively, while the DPOAE change diverged from 80% to 68%, respectively. Changes in TFI and VSS were less divergent between studies. In addition, SPI-1005 is well tolerated and no significant changes in AE number and severity have been observed.
Conclusions: SPI-1005 treatment for 21 days may reduce the incidence of ototoxicity measured 4 weeks after the cessation of IV tobramycin in this CF study population. Additional analyses including an unblinded comparison between active groups and placebo will be presented.

SU64. Unsupervised Machine Learning for Automated Image Segmentation of CT Temporal Bones: A Scoping Review

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Category: Clinical Otolaryngology and Pathology
Background: Convolutional neural networks (CNNs), a type of unsupervised machine learning, have been harnessed within medicine to assist in complex image classification tasks. By harnessing the power of big

data, CNNs have demonstrated potential in solving diagnostic conundrums that even experienced radiologists struggle to decipher. Their use in computed tomography (CT) of the temporal bones to segment structures of the ear has been successfully demonstrated using numerous different architectures. This scoping review aims to provide an overview of the literature surrounding CNNs for automated segmentation of CT temporal bones, critically appraising and discussing their strengths and limitations, and identifying any gaps in knowledge.
Methods: The PRISMA extension for scoping reviews (PRISMA-SCr) was used. Data were extracted from Medline, EMBASE, Scopus, Web of Science and Cochrane. MeSH terms and keywords were inputted into each database from inception until August 2022. Inclusion criteria were all papers on adult patients using CNNs to automatically segment CT temporal bones. Papers using alternative imaging modalities such as magnetic resonance imaging (MRI) or not specific to the temporal bone region, in addition to those which included paediatric patients, were excluded. Titles, abstracts and full text were screened by two reviewers, with conflicts resolved by a third reviewer after reaching a consensus.
Results: 1,611 studies were identified for screening. Architectures that were able to accurately segment structures on CT temporal bones included PWD-3D-net, 3D-U-net and UNETR. Performance metrics were high in all included studies in performing accurate image segmentation, suggesting that algorithm performance was equivalent to that of a radiologist. All architectures identified that the spatial resolution provided by CT temporal bones was problematic in further ameliorating algorithm performance.
Conclusions: This novel review summarises the existing literature surrounding the use of CNNs in otological imaging. Existing CNN architectures display good performance in automatically segmenting structures on CT temporal bones. We recommend the incorporation of high spatial resolution cone beam CTs into an automated image segmentation pipeline to further improve performance and help to translate this innovation into clinical practice.

SU65. The Endoscopic Transtympanic Cartilage Myringoplasty Without Freshed Perforation Margin and Distal Malleus Handle for Repairing Medium Sized Perforation

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Category: Clinical Otolaryngology and Pathology
Background: The freshed perforation margins and handle of malleus is considered the gold standard of myringoplasty for repairing chronic perforation involving the malleus.The objective of this study was to evaluate the outcome of endoscopic transtympanic cartilage myringoplasty without freshed perforation margin and distal malleus handle for repairing chronic medium sized perforations involving the malleus.
Methods: 46 patients with chronic medium sized perforations involving the malleus underwent endoscopic transtympanic cartilage myringoplasty, the perforation edges weren't de-epithelialized and the epithelium wasn't removed from the distal malleus handle. All the patients were followed up at least 12 months, and temporal bone computerized tomography were performed at postoperative 12 months. The graft success rate, hearing improvement, and complications were evaluated at 12 months postoperatively.
Results: At 12 months, the graft success rate was 95.7% (44/46), the mean ABG improved from 21.46±8.39 dB preoperatively to 9.84±2.41 dB postoperatively. All the ears exhibited endoscopic graft neovascularization at postoperative 2-4 weeks.No graft-related complications (e.g., graft lateralization, significant blunting, graft atelectasis, graft adhesions, or effusion) were encountered during the follow-up period. Temporal bone imaging revealed a pneumatized middle ear and mastoid in all cases with no evidence of cholesteatoma.
Conclusions: This study suggested that endoscopic, transtympanic cartilage underlay myringoplasty without freshed perforation margin and distal malleus handle did not affect graft neovascularization and graft outcome for repairing chronic medium sized perforations involving the malleus. No middle ear cholesteatoma was found in short term. Longer-term outcomes and risk of cholesteatoma, require further study.

SU66. Repeat Dosing of Tobramycin Induces Delayed High Frequency Hearing Loss in Mice

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Category: Clinical Otolaryngology and Pathology
Background: Aminoglycosides (AG), such as tobramycin, are commonly used to treat CF patients with recurrent pulmonary infections and those infected with multi-drug resistant tuberculosis. AGs are highly ototoxic, resulting in auditory dysfunction including hearing loss, tinnitus and hyperacusis. Our prior studies have shown that a 14-day course of 200 mg/kg/d of tobramycin in mice induced transient ABR threshold shifts that peaked between 16-20 kHz with no observed damage to hair cells. The goal of this study is to test a higher dose of tobramycin (400 mg/kg/d) that will result in hair cell injury and death, and to repeat the course following a recovery interval.
Methods: Adult CBA/CaJ mice received tobramycin (400 mg/kg/s.c.) once daily for 14 days, followed by a 14-day recovery interval before repeating the 14-day course at the same dose and schedule. ABRs were tested at 8, 16, and 20 kHz were performed at baseline, 6, 12, 17, 18, and 22 weeks from the start of tobramycin. Hair cell morphology and damage were determined by immunofluorescence (Prestin/Calretinin) microscopy on whole mount sections of the cochlea. DAPI staining confirmed the loss of cell nuclei in the organ of Corti.
Results: ABR threshold shifts initially peaked at 8 kHz (15 dB) at 6 weeks and then began to peak at 16 kHz from 6 to 17 weeks after the start of tobramycin dosing. ABR threshold shifts began at 20 kHz from 12 weeks after the start of tobramycin dosing or 6 weeks after the last tobramycin injection. Threshold shifts remained elevated across all tested frequencies at 22 weeks or 16 weeks after the last tobramycin injection. Cochlear histology revealed significant damage and loss of OHCs and IHCs in the basal turn of the cochlea corresponding to regions >20-24 kHz.
Conclusions: The administration of a repeat course of AGs induced an initial threshold shift at lower (8 kHz) vs higher (16 KHz) frequencies, and a delay in the onset of a threshold shift at the highest frequency tested (20KHz), months after the last tobramycin injection. Unlike our previous findings following a single 14-day course of tobramycin (200 mg/kg/d), threshold shifts remained elevated 22 weeks after start of dosing or 4 months after the last tobramycin injection. Furthermore, OHC and IHC loss was significant at high frequencies above 20 kHz. These findings indicate that the peak in threshold shift occur at different timepoints for different frequencies, starting at lower frequencies, and a delay in the threshold shifts observed at higher frequencies. In addition, the threshold shifts at 20 kHz correspond with significant hair cell loss, while the threshold shifts at 8-16 kHz do not. These complex preclinical findings may correspond with some initial clinical findings recently detailed in tobramycin receiving CF patients.

SU67. Open Board

SU68. Cx26 Heterozygous Knockout Causes Hyperacusis-Like Hearing Sensitivity Paradoxically Increased and Susceptibility to Noise
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Category: Clinical Otolaryngology and Pathology
Background: Cx26 (GJB2) mutations are responsible for more than 50% of nonsydromic hearing loss. The recessive heterozygous mutation carriers are estimated up to 10-20% in the general population. These heterozygote carriers have no apparent hearing loss and are considered normal in hearing in the clinic. However, it is unclear whether the Cx26 heterozygous deficiency can cause pathological changes and has other hearing dysfunctions. In this study, we examined hearing functional changes in Cx26+/- hetero-deletion mice.
Methods: Cx26 conditional knockout (cKO) mice were created by a Cre-FloxP technique. One group of Cx26+/- heterozygous cKO mice were exposed to white noise (95 dB SPL) for 2 hours, one time. Another group of Cx26+/- heterozygous cKO mice without noise exposure served as control. ABR, DPOAE, and cochlear microphonics (CM) were recorded to assess cochlear and hearing function.
Results: Cx26+/- mice demonstrated significant increase in hearing sensitivity. ABR thresholds were reduced and amplitudes of ABR were increased. Cochlear microphonics (CM) in Cx26+/- mice was also increased. However, the endocochlear potential (EP) in Cx26+/- mice was reduced. DPOAE in Cx26+/- mice was increased rather decreased. Prestin expression in Cx26+/- mice was compensatively up-regulated. Finally, we found that Cx26+/- mice were also sensitive to noise. Middle level of noise exposure could cause Cx26+/- mice permanent threshold shift (PTS) in hearing.

Conclusions: Cx26+/- heterozygous deletion can cause hyperacusis-like hearing sensitivity and active cochlear mechanics increased and leads to sensitivity to noise. The data also suggest that Cx26 heterozygote carriers are not normal in hearing, are sensitive to noise, and should avoid noise exposure in daily life.
Supported by NIH R01 DC 017025 and R01 DC019687 to HBZ.

SU69. A Prospective Study of Etiology and Auditory Profiles in Infants With Congenital Unilateral Sensorineural Hearing Loss

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Category: Clinical Otolaryngology and Pathology
Background: Unilateral sensorineural hearing loss (uSNHL) is associated with an increased risk of delays in speech- and language development, impaired sound localization and speech recognition in noise, as well as academic difficulties. The difficulties vary within the group, and more research is needed that explains the large differences between children with uSNHL. The choice of diagnosis and intervention methods needs further research, especially for infants detected early through the neonatal hearing-screening programs, where comorbidity may not be noticeable yet.
Methods: Twenty infants with congenital uSNHL were recruited with consecutive sampling from the universal neonatal hearing-screening program in Region Stockholm during 2019-2020 to study hearing loss cause. Auditory brainstem responses (ABRs), transient-evoked and distortion-product otoacoustic emissions (TEOAEs and DPOAEs), tympanograms and acoustic reflex thresholds (ARTs) were measured. Magnetic resonance imaging (MRI) and congenital cytomegalovirus (cCMV) infection testing were conducted.
Results: ABR thresholds of ≤20 dB nHL and TEOAEs were recorded in all normal-hearing ears (NEs, n = 20). The median ABR threshold in the impaired ear (IE) was 55 dB nHL (inter-quartile range: 40 dB nHL-no response, n = 20), where 40% had no recordable ABR threshold. None of the subjects had TEOAEs in their IEs. None tested positive for CMV infection. Fourteen subjects agreed to participate in an MRI scan. Malformations were common for all degrees of uSNHL and found in 64% of all scans (n = 9/14). In subjects with profound uSNHL MRI revealed 86% malformations (n = 6/7), and for mild to severe uSNHL 43% malformations (n = 3/7). Half of the MRIs demonstrated cochlear nerve aplasia or severe hypoplasia and 29% showed inner ear malformations (n = 4/14). IE and NE ABR input/output functions on a group level for subjects with ABRthrs <90 dB nHL were parallel shifted. A significant difference in interaural ARTs existed.
Conclusions: In congenital uSNHL, MRI is powerful in finding a possible hearing loss cause, while congenital CMV infection may be relatively uncommon. ABRs and ARTs indicated an absence of loudness recruitment by neural firing, with implications for amplification and maximal output levels in hearing aids for children with congenital uSNHL.
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SU70. The Relation Between Cochlear Nerve Survival and Word Recognition Scores

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Background: Sensorineural hearing loss (SNHL) results from loss of, or damage to, sensory cells. Recent studies from animal models and human temporal bones show that hair cell loss can be preceded by loss of synaptic connections between inner hair cells and spiral ganglion neurons. The silencing of these neurons must degrade auditory processing and likely translates into a variety of perceptual abnormalities including speech discrimination difficulties, particularly in noisy environments, and tinnitus via an induction of central gain adjustment secondary to loss of afferent input to the CNS. This study provides the first estimates of the quantitative relation between cochlear nerve degeneration (CND) and word recognition scores (WRS), both in quiet and in difficult listening situations, by combining audiometric data with temporal bone histopathology from several unrelated sources.
Methods: We analyzed retrospective audiological data that included 96,000 ears from patients aged 18 - 103 yrs, with normal hearing, conductive hearing loss or a variety of SNHL etiologies including: age-related hearing loss (AHRL), vestibular schwannoma or neurofibromatosis type 2; Meniere's disease; sudden SNHL, ototoxic drugs or noise damage. WRS was obtained using monosyllabic word lists and compared to

"predicted" WRS based on the Articulation Index and the audiogram. In a separate ongoing study, we measured WRS from 279 normal-hearing subjects aged 18 - 75 on time-compressed (65%) words with added reverberation. Values for CND as function of age and etiology were extracted from the literature.
Results: Among those with ARHL, mean WRS in quiet stayed near 100% until age 50, then declined monotonically to ~60% by age 95. The small deterioration in WRS in conductive hearing loss (<5% by age 85) suggests that cognitive decline is not a major confound. In SNHL, larger intelligibility deficits were observed in etiologies known, or suspected, to cause greater CND. After accounting for age and degree of hearing loss, WRS were worst with Meniere's disease, acoustic tumors or sudden SNHL. WRS matched for audiometric losses were much better in those with exposure to noise or ototoxic drugs. This is consistent with the ranking of CND from the human temporal bone literature, at least with respect to normal aging, noise exposure, Ménière's and acoustic tumors. Combining data on WRS vs. age and CND vs. age suggests that nerve loss must exceed 60% before WRS in quiet fall below 90%. The computed effect of CND on the more difficult listening task was more pronounced, with mean performance starting to fall at an age at which mean nerve loss is only 25%.
Conclusions: These findings agree with a number of studies linking speech perception with peripheral neural deficits and implicate CND as a major contributor to the intelligibility challenges of hearing impairment.
Research supported by NIDCD (P50 DC015857) and the Lauer Fund.

SU71. Transcriptome Analysis of Nascent Hair Cells Identifies Ccer2 as a Novel Gene Upregulated During Differentiation

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Category: Development: Cellular/Systems
Background: Atoh1, a helix loop helix transcription factor, is necessary and sufficient for sensory hair cell formation and differentiation. These specialized sensory cells detect sounds and movements in the auditory and vestibular systems, respectively. To discover the genes that are downstream of Atoh1 and involved in hair cells formation we profiled the transcriptome of Atoh1-induced ectopic hair cells at the early stage of mammalian cochlear development.
Methods: We electroporated embryonic (E) day 13 mouse cochlear explants with an Atoh1 GFP reporter construct, or with an empty GFP vector as a control. At this stage of development, overexpression of Atoh1 results in a 100% conversion of electroporated cells into hair cells. To identify the earliest genes regulated by Atoh1 overexpression, we used fluorescence-activated cell sorting (FACS) and sorted the cells overexpressing GFP 24 h after electroporation. We extracted RNA from both Atoh1 GFP and control GFP cells and performed bulk RNA-sequencing (RNA-seq).
Results: We found more than 800 differentially expressed genes (~700 upregulated and ~100 downregulated), and our bioinformatic analysis detected several known hair cell genes (e.g., Gfi1, Jag2, Dll1) in the Atoh1 expressing cells. Furthermore, we identified Ccer2 (coiled-coil glutamate-rich protein 2), a novel gene that was significantly upregulated (6-fold change). CCER2 is an uncharacterized protein; there is no published information about its structure, localization, or function. We confirmed the expression of CCER2 in endogenous cochlear and vestibular hair cells and assessed that is one of the earliest markers expressed during hair cells development. We investigated its spatiotemporal expression during mouse cochlear and vestibular development and found that in the cochlea, CCER2 has a developmental base-to-apex gradient and is transiently expressed starting at E13 up to postnatal day 6, following the spatiotemporal expression of Atoh1. In the balance organs (utricle and saccule), the protein is expressed embryonically and throughout adult stages. We analyzed the function of Ccer2, in hearing and balance, by generating Ccer2 mutant mice (FVB/NJ background) using CRISPR/Cas9 technology, and performed ABR and DPOAE, as well as rotarod balance tests.
Conclusions: Our transcriptomic analysis is the first RNA-seq study that profiled up- and down-regulated Atoh1 downstream targets in the early stages of hair cell differentiation, which led to the discovery of CCER2, a novel and specific protein marker for inner ear sensory hair cells. The characterization of CCER2 will provide insights into both Atoh1 and other signalling pathways where it is involved, advancing our understanding of inner ear development.

SU72. The Role of the Gαi-Binding Protein Girdin in Non-Canonical Wnt Signaling and Planar Cell Polarity in the Inner Ear

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Category: Development: Cellular/Systems

Background: During cochlear development, a non-canonical Wnt signaling pathway regulates cochlear elongation, hair cell planar polarity (PCP), and core PCP protein localization through activation of heterotrimeric Gi proteins {1}. Daple (CCDC88C) is thought to play a central role in Wnt-induced Gi activation by serving as a guanine nucleotide exchange factor (GEF) for Gai. However, loss of function phenotypes of Daple are distinct and milder in some aspects compared to those caused by genetic blockade of Wnt signaling in the cochlea {2}. Girdin (CCDC88A) shares high sequence similarity with Daple, including microtubule and Gai binding domains, and also has a unique actin binding domain {3}. Girdin is also highly expressed in the developing cochlear epithelium, suggesting that it may play a role in regulating G protein signaling. This led us to hypothesize that other members of the CCDC88 protein family may function redundantly with Daple to mediate non-canonical Wnt signaling in the cochlea.

Methods: To investigate the potential function of Girdin in regulating G protein signaling, we generated a knock-in mouse expressing Girdin that is deficient for Gai binding (GirdinFA). To determine whether Girdin plays redundant to Daple functions in Wnt/G protein signaling, we also crossed the GirdinFA mice with a Daple knockout (KO) to generate GirdinFA/FA; Daple-/- double mutant mice.

Results: GirdinFA/FA mice are viable and fertile, with normal cochlear elongation and hair bundle polarity and orientation at P0, indicating that the Gai binding activity of Girdin is not required for normal cochlear development. Moreover, cochlear elongation is normal in the GirdinFA/FA; Daple-/- mutant, suggesting that non-canonical Wnt signaling can proceed in the absence of the GEF activities of Daple and Girdin. Interestingly, while hair bundle morphogenesis is severely disrupted in the Daple-/-, both hair bundle polarity and orientation was significantly improved in GirdinFA/FA; Daple-/- mutants. This finding indicates that Girdin deficient in Gai binding, but not wild-type Girdin, can partially compensate for Daple's function in hair cell PCP. Experiments are ongoing to test the hypothesis that Gai-interaction may allosterically regulate other functions of Girdin that can compensate for Daple loss, such as interactions with the dynein microtubule motor.

Conclusions: These findings indicate that Gαi-binding activity of Girdin by itself is not required for non-Canonical Wnt signaling or hair cell PCP. However, Girdin can functionally compensate for Daple loss in certain settings. Our genetic evidence suggests allosteric regulation of different functional domains of Girdin, and that multiple GEFs are involved in Wnt/G protein signaling during cochlear morphogenesis.

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SU73. The Role of Sox2 in the Formation and Maintenance of Sensory Regions in Inner Ear Development

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Category: Development: Cellular/Systems

Background: Understanding the development of the inner ear is extremely important for human health. There are a variety of harmful stimuli that can damage hearing and balance function of the inner ear, including excessively loud noises and ototoxic drugs. The mammalian inner ear has minimal regenerative capacity replacing the cells being killed by these stimuli, causing permanent hearing loss and balance problems. Beyond damage, there are a variety of possible mechanisms resulting in reduced hearing, balance issues, or deafness occurring in the global population. Understanding how the development of the inner ear can provide insights into the failure of inner ear functions in mammals and may reveal strategies to restore hearing and balance in humans. Sox2, a known Notch signaling downstream target in the ear, is expressed broadly during early inner ear development. However, as the ear continues to develop, Sox2 expression becomes restricted to prosensory domains, regions of the inner ear that will later develop into auditory or vestibular sensory organs. The rest of the embryonic inner ear will form nonsensory structures such as the semicircular canals. Data from our lab and others suggests that Sox2 is expressed in both prosensory and

nonsensory progenitors during early inner ear development but become restricted to prosensory regions over time. The aim of this study is to understand the role of Sox2 during early inner ear development.
Methods: To test our hypothesis that the developmental potential of Sox2-expressing progenitor cells changes over time, we are using MORF3 lineage tracing mice to test whether single Sox2-expressing cells in the early inner ear primordium can generate prosensory or nonsensory cells, or both. In parallel, we are testing whether transcriptional targets of Sox2 change over time during ear development. Using a Sox2-GFP reporter mouse line, we will isolate Sox2-expressing cells and study their transcriptome (RNAseq) as well as identify the targets of Sox2 by a chromatin immunoprecipitation-based assay (CUT and RUN). This will be done to analyze how Sox2 binding is changing and affecting the transcriptional output at early and late time points of inner ear development.
Results: With the MORF3 mouse line, we have been able to lower tamoxifen administration to generate singly marked Sox2 expressing cells, with the hope of understanding more about their developmental potential. Combining the results of methods like CUT and RUN and RNAseq will help us understand how Sox2 differentially targets genes over time, as well as how this affects the overall transcriptome of the developing otocyst.
Conclusions: Our work will reveal the functional shift of a key regulator of inner ear development, gain a more specific understanding of how cells specify to become hair cells through development, and identify new strategies to target cells for hair cell regeneration.

SU74. "Eat Me" Signals and Phagocytic Activity Mediating Cochlear Neuronal Circuitry Refinement
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Category: Development: Cellular/Systems
Background: During cochlear development the sensory hair cells (HCs) initially experience an excess of spiral ganglion neuron (SGN) innervations that are refined prior to hearing onset. The mechanisms by which this synaptic refinement occurs is an area of active investigation. Canonical "eat me" signal phosphatidylserine (PS) has been implicated in synaptic refinement of the central nervous system but has not been well-characterized in the cochlea. PS is generally localized to the interior leaflet of the cell membrane but its presentation at the cell surface is governed by floppases, scramblases, and flippases. Flippases are of particular interest because they shuttle PS back to the interior membrane leaflet, thus providing a sort of "save me" signature. Previous studies found flippase ATP8A2 is important for hearing function, and RNA sequencing shows ATP8A2 is expressed in both type II SGNs and HCs. We hypothesize that ATP8A2 expression marks active SGN/OHC connections by internalizing any exposed PS within these connections and thus preventing removal of those synapses. We further hypothesize that neighboring cochlear supporting cells (SC) are the agents of phagocytic removal of PS-presenting SGN/OHC connections, possibly through the TAM receptors Tyro3, Axl, and MerTK. The TAM receptors are well-defined phagocytic receptors for PS and are expressed in the SCs. To test these hypotheses, we firstly investigated the expression of ATP8A2 in the cochlea and tested its relevance for the development of appropriate neuronal circuitry and hearing function.
Methods: We have generated an Atp8a2-HA tagged knock-in mouse to investigate the expression and localization of ATP8A2 throughout postnatal development of the cochlea. We also created a hypomorphic mouse in which 33 amino acids are knocked-out (KO) of the C-terminal of ATP8A2 (A2delta33), and an Atp8a2-KO mouse to probe whether ATP8A2 is required for the development and refinement of type II SGN innervation of OHCs. Immunolabeling analysis was done to study ATP8A2 expression. HC innervation and synaptic refinement were assessed by staining for pre- and post-synaptic markers.
Results: At birth ATP8A2-HA is clearly observed in IHCs, OHCs, and SGNs. During postnatal cochlear development ATP8A2 expression becomes increasingly specialized. By P20, ATP8A2 expression is restricted to the OHC/type II SGN axis, with especially high localization at the en passant synapses of OHCs. A2delta33 mice, hypmorphic for ATP8A2 function, exhibit moderate levels of high frequency hearing loss.
Conclusions: The drastic change in expression of ATP8A2 during postnatal development implicates its importance in the refinement of type II SGN synaptic connections to OHCs. Our working model is that activity-dependent ATP8A2 expression prevents PS exposure and thus provides a "save me" signal. Synapses and neurites that lack ATP8A2 will externalize PS and be removed through a yet unknown phagocytic pathway that we suspect involves SCs and the TAM receptors.

SU75. Extracellular Matrix and Hearing Function: Role of Hyaluronic Acid

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Category: Development: Cellular/Systems

Background: Hyaluronic acid (HA), a non-sulfated glycosaminoglycan, is part of the ground substance, a gel-like structure that bathes all the ECM components. HA has been involved both in tissue biomechanics, as it awards visco-elastic properties, and in cell signaling, through cell-ECM receptors. Thus, HA takes part in several biological processes, including morphogenesis and inflammation. Multiple studies demonstrated the importance of ECM during inner ear development and in hearing and some HA-related genes have been associated with hearing impairments. As such, the gene Cemip (or KIAA1199), encoding a Hyaluronidase, has been reported to be mutated in deaf patients. We therefore aim at characterizing the role of HA in hearing function.

Methods: By combining specific immunostainings, RNAscope and qRT-PCR assays, we first explored the spatio-temporal distribution of cochlear HA as well as the expression profile of enzymes responsible for its synthesis and degradation. In addition, we generated a mutant mouse model in which Cemip gene has been invalidated. We took advantage of this model to investigate the impact of HA accumulation on cochlear morphogenesis and hearing function, by performing morphological analyses and Auditory Brainstem Response (ABR) recordings.

Results: We found that HA is highly enriched in the basilar membrane (BM), for which visco-elastic properties are instrumental in sound wave decomposition, frequency discrimination and mechanical sound wave conversion. The main enzymes involved in HA metabolism are present at embryonic and postnatal stages in the cochlear duct and in the spiral ganglion. Despite an accumulation of HA in the BM region below hair cells, the global morphology of Cemip-deficient cochleae is preserved, suggesting that Cemip has no prominent role in cochlear development. However, we evidenced that hearing function of Cemip KO mice is slightly impaired, as ABR recordings revealed an increase in peak 1 amplitude at some frequencies. Although unexpected, this result suggests that either more sensory cells are stimulated by sound, or that spiral ganglion neurons are over-activated compared to control mice.

Conclusions: Altogether, our data suggest that HA might be instrumental in cochlear biomechanics, by awarding visco-elastic properties to the BM. Cemip loss has no critical impact on cochlear development and hearing, although an overstimulation of the cochlear nerve has been observed. We are currently investigating further this phenotype to identify the cause of this neuronal over-stimulation and we particularly focus on BM morphology and cochlear perineuronal nets. In the future, we also plan to examine whether Cemip KO mice are more prone to noise-induced hearing loss due to neuron excitotoxicity, for example.

SU76. The Transcription Factor Pou4f1 Regulates Spiral Ganglion Neuron Peripheral Axon Guidance

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Category: Development: Cellular/Systems

Background: The afferent innervation to the cochlea is comprised of the spiral ganglion neurons (SGNs), which transmit mechanosensory input from the hair cells centrally to the cochlear nucleus. Despite the importance of precise connections between SGNs and hair cells, we know relatively little about the genetic pathways involved in their development. One transcription factor known to be critically involved in SGN development is Pou4f1. Pou4f1 is expressed broadly by immature SGNs but later becomes restricted to a subgroup of Type 1 SGNs. Germline knockout (KO) of Pou4f1 leads to defects in SGN survival and overall axonal patterning, although it is unclear whether the pathfinding defects arise from SGN or cochlear efferent axons. To address these questions, we focused on elucidating the specific role of Pou4f1 in SGN axon outgrowth using morphological and molecular techniques.

Methods: To assess SGN pathfinding defects in the absence of Pou4f1, we combined Ngn1creErt2;R26RZSGreen sparse labelling of SGNs with a Pou4f1 KO mouse. In addition, we used the same Ngn1creErt2;R26RZSGreen combination along with a Pou4f1flox allele to delete Pou4f1 from SGNs, but not cochlear efferents. Finally, to examine the pathways mediated by Pou4f1, we examined changes in gene expression between Pou4f1 KO and wildtype SGNs at E14 using bulk RNASeq.

Results: Pou4f1 KO mice displayed SGN defects at birth which were consistent with previous results, in particular, a loss of basal SGNs and substantial axon pathfinding defects, in the apical regions. Sparse

labelling confirmed that at least some of these misrouted axons arose from SGNs. In addition, by using the Ngn1creErt2;R26Rzsgreen;Pou4f1flox combination to simultaneously label and knock out Pou4f1 specifically from SGNs, we were able to demonstrate a direct role for this transcription factor in SGN axon guidance. These results suggest that the efferent patterning defects that have been reported in Pou4f1 KOs are likely a secondary result of disruptions in SGN pathfinding. Analysis of changes in gene expression in Pou4f1-negative SGNs indicated significant decreases in known "axon guidance" genes. Simlarly, GO analysis of the top 50 altered genes also identified axon guidance and pathfinding as disrupted in the absence of Pou4f1. In particular, both approaches highlighted Sema3a signaling, a known pathway involved in axon pathfinding, as disrupted in Pou4f1 KO SGNs.
Conclusions: In this study we have shown that Pou4f1 directly regulates axon pathfinding in developing SGNs. We have also identified Sema3a as a candidate axon guidance factor likely acting downstream of Pou4f1. Future work will focus on examining the role of Sema3a in SG development, and on further analysis of the RNA-Seq dataset to identify additional Pou4f1 targets. Overall, this work enhances our understanding of SGN axon guidance which has potential long term applications to the development of successful strategies for cochlear regeneration.

SU77. Building a Cochlea-On-A-Chip Model

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Category: Development: Cellular/Systems
Background: Cochlear implants (CIs) operate by bypassing the defective or lost cochlear hair cells, a common cause of hearing loss, and electrically stimulating spiral ganglion neurons (SGNs)/ primary auditory neurons (PANs) directly. Thus, the outcomes of CIs depend on a population of excitable SGNs. A key limitation of cochlear implant (CI) performance is the electrical interface with SGNs. There is a pressing clinical need for a reliable model for testing strategies for the electrical stimulation of SGNs. Current in vivo clinical measures are very limited and animal models do not replicate the anatomical structure of the human cochlea. Therefore, we have been developing an in-vitro Cochlea-on-a-Chip model that combines 3D replication of the anatomy and core structures of the cochlea, along with embedded SGNs and multielectrode arrays (MEAs) to record neural responses.
Methods: Cochlea-on-a-Chip aims to replicate in vivo neural electrical responses expected in humans in a controlled and measurable in vitro system. The model consists of 3 main elements: 1) Rat SGNs/or human induced pluripotent stem cell (hiPSC)-derived SGNs (in separate models) 2) Custom microelectrode arrays (MEAs) to measure cellular electrical activity and 3) Custom-designed 3D printed microfluidic chips to replicate the structure of the human cochlea.
We focused on developing both the cellular and device aspects of the Cochlea-on-a-Chip. Our microfluidic device design has been iteratively optimised in order to ensure: accurate anatomical and electrical conductivity representation of the scala tympani(where CIs sit in the cochlea) and reproducible 3D printing and subsequent casting of the precise features of the PDMS cast model. The chips were tested with cells.
Results: A casted PDMS device from a 3D-printed mould demonstrated good casting of all required microfeatures. Rat SGNs and glial cells were seeded in initial Cochlea-on-a-Chip prototypes and survived in the SGN channel for over a month. SGNs extended neurites through the microchannels toward the CI channel. As a validation of cellular behaviour, we measured SGN firing activities in response to varying electrical stimuli using the patch-clamp technique. We also developed human auditory neuron-like cells from human induced pluripotent stem cells (hiPSCs) derived from human fibroblasts. These hiPSC-derived SGNs displayed both a similar morphology to rat SGNs and express a neuronal marker TUJ1. We were also able to culture these neurons onto commercial MEAs and measure both their spontaneous ability to develop action potentials, and their response to electrical stimulation, and show that the action potential profiles are very similar to rat SGNs also grown on MEAs.
Conclusions: Using our model, we are currently testing the relationship between SGN behaviour and varying electrical stimulation parameters such as pulse shape, amplitude, and duration. The cochlea-on-a-Chip model will enable the rapid evaluation of existing technologies and the development of new CIs and hearing loss treatment strategies.

SU78. Investigating the Role of a New Transcriptional Regulator in the Development of the Organ of Corti

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Category: Development: Cellular/Systems
Background: Hearing depends on the precise patterning of hair cells (HCs) and support cells (SCs) along the length of the organ of Corti; however, the signals that drive this developmental patterning are not fully understood. Recent work in our lab identified enrichment of the Ebf1 (Early B cell factor 1) motif in the open chromatin of prosensory cells collected from developing mouse cochleae. Subsequent scRNAseq, in situ hybridization, and immunostaining experiments involving embryonic day (E) 12-18 cochleae revealed that Ebf1 is expressed in the greater epithelial ridge, prosensory cells, HCs, and SCs. The transcription factor, furthermore, is expressed in base-apex expression gradient that coincides with HC differentiation. Such findings give us reason to believe that Ebf1 is an essential regulator of HC and SC patterning. To date, the role of Ebf1 has not been studied in the inner ear.
Methods: In our initial investigations of Ebf1, our lab studied an inducible conditional knockout (iCKO) model that used Sox2-CreER to excise Ebf1. When compared with Cre-negative controls, Ebf1-iCKO mice treated with tamoxifen at E11-12 demonstrated ~30% increase in inner HC (iHC) counts ($p < 0.001$) and no change in outer HC (oHC) counts. Our lab recently designed a non-inducible conditional knockout (cKO) model that allows for the study of postnatal stages. In our new cKO model, Slc26a9-Cre excises Ebf1 in the otocyst at E9.5, ~2.5 days before cochlear sensory cell development. We performed immunostaining experiments to examine Ebf1-dependent changes in sensory cell patterning of developing and adult cochleae. We also tested the auditory brainstem response to assess hearing in the adult mice.
Results: Compared with the Ebf1-iCKO model previously studied in our lab, our new Ebf1-cKO model demonstrates a much stronger phenotype. Loss of Ebf1 increases total iHC and oHC counts >2-fold in all regions of the Ebf1-cKO duct vs. those in littermate controls ($p < 0.001$). While the typical ratio of oHCs to iHCs is preserved in the basal turn of the Ebf1-cKO duct, there is a slight decrease in oHCs relative to iHCs in the middle and apical turns ($p < 0.05$). In addition to these supernumerary HCs within the organ of Corti, Ebf1-cKO cochleae possess supernumerary SCs as well as ectopic sensory patches. The supernumerary SCs express markers consistent with inner pillar cells, outer pillar cells, and Deiters' cells. The ectopic sensory patches appear medially, within the greater epithelial ridge and can be found throughout the length of the duct. Ebf1-cKO cochleae have abnormal innervation patterns with spiral bundles that appear less defined and neuronal projections that extend to the ectopic patches of HCs. In adult Ebf1-cKO mice, supernumerary HCs and SCs persist, and the mice are deaf.
Conclusions: Ebf1 regulates sensory cell patterning and is necessary for hearing.

SU79. The Cell Adhesion Molecule Nectin3 Regulates Peripheral Projection Patterns of Spiral Ganglion Neurons

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Category: Development: Cellular/Systems
Background: Our sense of hearing is critically dependent on the spiral ganglion neurons (SGNs), which connect the sensory hair cells (HCs) in the organ of Corti (OC) to the cochlear nuclei of the hindbrain. Type I SGNs innervate inner HCs (IHCs) to transmit sound signals, while type II SGNs (SGNIIs) innervate outer HCs (OHCs) to detect acoustic trauma. Despite their importance, our understanding of the molecular mechanisms that mediate wiring of the OC is still fragmentary.
It has been shown recently that the Planar Cell Polarity (PCP) pathway plays a non-autonomous role in cochlear supporting cells (SCs) in the guidance of SGNII peripheral projections. Intercellular PCP signaling mediates polarized cell behaviors within the plane of a tissue in a plethora of developmental processes. In the wild-type OC, SGNII afferents make a characteristic 90-degree turn toward the base of the cochlea and innervate multiple OHCs. In several PCP mutants, SGNII afferents turn randomly towards either the cochlear base or apex. Although it has been shown that PCP proteins localize asymmetrically to SC-SC junctions and act in the cochlear epithelium to guide SGNII afferents, the underlying mechanisms are currently unknown.
Methods: To investigate downstream effectors of PCP signaling in the OC that may serve as guidance cues for SGNII afferents, we immunolocalized several cell adhesion proteins in wild-type and Vangl2 KO OC and found that Nectin3 immunofluorescence signals were significantly decreased at both HC-SC and SC-SC

junctions in Vangl2 KO OC, suggesting that it is a potential downstream effector of Vangl2. Nectins are transmembrane cell adhesion molecules that engage in homophilic and heterophilic trans-interactions to regulate cell-cell adhesion in various cell types. In the cochlea, Nectin3 expressed in SCs interacts in-trans with Nectin1 expressed in HCs to generate the checkerboard pattern of HCs and SCs, but it is unknown if nectins also act at the SC-SC junctional level.
Results: To determine whether Nectin3 regulates SGNII projections, we generated two Nectin3 deletion alleles via CRISPR-Cas9. Initial analysis showed that Nectin3 mutants phenocopied the HC PCP phenotypes of reported Nectin3 null mutants, and importantly, displayed SGNII afferent misturning phenotypes. We hypothesize that Nectin3 fine-tunes the balance between SC-SGN and SC-SC adhesive forces to guide SGNII projections. Experiments are ongoing to test this hypothesis by perturbing Nectin3 trans-interactions in dissociated SGN cultures and ex vivo cochlear explants containing both the SGNs and the organ of Corti. Moreover, we have previously shown that the small GTPase Rac1 acts non-autonomously to guide SGNII afferent innervation. As nectins are known to activate Rac1/Cdc42 in other systems, we are testing whether they act in the same pathway to control SGNII afferent turning.
Conclusions: Overall, the new findings and continuing work will, for the first time, illuminate the role of Nectin3 in cochlear innervation.

SU80. The Impact of Otitis Media on Peripheral and Central Auditory Processing: A Systematic Review

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Category: Development: Human Subjects
Background: Otitis media (OM) is among the most common childhood diseases, with 80% of children having had at least one OM episode by the time they turn 8 years of age. Although OM is often self-limiting, many studies have suggested that recurrent OM episodes during early-childhood may have long-lasting negative effects on the ability to understand speech in noise. Studies investigating the peripheral and central mechanisms underlying these deficits have shown variable results. Hence, a need exists to summarize the evidence concerning the long-term effects of early-childhood OM on peripheral and central auditory processing.
Methods: A systematic review was conducted. Original studies were identified through systematic searches in the PubMed, Embase and Cochrane Library databases. Two reviewers independently screened the studies for inclusion. Studies comparing peripheral (pure-tone audiometry, otoacoustic emissions) or central (temporal, spectral, binaural) processing in children and young adults with a history of early-childhood OM to a control group were included. Both recurrent acute OM, as well as OM with effusion were considered.
Results: The search identified 14,762 records. After removal of duplicates, 11,301 records were screened based on the title and abstract, of which 276 were found to be eligible. Full text screening of all eligible records is currently underway.
Conclusions: Here, we will present the results of our systematic review on the consequences of early-childhood OM on auditory processing. Structuring the available evidence will inform clinical practice on the potential long-term consequences of OM, and will also identify the need for future research.

SU81. Bilateral Deafness in DFNB9 Mice Reversed by Single Unilateral Injection of Aav-Mediated Human Otof

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Category: Gene Therapy
Background: Mutations to the OTOF gene are among the most common reasons for auditory neuropathy, and there are currently no effective treatments for these patients. Although previous studies have shown the ability to rescue hearing in DFNB9 mice using OTOF gene replacement, the efficacy of such treatments has been limited.
Methods: To improve the efficacy, we developed a novel dual AAV-mediated gene therapy system based on the principles of protein trans-splicing

Results: We show that this system can reverse bilateral deafness in Otof –/– mice after a single unilateral injection. Immunohistochemistry showed that the system effectively expressed exogenous mouse or human otoferlin after injection on postnatal day 0–2. Auditory brainstem response (ABR) tests showed that human otoferlin restored hearing to near wild-type levels for at least 6 months and restored the release of synaptic vesicles in inner hair cells.
Conclusions: Our study not only provides a preferential clinical strategy for the treatment of OTOF-related auditory neuropathies, but also describes a route of development for other large-gene therapies and protein engineering techniques.

SU82. Large Gene Delivery to the Murine Cochlea by Triple AAV Vectors

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Category: Gene Therapy
Background: Adeno-associated virus (AAV)-mediated gene therapy for hereditary hearing loss is expected to become an unprecedented curative treatment. Among gene therapy strategies, the advantage of gene replacement is that it enables treatment regardless of the mutation position. However, AAV is limited in its transfer capacity by only being able to transfer 4.7 kb. Given that numerous diseases are caused by mutations in genes with coding sequences exceeding this capacity, packaging into a single AAV capsid is currently unfeasible for larger genes, which may require splitting the transgene into two or three parts. Although we previously reported the feasibility of a dual AAV vector approach (Omichi and Yoshimura et al., 2020), no triple AAV vector approach has been reported. Heretofore, we sought to investigate the transduction efficiency of triple AAV vectors in the adult murine cochleae.
Methods: As described by Maddalena et al., a reporter protein was created by fusing eGFP to mCherry under the CMV promoter. This was then split into three parts and each packaged in a separate AAV2/2 vector called AAV2/2.CMV.EM1, AAV2/2.CMV.EM2, and AAV2/2.CMV.EM3. C57BL/6J mice (4 to 5 weeks old) were used. One microliter of triple AAV vectors was co-injected into the cochlea using the round window membrane + canal fenestration method we have previously described. Auditory thresholds were assessed in all animals by auditory brainstem response 4 weeks post injection. Cochleae were then harvested and dissected to evaluate transduction efficiency. Single transduction was also studied using AAV2/2.CMV.EGFP or AAV2/2.CMV.mCherry.
Results: We observed robust transduction in inner hair cells (IHCs) with single AAV2/2 injection (AAV2/2.CMV.EGFP or AAV2/2.CMV.mCherry). On the other hand, up to 5% of IHCs were transduced by triple AAV vectors. Auditory thresholds in injected ears and uninjected ears were identical.
Conclusions: Herein, we reported the first study to test cochlear transduction mediated by triple AAV vectors in adult mice. Although the levels of transduction by triple AAV vectors might be less than that by a single vector, our findings suggest that by using triple AAV vectors, additional deafness genes could be potential future targets for cochlear gene therapy.

SU83. Development of a Platform for Parallel Functional Evaluation of Cell Type Specific Synthetic Promoters for Gene Therapy

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Category: Gene Therapy
Background: One of the key components adeno-associated viruses (AAVs) is a promoter that drives transgene expression. Optimal promoters are cell type-specific to avoid toxicity, compact to fit into AAV, and drive a precise level of expression. Unfortunately, identifying and functionally testing promoters is laborious and time consuming. To that end, we built a system to screen a library of synthetic promoters and read out specificity and expression levels for each promoter on a single-cell level and in a single experiment. Such a screen allows for rapid selection of cell type-specific promoters from a large number of candidates. Moreover, it can deliver a set of cell type-specific and compact promoters that drive a range of expression levels, allowing for precise control of transgene expression.
Methods: We created an AAV plasmid with a modified 3' UTR in the transgene cassette that contains a variable DNA barcode sequence compatible with single cell sequencing approaches. This vector served as a backbone to produce an AAV library, where each member contains a different synthetic promoter sequence

coupled with a unique barcode. We built and optimized this system via testing AAVs individually and as a library in mouse utricle explants, using immunohistochemistry to evaluate levels of a GFP reporter and to compare against single-cell RNAseq (scRNAseq) detection of the promoter barcode.
Results: We used previously internally characterized promoters to build and optimize our technology. Our scRNAseq pipeline detected barcodes for each promoter in our pools and enabled us to read out specificity and expression levels across the cell types and cell states ex vivo and in vivo in mouse inner ear. We compared the scRNA-seq results to immunohistochemistry data measuring GFP reporter expression and found that the results were consistent in matched single-vector control samples. Following this work, we applied our system to completely novel inner ear promoters and characterized their specificity and expression levels.
Conclusions: Our approach allows for fast and efficient synthetic promoter screening to deliver promoters for gene therapy that are compact and drive optimal expression levels of the transgene. This method should allow us to accelerate development and testing of novel gene therapy tools in the inner ear.

SU84. Dual Vector Gene Therapy Approaches for STRC-Related Hearing Loss

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Category: Gene Therapy
Background: Stereocilin (STRC) is a large structural protein expressed in outer hair cells (OHCs) of the cochlea, forming lateral links between adjacent stereocilia as well as attachments between the tallest row of stereocilia and the tectorial membrane. STRC is thought to be critical for cochlear amplification mediated by OHCs, and human patients with loss-of-function mutations in STRC exhibit moderate hearing loss in accordance with a defect in amplification. This autosomal recessive form of hearing loss, classified as DFNB16, is the second most prevalent cause of genetic auditory dysfunction in the US and EU and is a candidate for AAV-based gene replacement.
Methods: AAV-based gene therapy to restore STRC expression is constrained by the large size of the protein and the packaging limits of AAV. As such, we tested several dual vector strategies, utilizing both cell line and explant assays to measure STRC expression. In HEK293 cells, we used western blotting to quantitatively compare full-length STRC protein expression between recombination methods and varied split sites. Additionally, we developed an ex vivo assay using utricles explanted from adult Strc KO mice to assess the extent and localization of STRC expressed from dual vector candidates in vestibular hair cells. We then delivered the top candidates in vivo via injection through the round window membrane or posterior semicircular canal to better understand their relative OHC transduction efficiencies as well as their ability to restore hearing in Strc KO mice.
Results: Using both cell-based and explant assays, we show consequential differences in the recombination efficiencies of the dual vector methods tested. We also demonstrate that the localization of STRC protein produced via dual vector AAV can be effectively assessed ex vivo using utricle explants. In vivo, using multiple dual vector strategies, we restore expression of STRC in the stereocilia of OHCs in Strc KO mice and observe an improvement in ABR thresholds in these mice relative to baseline, as well as a restoration of DPOAEs, which are absent in KO animals. Finally, we show a correlation between the proportion of OHCs expressing STRC and hearing recovery, further supporting the importance of efficient dual vector strategies for OHC expression as well as the efficacy of this approach.
Conclusions: Our results indicate that multiple dual vector AAV gene replacement methods can be used to express full-length STRC in vivo, leading to meaningful auditory threshold improvements and recovery of DPOAEs by restoring outer hair cell function.

SU85. The Effects of Epigenetic Modifications on the Efficacy of Inner Ear Gene Therapy

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Category: Gene Therapy
Background: In most inner ear gene therapy studies, the therapy needs to be administered in the neonatal period for it to be effective. An example of this is seen in the whirler (Whrn^wi/wi) mouse. The Whrn^wi/wi mouse does not express whirlin and exhibits abnormally short stereocilia bundles on the cochlear and vestibular hair cells. In a previous study, we showed that treatment with AAV8-whirlin (long isoform) was effective at lengthening the stereocilia bundles when delivered in the neonatal age (P0-P5). However, when AAV8-whirlin was delivered to mature Whrn^wi/wi ears (P30), the stereocilia bundles remained abnormally short, despite the restoration of whirlin expression at the stereocilia tips. One possible explanation for this phenomenon is the difference in epigenetics between the neonatal and adult mouse inner ears. In this study, we explore whether epigenetic modification using the histone deacetylase (HDAC) inhibitor SAHA can help to make the adult mouse cochlea more amenable to the effects of inner ear gene therapy in the Whrn^wi/wi mouse model.
Methods: In vitro: Utricle cultures from 1–2-month-old Whrn^wi/wi mice were harvested and treated with three different conditions: culture media alone, culture media + AAV8-whirlin (long isoform, 1 × 10^13 GC), culture media + AAV8-whirlin + SAHA (5μM).
In vivo: Adult Whrn^wi/wi mice (1 month old) were injected with either AAV8-whirlin alone or AAV8-whirlin + SAHA (5μM) through the posterior semicircular canal (PSC). One month after the treatment, auditory brain stem responses (ABRs) were performed to assess auditory function. Confocal microscopy was used to examine cellular morphology.
Results: When adult Whrn^wi/wi utricles were cultured with culture media + AAV8-whilrin long isoform + SAHA, an increase in stereocilia length was observed in stereocilia expressing whirlin compared to adult Whrn^wi/wi utricles that were cultured with either culture media alone or with culture media + AAV8-whirlin. Lengthening of cochlear and vestibular stereocilia bundles was also observed when AAV8-whirlin + SAHA was administered into the adult Whrn^wi/wi mice through the PSC in vivo. The length of the stereocilia bundles expressing whirlin from the AAV8-whirlin + SAHA group was longer than the stereocilia expressing whirlin from just AAV8-whirlin + culture media alone. Morphologically, hair cell stereocilia architecture is partially restored, and there is a reduction in the supernumerary rows of stereocilia in the transduced hair cells. Unfortunately, there was no improvement in the auditory or vestibular function when compared to untreated Whrn^wi/wi mice.
Conclusions: Our study showed that combining AAV8-whirlin gene therapy with SAHA resulted in an increase in stereocilia bundle length in the adult Whrn^wi/wi hair cells in vitro and in vivo. Our data suggest that epigenetic modifications may be effective at alleviating structural abnormalities of stereocilia in adult mouse inner ears after the application of gene therapy.

SU86. Hybrid Cochlear Implantation With Gene Therapy in Chronically Deaf Mice

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Category: Gene Therapy
Background: While the cochlear implant (CI) is remarkably effective at restoring hearing for severe-to-profoundly deaf individuals, many CI recipients experience variable outcomes in their music perception or speech comprehension in noisy environments. Implant efficacy can be compromised by the etiology of deafness, intracochlear tissue response or delayed loss of residual hearing. We previously reported a fully implantable intracochlear electrode stimulator assembly designed for chronic implantation in mice. This model holds great potential in interrogating CI biology in transgenic deafness models and evaluating therapies to improve CI performance. The application of gene therapy to improve CI functionality is a potential therapeutic approach. Assessment of the efficacy of this therapy first requires refinement of the surgical approach, evaluation of the functional changes, and impact of electrical stimulation on virus transduction.
Methods: Pou4f3-DTR or kanamycin-deafened mice were used. Deafened animals received a CI via the round window membrane and gene therapy (AAV-GFP) via the posterior semi-circular canal. To confirm successful stimulation of the auditory pathway with the electrode array, electrically evoked auditory brainstem responses (EABRs) were recorded, and thresholds obtained. The animals were chronically stimulated 4 hours per day for 3 weeks at levels up to 6 dB above their electrical thresholds. Auditory brainstem responses (ABRs) were recorded to measure residual hearing thresholds and histological analyses of implanted cochleae were performed to evaluate vector transduction.

Results: Implant functionality was maintained throughout the duration of the treatment with chronic stimulation (up to 28 days) and reliable EABRs recorded. Elevated residual hearing thresholds were recorded after cochlear implantation mainly in the high frequencies. Viral vector transduction was observed in hair cells and supporting cells.
Conclusions: The hybrid CI-gene therapy approach is a technically feasible method to develop gene therapy approaches to improve residual hearing following cochlear implantation. Our findings and the methods applied here can provide valuable information useful for investigation of CI mechanisms in genetic or complex etiology deafness models and evaluation of therapies to improve CI performance.

SU87. The Helios Transcription Factor is Necessary for Both Outer Hair Cell Development and Maintenance

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Category: Genetics A: Genomics and Gene Regulation
Background: Transcription factors are key regulators of gene expression. In 2018, our laboratories demonstrated that the transcription factor helios is essential for OHC maturation. In mice, we showed that a mutation in the Ikzf2 gene, which encodes helios, resulted in a reduction in prestin-dependent electromotility and early-onset hearing loss. Moreover, ectopic expression of helios in inner hair cells (IHCs) led to the downregulation of markers specific to IHCs and a transcriptional shift towards an OHC-like state. In this current study, we have tested whether helios also plays a role in maintaining OHC function after the onset of hearing. Furthermore, we describe the transcriptional cascade downstream of helios in developing hair cells.
Methods: Exon eight of Ikzf2 was conditionally deleted by crossing Ikzf2 floxed mice with either Gfi1-Cre (depletion beginning at ~E16.5) or Prestin-CreERT2 (tamoxifen-induced at P12/13/14) mice. Auditory function of these Ikzf2 cKO mice was evaluated at 6-weeks of age by distortion product otoacoustic emission (DPOAE) and auditory brainstem response (ABR) threshold measurements. To evaluate OHC loss, cytocochleaograms were performed on whole mount cochlear preparations from 6-week old mice, stained with phalloidin, DAPI and an anti-prestin antibody. To define the regulatory transcriptional cascade downstream of helios in the developing OHC, OHCs from P8 Ikzf2fl/fl;Gfi1Cre mice and their littermate controls were isolated by flow cytometry, and analyzed by bulk and scRNA-seq followed by a bioinformatic analysis.
Results: Mice conditionally deleted for Ikzf2, using either cre-driver, exhibit elevated ABR hearing thresholds and increased DPOAE thresholds across all frequencies tested. Similarly, aberrant OHC morphology and OHC loss is observed in both mouse models, with the fewest OHCs remaining in the Ikzf2fl/fl;Gfi1Cre mice. Lastly, transcriptional changes were recorded in the helios-deficient OHCs.
Conclusions: Our data reveal that in addition to OHC development helios is also critical for their maintenance. We also describe the transcriptional impact of depleting helios in developing OHCs.

SU88. Epigenetic Analysis of Mature Inner and Outer Hair Cells to Identify Cell Type-Specific Regulatory Elements

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Category: Genetics A: Genomics and Gene Regulation
Background: Inner and outer hair cells in the organ of Corti are both mechanoreceptors required for our sensory perception of sound, but they are distinct cell types that differ in morphology and function. Hundreds of genes are differentially expressed between inner and outer hair cells, but the distal regulatory elements (enhancers) controlling the expression of those cell type-specific genes remain largely unidentified. Previously, combining FACS-purification and epigenetic assays, we profiled the epigenetic landscapes of hair cells from neonatal animals and identified enhancers responsible for hair cell gene expression in the developing organ of Corti. However, it is difficult to FACS-purify hair cells from mature organs due to the fragility of hair cells after dissociation. Here, we utilized the suction pipette technique to collect inner and outer hair cells and then performed epigenetic analysis to identify enhancers governing inner and outer hair cell type-specific gene expression.
Methods: Cochleae were dissected from 1-month-old CBA/J animals and dissociated with enzymatic digestion and mechanic trituration. Inner and outer hair cells were separated and collected based on their

distinct morphologies using the suction pipette technique. With a few hundred inner or outer hair cells, genome-wide chromatin accessibility was analyzed by ATAC-seq and histone modification landscapes were profiled by CUT and RUN.
Results: We were able to profile chromatin accessibility and active enhancer histone modifications (H3K4me1 and H3K27ac) in inner and outer hair cells. Comparison of the epigenetic landscapes between inner and outer hair cells revealed several hundred cell type-specific enhancers.
Conclusions: Epigenetic analysis of mature cochlear cells is feasible using the suction pipette technique and available epigenetic analyzing tools. Identification of cell type-specific enhancers will help us understand the regulatory networks governing the expression of inner and outer hair cell-specific genes, providing potential targets for sensory hair cell subtype fine-tuning in the context of gene therapy and regeneration.

SU89. Plucked From Obscurity: Molecularly Distinct Otic Mesenchyme Cell Subpopulations Exhibit Unique Transcriptional Changes After Loss of Pou3f4

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Category: Genetics A: Genomics and Gene Regulation
Background: The cochlea consists of diverse cell populations working in harmony to convert mechanical stimuli into electrical signals for the perception of sound. One such cell type is otic mesenchyme (OMCs), which is a specialized type of neural crest and cranial paraxial mesoderm that expresses multiple unique transcription factors, including Pou3f4. OMCs terminally differentiate into spatially and functionally distinct cell types, including fibrocytes of the lateral wall and spiral limbus, modiolar osteoblasts, and specialized tympanic border cells of the basilar membrane. Interestingly, consequences of Pou3f4 mutations are diverse and include a complete loss of endocochlear potential, shortening of the cochlear duct, and defective pathfinding and survival of spiral ganglion neurons (SGNs), indicating diverse roles of Pou3f4 in each OMC-derived cell type. Here, we aim to elucidate the molecular distinctness and functionality of OMCs and reveal how loss of Pou3f4 impacts cochlear development.
Methods: OMC enriched single-cell RNA-sequencing (scRNA-seq) datasets were generated from Pou3f4 wildtype and mutant whole cochleae at embryonic day (E) 15 and postnatal day (P) 7. Seurat v3 was used to process the data, including identification of OMC subpopulation marker genes, integration of datasets and differential expression analyses between Pou3f4 wildtype and mutant OMCs. To elucidate the role and contribution of OMCs in paracrine signaling within the developing cochlea, E15 and P7 OMC enriched datasets were merged with previously published epithelial enriched datasets to produce cell-cell communication networks that encompass all cochlear cell types. Ligand-receptor expression and cell interactions was then ascertained by using CellChat v1.1.3.
Results: We determine that OMCs divide into four transcriptionally distinct subpopulations well before the onset of hearing, each of which corresponding to one of the OMC-derived cochlear structures. Furthermore, we show OMC subpopulations display distinct functional roles corresponding to their spatial localization. We also determine how loss of Pou3f4 alters the transcriptome of each OMC subpopulation, revealing divergent roles of Pou3f4 during cochlear development. Additionally, we decipher the cochlear cellular communication pathways and suggest OMCs are the main contributors of outgoing signaling during cochlear development, including both global and subpopulation specifying signaling pathways. Finally, we indicate which outgoing signaling pathways are lost in Pou3f4 mutants which may be the cause of the defects in surrounding cell types.
Conclusions: We show that OMC are heterogeneous, both spatially and transcriptionally, during early cochlear development. Additionally, we reveal how loss of Pou3f4 effects the transcriptional profile of OMCs and propose signaling pathways that could be influencing the development of surrounding cell types. Without cochlear OMCs and their later terminally differentiated cell types, normal auditory function would not be feasible highlighting the importance of tissue specific mesenchymal cells in cochlear development.

SU90. Race, Ethnicity, and Genetic Ancestry in Exome Sequencing for Pediatric Hearing Loss

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Category: Genetics A: Genomics and Gene Regulation
Background: There is growing acknowledgement of disparities in genetic testing for hearing loss (HL) based on race and ethnicity. The diagnostic rate and characterized genes and variants vary widely by race/ethnic group, with White and Asian populations consistently having higher diagnostic rates than Black and Hispanic/Latino populations. This disparity is likely multifactorial, including discrepancy in inclusion in genetic testing, limiting what is known of HL genetics in diverse populations. Our goal was to assess diagnostic efficacy of exome sequencing (ES) by race/ethnicity as well as to analyze genetic ancestry in a clinically heterogeneous cohort of children with HL.
Methods: 256 pediatric patients with HL of unknown etiology at Boston Children's Hospital underwent ES between 2019 to 2022. ES analysis and variant calling used the DRAGEN pipeline and a custom Genomic Learning System. Primarily 366 known and candidate deafness-causing genes were evaluated. Parent-reported race/ethnicity were extracted from medical records. Genetic ancestry was computed by ADMIXTURE analysis, using LD pruned dataset, with cv options for k=3-5. For this analysis, genetic ancestry was based on k=3 grouping and 0.1 fraction of ancestry was the threshold for inclusion within an ancestry group. The total number of HL gene variants, including causative variants, number and type (Benign/Likely Benign/Variant of unclear significance/Likely pathogenic/Pathogenic) of ClinVar variants, and carrier status were calculated for each proband.
Results: Overall, 33.2% of patients had positive findings in 42 genes, with variability across self-reported racial/ethnic groups. Asian (41.2%, 7/17) and White (36.7%, 65/177) groups had higher diagnostic rates than Black (25.0%, 3/12), Hispanic/Latino (16.7%, 3/18), and Other/Unknown (21.2%, 7/32) children, consistent with previous reports. The average number of total variants per proband was higher in Black patients (9.08) as compared to White patients (5.08). Similarly, the average number of VUS per patient was higher in Black (3.42) compared to White patients (1.96).
Genetic ancestry analysis showed that 22.6% of the cohort surpassed the 0.1 threshold for inclusion in an ancestry group for multiple groups. Genetic diagnosis was found in 26.2% (11/42) of 2-way and 38.9% (7/18) of 3-way admixed genetic populations. Of those who surpassed the 0.1 threshold for only one admixture group, a genetic diagnosis was found in 36.4% of Asian ancestry, 32.2% European ancestry, and 10% of those with African ancestry. Analysis of variant distribution by ancestry, linkage disequilibrium, and cumulative carrier status is ongoing.
Conclusions: ES allows for analysis of genetic ancestry to help identify genetic variation within and between populations, thus increasing opportunities to characterize novel variants and genes, contributing to our understanding of the race/ethnicity and ancestry in pediatric HL. As genetic testing for HL continues to expand, availability across all populations is imperative. This requires nuanced appreciation of the genetic causes as well as environmental and social contributors.

SU91. Systematic Functional Characterization of Causal Genetic Variants in Hearing and Vestibular Disorders

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Category: Genetics B: General
Background: Low-cost next-generation sequencing technologies have facilitated many genome-wide association studies (GWAS) and exome sequencing projects identifying hundreds of variants and genes associated with hearing loss. More than 150 loci and over 100 genes are currently associated with non-syndromic hearing loss. Human geneticists now face the immense challenge of functionally testing these candidate disease genes and variants to understand disease pathophysiology. Identifying which candidate genes or variants are pathologically relevant represents a critical barrier that limits our ability to generate relevant human disease models. Our long-term goal is to understand how gene function contributes to human hearing loss. We aim to functionally test the candidate genes or variants using zebrafish as a model system. Model organisms have revolutionized our understanding of human disease; inactivation of candidate human disease genes in animals (i.e., creating gene "knockouts") often triggers similar phenotypes and provides a valuable disease model. Zebrafish provide an ideal model organism to study hearing loss because of their external embryonic development, transparent body, accessible inner ear, and the presence of lateral line neuromasts (functionally analogous to mechanoreceptors of the mammalian inner ear).

Methods: We have optimized the CRISPR/Cas9-based method to screen for phenotypes in F0 (the founding generation) by generating biallelic mutations in a high-throughput manner. Moreover, our approach is not only limited to gene knockouts but is also focused on studying single nucleotide variants (SNVs) to identify which SNVs are pathologically relevant. We further optimized the CRISPR/Cas9-mediated targeted base editing approach to induce single nucleotide changes in the genome. We combined these optimized mutagenesis approaches with morphological and behavioral phenotyping as well as functional hair cell screening strategies to functionally characterize genes associated with hearing.
Results: We screened over one hundred genes and identified around thirty genes involved in hearing loss and inner ear development, these including genes without any functional study, no in vivo model, previous unknown hearing behavior or unknown hair cell function.
Conclusions: With the optimized methods, it is now possible to rapidly screen for hearing-associated genes and functionally test the single nucleotide variants in a high-throughput, cost-effective manner.

SU92. TMPRSS3 Limited Expression in Spiral Ganglion Shows No Impact on Auditory Neuron Differentiation: Implications for Cochlear Implantation

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Category: Genetics B: General
Background: Biallelic mutations in the transmembrane protease, serine 3 (TMPRSS3) gene are one of the most common causes of hearing loss in children and adults undergoing cochlear implantation (CI). Previous studies reported controversial CI outcomes in TMPRSS3-associated hearing loss. Lack of TMPRSS3 does not impact sensory hair cell development but leads to rapid hair cell degeneration at the onset of hearing in genetic mouse models. The spatial expression of TMPRSS3 within the inner ear and spiral ganglion neurons (SGNs) along with the role of TMPRSS3 in hair cell function remains to be elucidated.
Methods: TMPRSS3 expression in mouse cochlea and human auditory nerves (HAN) was determined by using hybridization chain reaction (HCR), RT-PCR, and single-cell RNA-sequencing (scRNAseq) data analysis. SGNs were immunolabeled with antibodies specific for SGN subtypes (IA, IB, IC, and II) in the cryosections of wild-type and Tmprss3Y260X/Y260X mice at P11. The SGN subtype cell counts were analyzed to determine the SGN differentiation. The sensory hair cells (HC) mechanotransduction was analyzed with FM1-43 uptake assay at P7.
Results: Robust Tmprss3 expression occurs throughout the mouse organ of Corti including inner and outer hair cells, support cells, the spindle, and root cells of the lateral wall. Faint staining within <5% of the HAN and SGN was observed. scRNAseq datasets from the publicly available database (gene Expression Analysis Resource, gEAR) also show limited Tmprss3 expression in type II SGN and no expression in type I SGN. The SGN subtype cell counts show a similar differentiation profile in both wild-type and Tmprss3Y260X/Y260X mice at P11. The FM1-43 indicated functional HC in Tmprss3-mutant mice at P7.
Conclusions: Tmprss3 expression is limited in auditory neurons suggesting that the mechanistic role of TMPRSS3 is within the organ of Corti. In addition, TMPRSS3 plays no role in the spontaneous firing activity and mechanotransduction of hair cells. Further studies are necessary to elucidate the mechanism of TMPRSS3- associated hearing loss, but these data suggest CI outcomes in patients with TMPRSS3-associated hearing loss should be similar to patients with hearing loss caused by genes expressed within the organ of Corti.

SU93. Disrupted GRHL2 Transcriptional Activity as a Mechanism of Autosomal Dominant Hearing Loss Development (DFNA28)

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Category: Genetics B: General
Background: GRHL2 is one from over 50 genes causative of autosomal dominant hearing loss (ADHL); it is also implicated in other disorders, including cancers. GRHL2 encodes a transcription factor but up to now only a handful of ADHL-related GRHL2 pathogenic variants have been reported. Their mode of action leading to ADHL development remains unknown. The aim of the study was to identify the genetic basis of

ADHL in a multigeneration family with postlingual, progressive HL and to gain insight into the molecular mechanism of the ADHL-related (DFNA28) GRHL2 mutations.
Methods: Genomic DNA was isolated from the peripheral blood samples of the proband and other family members (n = 8). Next-generation sequencing was performed using a multi-gene panel with 237 HL-related genes. Segregation analysis of the selected GRHL2 variant with HL in the family was performed by Sanger sequencing. For four different ADHL-related GRHL2 variants expression vectors were prepared and luciferase reporter gene assay was conducted in HEK293T cells.
Results: In the family a novel heterozygous GRHL2 variant (NM_024915.4:c.1061C>T; NP_079191.2:p.(Ala354Val)) segregating with HL was idnetified. It localizes in the region corresponding to the DNA binding domain. The functional effect of the variant as well as of the other two GRHL2 variants located in the DNA-binding domain (i.e. c.1258-1G>A, p.(Gly420Glufs*111) and c.1276C>T, p.(Arg426*)) was a reduction in GRHL2 transcriptional activity. In contrast, the c.1609–1610insC (p.(Arg537Profs*11)) variant affecting the DNA dimerization domain of the GRHL2 protein acted in a different way leading to a strong activation of the GRHL-responsive promoter.
Conclusions: Our data show that only truncating GRHL2 mutations can cause ADHL. The pathogenicity of the novel missense ADHL-related GRHL2 variant was strengthened by the results of functional assays. GRHL2 mutations causing ADHL demonstrated both suppression and activation of GRHL2 transcriptional activity and the effect seems to depend on where the variant is located. While the variants located in the DNA-binding domain showed haploinsufficiency, the variant located in the DNA dimerization domain presented a gain of function effect. Our study sheds new light on the mechanism of GRHL2 mutations leading to hearing loss.
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SU94. Generation of Isogenic Control iPSC Line From the Hearing Loss-Causative ATP2B2 p.(C666) Variant*

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Category: Genetics B: General
Background: ATP2B2 encodes the plasma membrane Ca2+ ATPase 2 (PMCA2) in the outer hair cells (OHCs), a protein component shown to be essential for hearing and balance. Mutations in the PMCA2 have been associated with human dominant genetic deafness. ATP2B2 highlights a critical role played by PMCA2 in maintaining Ca2+ ion homeostasis and control of hair cell function and survival. Human induced pluripotent stem cells (hiPSCs) have emerged as a potential model system to study the impact that specific genetic variants play in the development of many disease states, including HL. These will be important for understanding the molecular mechanisms underlying genetic hearing loss and the development of therapeutic strategies.
Methods: hiPSCs were reprogrammed from a patient-derived lymphoblastoid cell line carrying a point mutation, c.1998C>A (p.C666*), in the ATP2B2 gene using an engineered Epstein Barr Virus (EBV) based OriP/EBNA-1 episomal vectors expressing pluripotent factors – Oct4, Sox2, Nanog, Lin28, Klf4 and L-Myc. Once reprogrammed, there are several tests performed to validate the cell line. These tests include sanger sequencing to validate the mutation, karyotyping to assure the chromosome normality, STR analysis, and mycoplasma detection. Pluripotency of the reprogrammed cell line was validated by immunocytochemistry (ICC) and the differentiation capacity assessed by trilineage differentiation followed by ICC for germ layer-specific markers. Isogenic lines were derived through the CRISPR/Cas9-mediated genetic correction of the ATP2B2 variant.
Results: hiPSC lines were successfully derived from the patient lymphoblastoid line carrying the hearing loss-related mutation in the ATP2B2 gene. These lines maintained the genomic stability as assessed by G-band karyotyping and stained positive for the pluripotency markers when assessed by ICC. The cells were monitored for EBV gene expression by PCR analysis and were negative for both the EBV genome and

EBNA1-based reprogramming vectors. Our results showed the capacity of differentiating into the three primary germ layers following trilineage differentiation. Treatment of the iPSC line with recombinant Cas9 protein complexed with short guide RNAs targeting the point mutation and a single stranded oligodeoxynucleotide (ssODN) template bearing the wildtype ATP2B2 gene sequence was used to produce isogenic iPSC lines. Individual clonal CRISPR-treated lines were screened for correction of the variant by Sanger sequencing. The absence of off-target CRISPR events was assured by Sanger sequencing.
Conclusions: These isogenic pairs of iPSC lines (bearing the c.1998C>A (p.C666*) ATP2B2 variant or genome corrected) provide a valuable cell model for studying the effects of this variant in ATP2B2 on the cellular and molecular functionality of cells of the inner ear, which can benefit hearing loss patients who have ATP2B2 mutations, and other hearing loss mutations. In addition, these cells can be used as a platform for therapeutic development and testing.

SU95. Prestin Knock-In Mouse Models Carrying Deafness-Associated Missense Variants Display Early and Progressive Hearing Impairment

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Category: Genetics B: General
Background: SLC26A5, also known as prestin, mediates electromotility of cochlear outer hair cells (OHCs). Although prestin is known to be essential for mammalian hearing, only a limited number of deafness-associated variants have been reported, including p.A100T (c.298G>A) and p.P119S (c.355C>T). Our previous in vitro study revealed that these missense changes negatively affected prestin function. Subsequently, we generated A100T- and P119S-knock-in (KI) mouse models and showed that homozygotes of both lines exhibited impaired hearing. In this study, we examine compound heterozygous (cHet) mice that carry one copy of each allele. i.e., A100T and P119S, to better understand the disease progression in the patient with the same genotype.
Methods: A100T- and P119S-prestin-KI homozygotes were crossed to generate cHets. Distortion product otoacoustic emissions (DPOAEs) were measured at f2 frequencies ranging from 2 to 47 kHz. Auditory brainstem responses (ABRs) were also acquired. Electrophysiological measurements on OHCs isolated from the cHet mice were also used to assess prestin function. Immunofluorescence, obtained using a prestin antibody on whole-mount tissue samples from cHets, documented OHC loss.
Results: Although cHet mice exhibited significantly elevated ABR and DPOAE thresholds across frequency at ~5 weeks of age, the shifts at low frequencies were smaller at weaning. Electrophysiological measurements obtained in vitro showed impaired prestin function in OHCs isolated from cHets. In addition, anatomical evaluations demonstrated that OHC loss in cHet mice was limited to the basal region of the cochleae, similar to A100T homozygotes. In contrast, the OHC loss in P119S homozygous mice with more widespread than that observed in the cHets.
Conclusions: Our results demonstrate that both A100T and P119S missense variants are disease-causing alleles of SLC26A5, although they seem to differentially affect OHC survival. Further examination is needed to elucidate how each prestin variant interacts with one other and affects OHC function. The results will provide insights into possibilities for interventions that mitigate or prevent hearing loss due to OHC dysfunction.

SU96. Functional Analysis of the Connexin26 V37I Variant in hiPSC-Derived Human Tissue

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Category: Genetics B: General
Background: Hearing loss (HL) is the most common sensory disorder. There are 120+ genes that have been implicated in genetic HL. The most common is Gap Junction Beta 2 (GJB2). It codes for the gap junction protein Connexin26 (Cx26), expressed by the supporting cells of the inner ear. Cx26 is vital for nutrient and signaling molecule distribution in the inner ear. A common GJB2 variant is c.109G>A which causes a missense mutation and a single amino acid change (p.V37I), leading to mild-to-moderate hearing loss. As human cochlear tissue is hard to obtain, previous in vitro studies to characterize GJB2 variants have been carried out in non-hearing relevant expression systems. These systems have greatly aided our understanding

of Cx26, however do not recapitulate the genomic environment of individuals with variants and factors native to the otic epithelium.
Methods: To better account for these factors, we have used an established model of Cx26-espressing otic epithelium (Cx26-OE) differentiated from human induced pluripotent stem cells (hiPSCs). We reprogrammed an hiPSC line from a Cx26-V37I individual using Sendai virus reprogramming. The line was then corrected to Cx26-WT using CRISPR. The Cx26-V37I and isogenic control lines were differentiated into early otic epithelial aggregates. These were platted on laminin for functional analysis. Fluorescent recovery after photobleaching (FRAP) was performed to assess gap junction functionality, in the presence and absence of the pan-gap junction inhibitor CBX. Inner ear organoids were differentiated from the V37I and WT lines and immunocytochemistry was performed for Cx26 and hair cell markers at day 60.
Results: The Cx26-V37I and WT lines were shown to be pluripotent by qPCR and ICC for pluripotency markers. CRISPR correction had a 37% editing efficiency. FRAP curves showed full and equivalent recovery between the uninhibited V37I and WT lines ($p>0.05$). When CBX was added, the WT line showed no recovery and the V37I line showed ~50% recovery ($p=0.004$). Inner ear organoids express Cx26 and work on gene editing and delivery for functional recovery is ongoing in our lab.
Conclusions: Variants in GJB2 are the most common cause of genetic hearing loss. There are several proposed mechanisms of dysfunction, but a precise understanding of these factors in human tissue is still elusive. We are able to differentiate Cx26-OE from hiPSCs harboring naturally-occurring Cx26 variants. This model can be used for FRAP and other functional assays. We are also able to derive inner ear organoids that express a variety of cell types including hair cells and supporting cells. The use of these model systems will be valuable to understanding the effects of Cx26 variants in human tissue and to develop gene and cell-based therapies.

SU97. GRHL2 Gene as an Important Cause of Slowly Progressive Autosomal Dominant Hearing Loss in Koreans

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Category: Genetics B: General
Background: Grainy head-like 2 (GRHL2) is an encoding transcription factor, which is associated with the autosomal dominant form of hearing loss (DFNA28). Since the first report in 2002, seven SNVs which include four nonsense or frameshift variants, one splice region variant and one missense variant, and one CNV (deletion) were reported to be associated with hearing loss. GRHL2 gene-related hearing loss has been reported to be associated with age-related hearing or potentially noise-induced hearing loss. Hearing loss is thought to be caused via a mechanism of haploinsufficiency or loss of function. However, there still is a scarce of related literature and there has been no report in Koreans. In the study, we first report the GRHL2-related hearing loss in Koreans and suggest the 1609th residue of GRHL2 DNA sequence might be a mutational hotspot.
Methods: A 50-year-old woman visited the clinic presenting with slowly progressive hearing loss, which had started in her forties. She had a family history of hearing loss in her mother side. Her mother and mother's older sister with her son all benefitted from wearing hearing aids, which showed autosomal dominant inheritance pattern. Audiogram showed bilateral moderate degree sensorineural hearing loss (~40dBHL). Exome sequencing followed by filtering steps using bioinformatics tools identified potential causative variants in this hearing-impaired pedigree and segregation study using sanger sequencing further confirmed the causative variants.
Results: Exome sequencing and following bioinformatics tools identified one nonsense variant of GRHL2 gene: c.1609C>T;p.Arg537* as a deafness causing variant, which segregated well with hearing loss in the family. Arginine on 537th amino acid of GRHL2 was well conserved down to zebrafish, which further confirmed the pathogenicity of the variant. According to the ACMG/AMP variant interpretation guidelines for genetic hearing loss, PVS1 and PM2 rule were applied to the variant, which classified the variant as 'Likely Pathogenic'.
Conclusions: Although alterations of GRHL2 gene have been assumed to be associated with age-related hearing loss or noise-induced hearing loss, little has been reported in the literature. Here we add the novel variant to the literature by reporting the first DFNA28 pedigree in Koreans. Functional study revealing the underlying molecular mechanism needs to be performed in the near future.

SU98. Closing the Gap in Racial and Ethnic Disparities in Genetic Testing for Hearing Loss

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Category: Genetics B: General

Background: Hearing-loss gene panel testing is the current standard to investigate the genetic etiology of sensorineural hearing loss (SNHL). We previously showed that under-represented minority (URM, comprising Hispanic and non-Hispanic Black or Native American) children with SNHL have 5-fold lower odds of receiving a genetic diagnosis with existing hearing-loss gene-panel testing and that rare genetic variants identified in these URM individuals are more likely to be Variants of Uncertain Significance (VUS) compared with pathogenic or likely pathogenic (P/LP) variants. This disparity is likely related to the historic vast underrepresentation of these populations in gene-discovery studies for hearing loss.

Methods: We studied 927 total variants from 283 URM patients with SNHL. Only 132 variants were P/LP, whereas 697 variants were VUSs. We sought to investigate pathogenicity of these VUSs to improve genetic diagnosis. From these 697 VUSs, 26 variants were prioritized for characterization, as they appeared at least 2 times in unique probands in our dataset. We used ACMG variant interpretation guidelines with hearing-loss expert specification to attempt re-classification of these multiple-hit VUSs, focusing especially on case-control analysis relative to ancestry-matched control populations and computational prediction using REVEL.

Results: 10 out of 26 of these VUSs, which included variants in OTOG, TJP2, COL11A2 and other genes, were able to be re-characterized as Likely Pathogenic, which may enable the probands to receive a genetic diagnosis. For the remaining 16 VUSs, re-classification would require parental testing and segregation analysis. In addition to these VUSs that appeared at least twice in our dataset, many additional VUSs appeared only once, but were extremely rare or absent from ancestry-matched databases and could be re-classified with additional information.

Conclusions: This study demonstrates the necessity and potential impact of increasing representation of URM individuals in hearing-loss genetic testing to address disparities in hearing healthcare utilization and outcomes.

SU99. Making a Case for Genetic Testing Prior to Cochlear Implantation for Improved Surgical Outcomes and Prognostic Counseling

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Category: Genetics B: General

Background: The clinical utility of next generation sequencing gene panels for hearing loss has been well established in the genomic era. We hypothesize that genetic testing may contribute unique insight to the pre-operative cochlear implant (CI) evaluation, beyond current clinical practices, and influence clinical decisions.

Methods: Medical history, surgical and audiologic reports and medical imaging were reviewed for a 10-years old male who underwent sequential cochlear implantation and was subsequently diagnosed with a pathogenic POU3F4 variant. Genetic testing was performed using OtoSCOPE v5, a targeted gene panel to screen all known hearing loss-associated genes. Genetic data were discussed in the context of the individual's medical and family history in a multidisciplinary meeting including clinicians, geneticists, scientists, bioinformaticians and a genetic counselor.

Results: The patient presented for cochlear implant evaluation at 10 years of age with bilateral profound hearing loss. He primarily signed and gestured to communicate. Pre-operative CT imaging showed bilateral enlarged vestibular aqueducts but did not identify a risk for gusher. He underwent left cochlear implantation, complicated by cerebrospinal fluid 'gusher'. Sequential right cochlear implantation was performed three years later with upfront ear canal closure. Subsequent genetic testing diagnosed a hemizygous pathogenic stop-gained variant in POU3F4 gene: p.(Arg167Ter). At last follow-up at age 25, the patient is a bilateral CI user, utilizing total communication (CI, sign, lip-reading), primarily communicating with non-verbal signs.

Conclusions: Pre-surgical knowledge of a pathogenic POU3F4 variant would have allowed anticipation of cerebrospinal fluid 'gusher' at the time of surgery, which was not expected from the limited imaging findings. Further, appropriate counseling, informed by genetic diagnosis, regarding the potential for associated developmental delays may proactively direct additional post-implantation interventions. This case highlights the need for comprehensive genetic testing as part of the pre-cochlear implantation evaluation in those with congenital hearing loss.

SU100. Identifying Pathogenic OTOF Variants in Auditory Neuropathy Spectrum Disorder Patients Using Targeted Next-Generation Sequencing and Minigene Assays

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Category: Genetics B: General

Background: Auditory neuropathy spectrum disorder accounts for ~10% of pediatric sensorineural hearing impairment. The etiologies of auditory neuropathy spectrum disorder are multiple, including both genetic and acquired risk factors. Recessive pathogenic OTOF variants are the most common cause responsible for non-syndromic auditory neuropathy spectrum disorder. In this study, we leveraged targeted next-generation sequencing and functional minigene assays to dissect the landscape of OTOF variants in Taiwanese patients with non-syndromic auditory neuropathy spectrum disorder.

Methods: Sixty-six unrelated patients with non-syndromic auditory neuropathy spectrum disorder were enrolled from 2013 to 2022. Comprehensive genetic analyses were performed in all patients using next-generation sequencing targeting 220 deafness genes. Sanger sequencing was performed to verify candidate variants, and minigene assays were applied to confirm the pathogenicity of identified splice-site variants.

Results: A total of 32 (48.5%) patients were identified with homozygous or compound heterozygous OTOF variants and 9 (13.6%) with heterozygous OTOF variants. Of them, c.5098G>C is the most common variant identified in our cohort, being homozygous in 12 patients and compound heterozygous in 20 patients. Of note, we identified 6 (9.1%) patients with splice-site variants, including c.3864G>A, c.3894+5G>C, c.4023+1G>A, c.4227+5G>C, c.4961-1G>A, and c.5813+2T>C, and each was in compound heterozygosity with another pathogenic OTOF variant in the affected probands. All the 6 splice-site variants were then proved to be spliceogenic by minigene assays.

Conclusions: OTOF variants are the most common etiologies for non-syndromic auditory neuropathy spectrum disorder, and c.5098G>C is the most prevalent variant in the Taiwanese population. Targeted next-generation sequencing followed by minigene assays is useful in deciphering the genetic underpinnings in patients with non-syndromic auditory neuropathy spectrum disorder.

SU101. RNA-seq Transcriptome Profiling of Cochlear Genes Reveals Significant Sex Differences in Cochlear Biology in Mice

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Category: Genetics B: General

Background: Males and females differ in their hearing sensitivity and susceptibility to otologic disorders. Males are more susceptible to acoustic overstimulation, and their hearing deteriorates earlier and faster as age increases. In contrast, females are more prone to immune-related hearing loss. At present, the underlying

mechanisms for these differences are poorly understood. Here, we report the sex differences in the expression profiles of cochlear genes and the functional implications of these sex-biased genes in mice.
Methods: Male and female mice with the C57BL/6J background were used, and their cochleae were collected at the age of two months. All female cochleae were collected at the estrus stage in the estrous cycle, determined by a vaginal cytology assay. Sensory epithelia, spiral ganglions, and lateral wall tissues were isolated with two cochleae from an animal generating one sample (n=4 samples for each sex). Total RNAs were extracted and then used for RNA-sequencing analyses to determine the expression levels of cochlear genes. The expression profiles of cochlear genes were compared between male and female samples. Differentially expressed genes were further analyzed for their functional implications using multiple bioinformatics tools, including GSEA, DAVID, QuickGo, and String.
Results: Using adjusted $p < 0.05$ and Log2 fold difference > 0.5 or < -0.5 as the cutoff for significant difference, we identified a total of 1449 protein-coding genes that differ in their expression levels between sexes, among which 907 genes are male-biased, and 542 genes are female-biased. Most differentially expressed genes are autosomal (1378 genes, 95.1%). Using FDR < 0.05 as the cutoff criterion, we revealed eight biological process gene ontology (GO) terms for male-biased genes. These terms are related to ATP production, mRNA processing, and stress. The cellular component GO terms for male-biased genes are primarily associated with the mitochondrion and nucleus. In contrast, the top biological process GO terms for female-biased genes are related to ion transport, neurotransmitter transport, cell adhesion, and neurogenesis. The cellular component terms are mainly associated with the synapse, cell membrane, and cell junction. These results are further supported by the phenotype enrichment analysis (GSEA). The male-biased genes are enriched in gene sets of oxidative phosphorylation, aerobic respiration, and ATP-biosynthetic process. In contrast, female-biased genes are enriched in gene sets of nerve impulse transmission, multicellular organismal signaling, neuronal action potential, and vesicle-mediated transport in the synapse.
Conclusions: Our data reveal significant sex differences in the expression profiles of cochlear genes. Male cochleae have stronger gene activities with functions in mitochondrial energy production and mRNA processing. Female cochleae have robust gene activities with functions in intercellular signaling. These findings provide new insights into molecular mechanisms for sex differences in cochlear susceptibility to otologic disorders, including acoustic injury, age-related hearing loss, genetic hearing loss, and hidden hearing loss.

SU102. Identification of the First Pathogenic Synonymous DFNA5 Variant

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Category: Genetics B: General
Background: Variants in the gene DFNA5 (also known as GSDME) are associated with autosomal dominant non-syndromic hearing loss (ADNSHL). All reported cases of DFNA5-related HL involve skipping of exon 8, resulting in the expression of a constitutively active truncated protein that induces apoptosis of cochlear hair cells. Currently, all known pathogenic DFNA5 variants are in the intronic regions flanking exon 8, are missense variants within exon 8, or are structural variants. We hypothesized that some cases of DFNA5-related HL are caused by splice-altering synonymous variants.
Methods: Candidate variants were identified in persons tested using comprehensive genetic testing (OtoSCOPE). Variants were filtered and prioritized based on minor allele frequency, in silico predictions, family history, and clinical correlation. Functional impact of candidate variants was assessed using minigene splicing assays. Patient DNA containing exon 8 of DFNA5 and a portion of the flanking intronic regions was subcloned into a pre-constructed pET01 Exontrap vector encoding 5' and 3' exons separated by a multiple cloning site. Vectors were transfected into HEK293 cells. RNA harvest was performed 36-48 hours post-transfection. Changes in splicing were assessed using gel electrophoresis and Sanger sequencing.
Results: We identified a variant in DFNA5; NM_004403.3:c.1161C>T, p.(Tyr387Tyr) in a 9-year old non-Hispanic proband with down sloping mild hearing loss. Family history indicates father, paternal aunt, and paternal grandfather have hearing loss. The c.1161C>T transition is absent from gnomAD, conserved and has a low CADD score of 7.4. It is located 22 base pairs from the 3' end of exon 8. Human Splicing Finder

predicts that this variant affects splicing by altering exonic splice enhancer and silencer motifs. Minigene splicing assay confirmed that the c.1161C>T variant results in complete exon 8 skipping.
Conclusions: This work expands the mutational landscape of DFNA5-related HL to include synonymous variants and highlights the importance of assessing persons for splice-altering synonymous variants.
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SU103. Asymmetric Mechanotransduction by Hair Cells of the Zebrafish Lateral Line

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Category: Hair Cells: Anatomy and Physiology
Background: In the lateral line system, water motion is detected by neuromast organs, fundamental units that are arrayed on a fish's surface. Each neuromast contains hair cells, specialized mechanoreceptors that convert mechanical stimuli, in the form of water movement, into electrical signals. The orientation of hair cells' mechanosensitive structures ensures that the opening of mechanically-gated channels is maximal when deflected in a single direction. In each neuromast organ, hair cells have two opposing orientations, enabling bi-directional detection of water movement. Interestingly, Tmc2b and Tmc2a proteins that constitute the mechanotransduction channels in neuromasts distribute asymmetrically so that Tmc2a is expressed in hair cells of only one orientation.
Methods: Here, we advance our understanding of lateral line function using both in vivo recording of extracellular potentials and calcium imaging of neuromasts.
Results: We demonstrate that hair cells of one orientation have larger mechanosensitive responses. The associated afferent neurons that innervate neuromast hair cells faithfully preserve this functional difference en route to the brain. Moreover, Emx2, a transcription factor required for the formation of hair cells with opposing orientations, is necessary to establish this functional asymmetry within neuromasts. Remarkably, genetic removal of Tmc2a, which leaves hair cell opposing polarities preserved, abolishes the functional asymmetry as measured by recording extracellular potentials and calcium imaging.
Conclusions: Overall, our work indicates that oppositely oriented hair cells within a neuromast employ different proteins to change the quality of mechanotransduction in order to sense water motion direction.

SU104. Membrane Curvature is Not Responsible for Hair Cell Soma-Stereocilia Differences in Membrane Fluidity

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Category: Hair Cells: Anatomy and Physiology
Background: Hair cell mechanotransduction (MET) requires the interaction of numerous proteins clustered at the upper and lower insertion points of stereocilia. Many of these proteins are transmembrane proteins that interact with each other and/or with the cytoskeleton. Thus, the membrane can influence these molecules either directly or indirectly. The energy associated with conformational changes happening within the membrane will be directly impacted by the mechanical properties of that membrane.
Previous work in hair cells has demonstrated that membrane diffusivity was much faster in stereocilia than in the soma and that the stereocilia membrane was selectively sensitive to membrane potential and divalent ion concentrations in a manner consistent with how MET channel open probability was regulated by these manipulations. These results led to the hypothesis that membrane curvature due to the small diameter of the stereocilia could underlie the differences reported in diffusivity.
Methods: To test this hypothesis, we took two approaches. The first was to compare membrane properties in cell types with different membrane shapes to determine if membrane properties correlated across cell types with curvature properties. The second approach was to develop an in-vitro system to study the effects of curvature in different lipid environments with liposomes of different diameter.
We used a novel viscosity sensor BODIPY-1c to monitor membrane viscosity; this sensor provides better spatial and temporal resolution as compared to previous FRAP experiments. We compared hair cell stereocilia and soma as well as HeLa cells, MDCK cells and brush border cells.
Results: We found that stereocilia were unique in having very low viscosity as compared to each of these cell types, in particular brush border cells whose microvilli were much more viscous than soma.

Liposome assays revealed a predicted relationship with liposome diameter and viscosity that was augmented by the presence of cholesterol and/or sphingomyelin. Characterization of liposomes with a comparable makeup of lipids as reported in stereocilia show a weak sensitivity to diameter and these differences could not explain the stereocilia measurements.
Conclusions: Together these data suggest that stereocilia represent a unique compartment where membrane viscosity is maintained at low levels.

SU105. Temporal Changes in Morphology of the Auditory and Vestibular Organs in C57BL/6J Mice

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Category: Hair Cells: Anatomy and Physiology
Background: A C57BL/6J mouse model of progressive hearing loss (HL) carries the homozygous splice-site variant c.753A>G in the cadherin 23 gene (Cdh23), known as an ahl allele (Cdh23ahl/ahl). The Cdh23 gene encodes a protein called cadherin 23, which has shown to connect to protocadherin 15 and make up the tip link of hair cell stereocilia in the cochlea and vestibule. Pathogenic variants in the Cdh23 gene cause HL because they disrupt the formation and stability of the tip link and weaken the connection between sensory hairs. HL in C57BL/6J mice progresses gradually from high-frequency range and spreads toward low frequencies, resulting in severe to profound HL, meaning that they possess a similar phenotype as in humans with CDH23-related HL. Currently, there are few reports describing temporal changes in the morphology of cochleae and vestibules of C57BL/6J mice. To address this, we examined the auditory function and number of hair cells in the inner ear in C57BL/6J mice longitudinally.
Methods: We used C57BL/6JJmsSlc - Cdh23ahl/ahl (C57BL/6J) mice and C3H/HeNJcl (C3H) mice Cdh23+/+ as control. Auditory testing in the form of auditory brainstem response were measured at 4, 12, 24, 36, 48 and 60 weeks of age. Cochleae and utricles were harvested and dissected at the same time points. Tissues were stained with anti-Myosin 7A antibody for hair cells (HCs) and counterstained with Alexa Fluor 488 phalloidin. Morphology of auditory and vestibular organs were evaluated by counting the number of inner HCs (IHCs) and outer HCs (OHCs) expressed in the cochleae from the apical to the basal turn and utricular HCs temporally on confocal fluorescence microscopy.
Results: The progression of HL in C57BL/6J mice begun in high frequencies at 12 weeks of age, leading to profound HL at 48 weeks of age, indicating that auditory thresholds in C57BL/6J mice were elevated relative to those in C3H mice. Following changes in the auditory function, reductions in the number of OHCs were observed in the base as early as 24 weeks of age and spread apically before those for IHCs. In terms of changes for utricular HCs, we had preliminary results. Therefore, we will discuss in detail.
Conclusions: We highlighted the natural degradation in morphology of the auditory and vestibular organs for C57BL/6J mice. Our findings may be useful for studies using gene therapies in murine models exhibiting similar progressive HL to that in humans.

SU106. Cholesterol as a Tool to Probe the Role of Membrane in Cochlear Hair Cell Mechanotransduction

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Category: Hair Cells: Anatomy and Physiology
Background: Most mechanically gated channels are sensitive to force translated through the membrane. The lipid bilayer can modulate channel function directly through lipid/protein interactions or indirectly based on membrane mechanical properties. Cholesterol is an important component of the membrane and a major modulator of membrane mechanical properties. While other ion channels and mechanically gated channels can be activated or modulated by changes to the membrane cholesterol, the functional role of membrane cholesterol in regulation of mammalian cochlear mechanotransduction (MET) channels has not been closely investigated.
Methods: Using whole-cell patch clamping and live-cell fluorescence lifetime imaging (FLIM) of a viscosity-sensitive molecular rotor BODIPY 1c for the first time in the inner ear, we examined the role of membrane cholesterol in modulating the MET response properties of rat cochlear hair cells. Molecular rotors are fluorophores for which the fluorescence lifetime (the average time a fluorophore remains in the excited state) increase with increasing viscosity of their immediate environment.

Results: We confirmed extraction of cholesterol, using methyl β cyclodextrin (MβCD), with reduced filipin staining in both inner and outer hair bundles. MβCD results in reversible reduction in fluorescence lifetime in hair bundles, suggesting initial reduction followed by gradual recovery in the stereocilia membrane viscosity. MβCD reversibly increases the MET channel resting open probability, suggesting that cholesterol depletion increases force transfer to the MET channel.
Conclusions: Together this data suggests that the cell membrane is part of the force relay machinery to the MET channel and could possibly interact directly with components of the MET machinery. Further studies are needed to generate causal link between MET channel gating and membrane mechanics.

SU107. Length Regulation of Mechanotransducing Rows of Stereocilia by Formin Inhibitor SMIFH2

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Category: Hair Cells: Anatomy and Physiology
Background: Stereocilia are actin-based protrusions at the apical surface of auditory sensory hair cells. Several stereocilia of precisely regulated length and width form a bundle with a staircase-like structure that is required for detecting sound. Stereocilia are formed around a core of parallel, unbranched actin filaments. We speculated that formin proteins may contribute to elongating these filaments. To test this idea, we studied the effect of the formin inhibitor SMIFH2 on stereocilia length.
Methods: Cochlear explants from mice at postnatal day 4 were cultured with SMIFH2. Scanning electron microscopy, immunofluorescent staining and actin incorporation levels were analyzed after SMIFH2 treatment.
Results: If formins elongate the F-actin in the core, then inhibition would result in shorter stereocilia. Instead, we observed a rapid and dose-dependent increase in the length of stereocilia in the shorter rows of the bundle. Actin incorporated at stereocilia tips, suggesting that F-actin in the stereocilia core elongated from their barbed ends. FMN1 and DAAM1 are the most highly expressed members of the formin family in auditory hair cells according to published RNAseq data. Immunostaining revealed that FMN1 and DAAM1 localize just above the base of stereocilia and that each is mislocalized following SMIFH2 treatment. We also observed that actin binding proteins that normally localize to stereocilia tips including EPS8, MYO3A, MYO15, ESPNL, and CFL1 were reduced when formins were inhibited.
Conclusions: Together, these data suggest that formins can decrease actin polymerization and indirectly regulate the protein composition of stereocilia tips.

SU108. Role of the Long Isoform of Myosin 15 in the Activity-Driven Plasticity of the Auditory Stereocilia Cytoskeleton

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Category: Hair Cells: Anatomy and Physiology
Background: Modified microvilli in the inner ear, known as stereocilia, detect sound-induced deflections through the opening of mechano-electrical transduction (MET) channels at their tips. At rest, a small MET channel current results in a constant calcium influx. It has been demonstrated that this entry of calcium ions at rest regulates the morphology of the stereocilia cytoskeleton (Velez-Ortega, et al. Elife, 2017). However, the molecular mechanisms involved in this activity-driven cytoskeleton plasticity are currently unknown. Given that myosin 15 is required for stereocilia elongation and the maintenance of the stereocilia bundle, we wondered whether myosin 15 isoforms are involved in the MET-dependent stereocilia cytoskeleton remodeling.
Methods: Organ of Corti explants were isolated from mice that lack the long isoform of myosin 15 (Myo15 ΔN/ΔN) and their heterozygous and wildtype control littermates at postnatal days 4 and 7. Explants were treated for 4, 6, or 24 hours in medium supplemented with the known MET channel blockers benzamil or tubocurarine, or their vehicle controls. Next, samples were fixed in a solution of 4% paraformaldehyde (PFA) or 3% glutaraldehyde/PFA supplemented with CaCl2. Samples were either immunostained against ESPNL or prepared for scanning electron microscopy and were imaged with a Leica SP8 confocal microscope or a FEI Helios NanoLab Dual Beam, respectively. Auditory brainstem responses (ABRs) were measured in 4 to 7-week-old mice anesthetized with 2,2,2-tribromoethanol, before and after exposure to broadband noise at 100 dB SPL for 30 min.

Results: After MET channel blockage, inner and outer hair cells from Myo15 ΔN/ΔN mice exhibited greater remodeling of transducing stereocilia (i.e. middle and shortest rows) than heterozygous or wild-type controls. However, stereocilia regrowth after MET-blockage washout was not impaired. In addition, in inner hair cells from Myo15 ΔN/ΔN mice, we also observed significant shortening of stereocilia from the tallest row, which are normally unaffected in wild-type hair cells. Thus, we are currently testing the expression of stereocilia row-identity proteins (like ESPNL) in hair cells from Myo15 ΔN/ΔN mice. While heterozygous mice develop normal hearing, the remodeling of their stereocilia cytoskeleton after MET channel blockage was greater than in wild-type littermates. Therefore, we are also evaluating the recovery of hearing thresholds after noise exposure in heterozygous and wild-type littermates.
Conclusions: Hair cells lacking either one or two alleles of the long isoform of myosin 15 have lower stereocilia cytoskeleton stability. Moreover, individuals carrying "recessive" deafness-causing mutations affecting the long isoform of myosin XVA could potentially have a greater susceptibility to noise-induced hearing loss.

SU109. Repair of the Stereocilia Actin Core is Facilitated by the Mechanosensor Function of XIRP2
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Category: Hair Cells: Anatomy and Physiology
Background: Sensory hair cells of the inner ear experience continuous mechanical stress. Because mammalian auditory hair cells do not regenerate, the repair of hair cell damage is important for continued auditory function. Recent studies have concluded that the actin core of the vibration-sensing organelle, the hair bundle and its stereocilia, is stable over months, implying that any structural damage must be actively repaired. The stereocilia F-actin core can sustain damage, most notably by noise exposure, which was shown to cause "gaps" in phalloidin labeling of F-actin in stereocilia. In our studies, we found that these gaps are repaired in days. We therefore investigated the molecular mechanisms by which the F-actin lesions are sensed and repaired. According to an emerging concept in mechanobiology, F-actin possesses intrinsic mechanosensor properties. In this model, mechanical strain reveals cryptic binding sites on actin filaments, to which effector proteins are recruited, providing an on/off switch for downstream processes. These processes were implicated in the recruitment of actin repair substrates and in the prevention of F-actin fiber breakage. Here, we present evidence that hair cells employ a similar strategy to repair its F-actin-based stereocilia, and that the protein XIRP2 (Xin Actin Binding Repeat Containing 2) is critically involved.
Methods: We generated KO and KI mouse models for (isoform-specific) loss-of-function and expression/localization studies, respectively. We used laser ablation of stress fibers and stereocilia to test the mechanosensory properties of XIRP2. Finally, we devised a strategy, using the split-GFP approach, to image the movement of endogenous XIRP2 in the living hair cell.
Results: Evidence indicates that stereocilia gaps in mouse auditory hair cells are largely repaired within one week of traumatic noise exposure through the incorporation of newly synthesized actin. We find that XIRP2 is directly involved in this form of repair. XIRP2 localized to stereocilia gaps in a manner dependent on its C-terminal domain (CTD). In fibroblasts, XIRP2 is recruited to laser ablated stress fiber strain sites in a CTD-dependent manner. Analysis of a mouse line lacking XIRP2's CTD provided evidence that the CTD is required for the repair process in vivo. In our working model, we propose that mechanical stress (induced e.g. by noise) creates a partial and localized depolymerization of stereocilia F-actin that leaves the remaining actin fibers under increased strain. XIRP2, through its CTD, is then recruited to the lesion in a force-dependent manner and mediates its repair. We are in the process of investigating the recruitment of GFP-tagged XIRP2 to stereocilia gaps in living hair cells.
Conclusions: Our study describes a novel repair process in hair cells, with potential implications for identifying strategies to prevent and reverse noise and age-related hearing loss.

SU110. Expression and Characterization of MYO7A Isoforms Localized to the Stereocilia Upper Tip-Link Density
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Category: Hair Cells: Anatomy and Physiology

Background: The molecular motor myosin 7a (MYO7A) is expressed in hair cells and photoreceptors, and mutations in MYO7A cause Usher Syndrome type 1 (USH1B) and autosomal recessive hearing loss, DFNB2. MYO7A has multiple functions in cochlear hair cells, being essential for normal development of hair bundle architecture, as well as concentrating at the upper tip link density (UTLD) where it helps tension the mechanoelectrical transduction (MET) complex. Hair cells produce multiple isoforms of MYO7A that contribute to these diverse and essential functions. At the UTLD, a canonical isoform (MYO7A-C) is detected in addition to an isoform (MYO7A-N) that has an additional exon in the ATPase motor domain. In this study, we explore the hypothesis that altered ATPase activities of MYO7A-C and MYO7A-N contribute towards tuning mechanical activity at the UTLD.
Methods: Testing this hypothesis using biophysical approaches requires the isolation of highly pure MYO7A protein in milligram quantities. This is challenging to obtain from primary tissues, and thus recombinant expression systems are needed. Expression of MYO7A poses additional challenges as it has five light chain binding sites (LCBS) that bind to an unknown assortment of light chain proteins. These light chains likely regulate MYO7A motor activity and are thus key for understanding functional differences between MYO7A-C and MYO7A-N at the UTLD. We have addressed this problem using a multiple promoter baculovirus system (biGBac) to express MYO7A in Sf9 insect cells. Utilizing the biGBac expression system, we engineered baculovirus that contains the ATPase domain of either MYO7A-C or MYO7A-N, in addition to co-expressing a myosin specific chaperone (UNC45A), and candidate light chains: calmodulin (CALM1), calmodulin-like protein 4 (CALML4), essential light chain (MYL6) and regulatory light chain (MYL12B).
Results: MYO7A-C and MYO7A-N baculovirus were used to produce recombinant protein in Sf9 cells. MYO7A-C protein was captured from whole cell lysates using FLAG-affinity chromatography and further purified with sequential anion exchange and size exclusion chromatography. SDS-PAGE shows that recombinant MYO7A-C is > 99% pure and forms a pentamer, stably binding to all four light chains (CALM, CALML4, MYL6, MYL12B). These results confirm previous co-immunoprecipitation of endogenous MYO7A from hair cells (Morgan, 2016), by showing that CALM and CALML4 bind to MYO7A. We are presently isolating MYO7A-N and performing functional assays to compare its activity with MYO7A-C.
Conclusions: In conclusion, we have established a scalable pipeline to express MYO7A protein isoforms present at the UTLD. This will allow us to perform biophysical and structural studies to understand its contribution to MET, and the effects of pathogenic USH1B / DFNB2 deafness mutations.
Funded by R01 DC018842.

SU111. Myosin Light Chain Kinase Regulates Cellular Shape Changes in Cochlear Hair Cells via Phosphorylation of Myosin Regulatory Light Chain

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Category: Hair Cells: Anatomy and Physiology
Background: The organ of Corti, or sensory epithelium of the cochlea, comprises hair cells (HCs) and various types of supporting cells. Cellular shape changes of HCs are important both during the development of auditory epithelia and in normal hearing. It was previously observed that HCs and inner sulcus cells (ISCs) undergo shape changes similar to the apical constrictions of neural epithelia. Apical constriction, which is induced by contraction of actomyosin cables at the apical junctional complex, is necessary for the physiological function of the epithelium. This contraction is regulated primarily by phosphorylation of myosin regulatory light chains (MRLCs) by myosin light chain kinase (MLCK). Because the MRLC and MLCK isoforms expressed in HCs and ISCs are unknown, we investigated the expression patterns and roles of these proteins in the mammalian organ of Corti.
Methods: Postnatal Wister rats were used for experiments at 0, 7, and 21 days of age. To investigate the isoforms of MRLC and MLCK in the organ of Corti, we performed droplet digital PCR (ddPCR) and immunofluorescence labeling of whole-mounted specimens. We also expressed and purified MRLC and MLCK proteins and confirmed their expression by ddPCR. Furthermore, in vitro kinase reactions were performed to confirm the phosphorylation of MRLC by MLCK. Finally, we measured the changes in shape of outer HCs of newborn rats after inhibition of MLCK and of protein kinase A (PKA) and protein kinase C (PKC).

Results: ddPCR revealed that HCs expressed MYL12A/B and MYL9, a non-muscle MRLC, as well as smooth muscle MLCK (smMLCK). Immunofluorescence labeling throughout the organ of Corti demonstrated that only MYL12 was expressed in the apical portions of HCs, whereas MYL12 and MYL9 were expressed in ISCs. Phosphorylation of purified MYL12B by smMLCK was observed by in vitro kinase reactions, and immunolabeling confirmed that harvested HCs contained phosphorylated MYL12. Furthermore, in conjunction with expansion of the apical surface areas of outer HCs, MYL12 phosphorylation was reduced by ML 7, an inhibitor of smMLCK. In contrast, H89 and calphostin C, which inhibit PKA and PKC, elicited neither changes in the surface area of outer HCs nor phosphorylation of MYL12.
Conclusions: MYL12A/B and MYL9 are the predominant isoforms of MRLC in the organ of Corti, and among the MLCK family proteins only smMLCK is expressed. In addition, only MYL12 occurs along the actomyosin cables of HCs. MYL12 is phosphorylated by smMLCK and the phosphorylated protein is observed in HCs. Furthermore, the apical surface area of HCs is enlarged by the inhibition of smMLCK. We conclude that MYL12 phosphorylation by smMLCK contributes to the apical shape changes of HCs during the development of auditory epithelia and in normal hearing.

SU112. Nanomechanics of Dimeric Protocadherin 15
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Category: Hair Cells: Anatomy and Physiology
Background: Mechanical force transmitted through elements called gating springs opens and closes mechanotransduction channels at the stereociliary tips of cochlear hair bundles. Although the molecular components of the gating spring remain uncertain, one candidate is the filamentous tip link that interconnects adjacent stereocilia. Each tip link comprises four molecules: a dimer of protocadherin 15 (PCDH15) and a dimer of cadherin 23. PCDH15 is composed of 11 extracellular cadherin domains and is stabilized by Ca2+ binding at its interdomain linkers. Underscoring the importance of the tip link in hearing, there are hundreds of mutations in the tip link that result in deafness. Our previous measurements with a high-precision optical trap showed that an individual monomer of PCDH15 acts as an entropic spring much softer than would be expected from its enthalpic elasticity. We accordingly hypothesized that dimeric PCDH15 has the appropriate physical properties to be a component of the gating spring. We have used the same optical trap to assess the response PCDH15 dimers over a physiologically relevant range of forces and Ca2+concentrations.
Methods: In the optical-trap apparatus, a single PCDH15 dimer is tethered between a pedestal bead covalently attached to a coverslip and a probe bead diffusing in solution. A weak laser measures the position of the probe bead while a strong laser exerts controlled forces on the bead, and thus on the tethered molecule. Because the force can be increased at a controlled rate, we can evaluate the extension of the dimer as a function of forces within the physiological range.
Results: Our data acquired at a relatively high Ca2+ concentration of 3 mM show that dimeric PCDH15 undergoes infrequent unfolding events of magnitudes less than would be expected for the unfolding of an entire cadherin domain. When the Ca2+ concentration is lowered to a physiological value of 20 μM, the dimer is softer than at high Ca2+. In contrast with monomeric PCDH15 at that Ca2+ concentration, the dimer rarely exhibits unfolding, let alone unfolding corresponding to entire cadherin domains. This result suggests that dimeric PCDH15 within the ear can bear tension up to a level of 60 pN with minimal unfolding. In keeping with the dependence of PCDH15's structure on the Ca2+ concentration, the removal of Ca2+ elicits numerous unfolding events.
Conclusions: Although our results support a contribution of the tip link to the gating spring, further characterization of the PCDH15 dimer will be needed to confirm the hypothesis. Biophysical investigations of the remainder of the tip link will be critical to our understanding of normal and pathological human hearing.

SU113. Revisiting the Membrane Thickness Sensitivity of Prestin
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Category: Hair Cells: Anatomy and Physiology

Background: Membrane thickness dependence (Td) of a membrane protein arises from a difference of hydrophobic profile of the protein in different conformational states. For prestin, which undergoes changes in the surface area coupled with charge transfer Q across the membrane, membrane thickness dependence is expected if the volume of the protein is conserved during conformational changes.
Methods: Indeed, an experimental study confirms that is the case with mammalian prestin: A reduction of membrane thickness shifts the transition voltage of the protein in the positive direction and an increase in membrane thickness has an opposite effect [1]. This observation is consistent with the prediction that a decreased hydrophobic thickness of the protein is associated with an increase in the surface area on hyperpolarization [2].
Results: To test if thickness dependence is a useful indicator of conformational changes of prestin mutants, the experimental examination was extended to chicken prestin, which was, at the time, thought to be without motile activities. Contrary to the expectation, chicken prestin showed larger membrane thickness dependence accompanied by somewhat smaller charge transfer compared with mammalian prestin [3]. This result appeared to disprove the idea of membrane thickness dependence as a useful indicator of conformational changes. However, it turned out that chicken prestin is in the apical membrane and can drive hair bundles of short hair cells [4]. Thus, membrane thickness dependence can be a useful indicator of conformational changes of prestin after all.
Conclusions: If indeed membrane thickness dependence (Td) corresponds to the surface area changes during conformational transition, it serves as a good indicator for surface area changes, a quantity very difficult to obtain, and associated with the length change of outer hair cells (OHCs). In addition, the motile force should increase with Q/Td, where Q is charge transfer. Therefore Td is essential for assessing the functionality of prestin. However, Td should not affect the speed of conformational transitions of prestin as measured by membrane capacitance unless the cell is under mechanical load or conformational changes affect the viscoelastic factor.
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SU114. Rapid Assessment of Temporal Processing From the Peripheral and Central Auditory Pathway Using Dynamic Amplitude Modulated Stimuli

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Category: Hearing Loss: Consequences and Adaptation
Background: Envelope or amplitude modulation (AM) cues in a signal are critically important for the perception of complex signals, e.g., speech. Neural coding of AM can be noninvasively probed using the envelope following response (EFR), which receives contributions from both cortical (slower fluctuations, <40 Hz) and subcortical (faster fluctuations, >100 Hz) generators. As subcortical versus cortical signatures of AM representations vary across hearing loss etiologies, the EFR has great diagnostic potential. AM representations are routinely evaluated by the temporal modulation transfer function (tMTF), which is the strength of the EFR as a function of AM frequency. Currently, tMTF is measured serially for discrete sinusoidally amplitude modulated (sAM) tones. This process is time -consuming and inefficient, impeding clinical translation. Here we present a dynamically varying AM tone (dAM) used to measure a tMTF. We compare the tMTF obtained from the dAM stimuli to traditional tMTFs across humans and multiple rodent models (Mongolian gerbils, Cba/CaJ mice, Fischer-344 rats), with and without hearing pathologies (aging, inflammation, noise exposure).
Methods: EEG responses were obtained using sub-dermal needle electrodes in rodent models, and 64-channel EEG caps in humans. Discrete EFRs to sAM tones were obtained for 250-ms long stimuli with 3 kHz carrier frequency amplitude modulated at 40, 110, 512, and 1024 Hz in humans. In rodents, carrier frequencies and amplitude modulation rates varied based on species-specific differences. Tones with dynamically varying AM, the frequency of which increased exponentially from 9 Hz to 1.5 kHz over one second, and identical carrier frequencies were also used to elicit EFRs and the tMTFs were compared. Fast Fourier transforms were used to calculate discrete EFR amplitudes. A spectrally specific frequency-demodulation-based analysis was used to calculate EFR amplitudes from dAM stimuli.

Results: Robust tMTFs were obtained for discrete sAM stimuli from all species tested. Preliminary results show strong tracking of dAM envelopes in rodents, which can be used to estimate the tMTF at a fine frequency resolution. These tMTFs are comparable to those obtained using discrete sAM tones. In humans, preliminary results suggest tracking of dAM envelopes was comparable to sAM stimuli, but only at lower modulation frequencies. Ongoing analysis is aimed at refining the dAM stimuli (trajectories, timescales) to optimize AM tracking while simultaneously reducing recording times in humans.
Conclusions: These results suggest that dynamically varying AM tones can be used to efficiently estimate the tMTF. However, further optimization is needed to obtain robust tMTF estimates in humans at higher AM frequencies. When combined with spectrally specific analysis, the dAM tone can be used to substantially speed up the tMTF measuring time, paving the way for potential clinical translation.

SU115. Auditory Brainstem Responses in Individuals With Extended High Frequency Hearing Loss

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Category: Hearing Loss: Consequences and Adaptation
Background: Many adults report speech-in-noise perception difficulties despite having clinically normal hearing. Emerging studies suggest that hearing in the extended high-frequencies (10, 12.5, and 16 kHz) may be impaired in some of these listeners. However, the mechanisms explaining this relationship between EHF hearing loss and speech-in-noise difficulties are not clear. One mechanism by which EHF loss could impair speech-in-noise perception is by affecting the neural encoding of sound at the brainstem. Although some previous studies measured EHF thresholds and auditory brainstem responses (ABRs) in the context of cochlear synaptopathy (e.g., Guest et al., 2018), these studies were not designed or did not examine the effects of EHF loss. The objective of the present study was to test the hypothesis that EHF loss could reduce the neural processing necessary for the optimal functioning of mechanisms acting at lower frequencies (re; EHFs).
Methods: A case-control design was used where the experimental group included individuals with EHF loss (n=17), and EHF-normal participants served as controls (n=28). EHF loss was defined as hearing thresholds >20 dB at any of the EHFs (10, 12.5, and 16 kHz). ABR was recorded with a two-channel system for 2048 sweeps using clicks stimuli at 80dB nHL at two stimulus rates (11.1/s and 90.1/s). Absolute and inter-peak latencies, amplitude, and amplitude ratios for waves I, III, and V were analysed and were input as response variables to several linear mixed effects models
Results: The percentage of EHF loss in the sample was 35.5%, with 12 bilateral and four unilateral EHF loss. Averaged EHF thresholds appear to have no effect on the ABR parameters studied. The effect of individual EHF thresholds is currently being examined. The effect of EHF loss and other demographic variables on ABR response characteristics will be presented.
Conclusions: Click-evoked ABR recorded using standard protocols may not be sensitive to potential deficits in neural synchrony induced by EHF hearing loss, provided averaged EHF thresholds and individual thresholds reveal similar results as predictors. Our recording technique primarily focused on getting reliable and robust wave I. As a result, the findings have implications for examining the claims that basal cochlear damage (likely manifested as EHF loss) could be associated with cochlear synaptopathy in lower frequencies in humans.

SU116. Evaluation of Neural Representation of Speech Syllables at the Brainstem and Cortical Levels in Normal and Impaired Ears

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Category: Hearing Loss: Consequences and Adaptation
Background: Behavioral studies have established that sensorineural hearing loss (SNHL), reduces audibility (hearing loss), speech discrimination ability (frequency selectivity), particularly in adverse listening conditions, and produces abnormal growth of loudness. In terms of remediation, behavioral studies have shown that hearing aid processed sounds improve both audibility and speech discrimination ability in most of these individuals, but not all. The neural bases for these perceptual deficits are not well understood. In an effort to address this knowledge gap we evaluate the consequences of cochlear hearing loss on the neural representation of these acoustic features as reflected in the scalp recorded brainstem frequency following-(FFR to evaluate temporal fine structure (TFS) encoding) and envelope following responses (EFR

to evaluate encoding of envelop periodicity). The concurrent recording of these brainstem responses (EFR and FFR) and the cortical acoustic change complex (ACC) allow us to compare the nature of neural encoding at two levels of processing along the auditory neuraxis. We also evaluate the influence of vision and presence of noise on these measures.
Methods: For our preliminary pilot data, simultaneous EFR-FFR and ACC responses were recorded from 5 normal-hearing young adults (18-55 years) and 5 hearing-impaired (mild to moderate SNHL) adults using unprocessed and processed versions of CV syllables /ba-da/. Based on the audiograms the hearing-impaired individuals present with, they were allocated to either of two hearing-impaired subgroups. These groups were categorized based on standard audiograms for the IEC 60118-15. Processed stimuli refers to the speech sounds processed using a 'WDRC tool' streamlit app created as a part of the open-source speech processing platform (OSP), which mimics the real-time master hearing aid. Post-acquisition, EEG responses were filtered 70-2500Hz to visualize subcortical EFR-FFR and 1-30Hz to visualize cortical ACC responses.
Results: Both the spectral data and the spectrogram data show robust f0 and its harmonics (EFR data), and the formant related peaks (FFR data) for both syllables for the unprocessed speech in both groups. For processed speech, normal-hearing show improved responses. Hearing-impaired individuals on the other hand, showed slight improvement in envelope and fine structure responses. Comparing unprocessed and processed conditions for the ACC revealed a large amplitude /ba/ offset and onset of /da/ for the processed condition. These initial results are encouraging in that these responses are clearly representing the acoustics features that will be evaluated in the SNHL group.
Conclusions: Our results are preliminary. We hypothesize that (1) SNHL will degrade the TFS representation without altering the envelope representation; (2) hearing-aid-processed speech will improve neural representation of F2 harmonics with no change in the representation of F1 harmonics and the envelope; (3) this improvement be positively correlated with behavioural measures; and (4) addition of visual cues will improve neural representation at both the brainstem and cortical levels.

SU117. Oncomodulin Alters ATP-Dependent Calcium Signaling and Increases Susceptibility to Noise in Adult Mice

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Category: Hearing Loss: Consequences and Adaptation
Background: Acoustic overstimulation leads to hearing loss and disrupts the Ca2+ homeostasis in hair cells. In the organ of Corti, ATP receptors potentially shunt the current away from mechanoelectrical transducer (MET) channels and thus, provide a purinergic-mediated protective purinergic hearing adaptation. Previous studies showed that in outer hair cells (OHCs), the lack of oncomodulin (OCM), an EF-hand calcium-binding protein (CaBP), alters Ca2+ signals during the early stage of development. Ocm-knockout (Ocm-/-) mice show a progressive hearing loss after 1 month age compared to Ocm wild-type (Ocm+/+) mice. Here, we investigated whether the lack of OCM influences Ca2+ signaling and changes the noise susceptibility in adult mice.
Methods: To measure Ca2+ signaling in OHCs, we generated Ocm+/+ and Ocm-/- mice with a genetically encoded calcium sensor (GCaMP6s). These mice conditionally express GCaMP6s after Cre recombination driven by the Atoh1 promoter. GCaMP6s positive (GCaMP6s+) hair cells show fluorescence-based changes in Ca2+ signaling. The apical organ of Corti was collected before and after exposure to sustained moderate broadband sound (95 dB SPL, 9 hrs) from 3-4 weeks (wks) mice. To induce Ca2+ transients in OHCs, 100 μM ATP was applied. Cochleae were collected for qPCR, western blot, and immunocytochemical analysis. OHCs' biophysical properties were investigated using single-cell patch clamp recordings.
Results: Mature OHCs from CBA/CaH Ocm-/- mice showed similar biophysical properties and electromotility compared to Ocm+/+ OHCs. GCaMP6s+ mice were utilized to measure the Ca2+ signaling in OHCs with and without noise exposure. GCaMP6s+ Ocm-/- mice showed increased vulnerability with higher threshold shifts of distortion product otoacoustic emissions (DPOAEs) and auditory brainstem responses (ABRs) compared to Ocm+/+ mice. Without noise stimulation, 3-4 wks GCaMP6s+ Ocm-/- mice showed an increased purinergic P2X2 receptor expression and higher fractional changes of Ca2+ signaling induced by extracellular ATP. Noise overstimulation upregulated P2X2 expression only in GCaMP6s+ Ocm+/+ cochlea but not in GCaMP6s+ Ocm-/- mice.

Conclusions: We conclude that the lack of OCM does not affect OHC electrophysiological function in adult mice. Without OCM buffering, the Ocm-/- exhibits an increased susceptibility to noise that is associated with purinergic signaling.

SU118. Relationships Between Hearing, Cognition and Social Activity for Older Adults Without Dementia

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Category: Hearing Loss: Consequences and Adaptation

Background: People vary widely in their ability to understand speech in adverse conditions, such as when background noise is present. Yet, we do not fully understand the factors that contribute to this variability. Social isolation might affect speech perception, because people who are socially isolated receive less spoken input. Social isolation may be more likely to occur in older people in general, or because age-related hearing loss makes it more difficult to communicate, which may increase the likelihood that someone will withdraw from social settings. The latter has been suggested as a possible explanation for why people with hearing loss are more likely to develop dementia. Here, we examined how social participation involving speech and non-speech activities relates to self-reported hearing ability, speech-in-noise performance, and sub-clinical symptoms of dementia.

Methods: We recruited adults age 60-85 years using the online recruitment platform Prolific. All participants performed a series of online tests and questionnaires, including a short form (15 item) version of the Speech Spatial and Qualities of Hearing Scale (SSQ), an adaptive speech-in-babble test, and the Ascertain Dementia 8-Item Informant Questionnaire (AD8). We also devised a social participation questionnaire to probe the frequency with which participants engage in social activities that involve spoken communication (e.g., visiting friends and relatives) and those that do not involve spoken communication (e.g., exchanging text messages, playing computer games with others). None of the participants had ever been diagnosed with dementia.

Results: Preliminary results (47 participants) suggest that self-reported hearing ability (SSQ score) correlates with sub-clinical dementia symptoms (AD8 score) and also with the frequency of social activities involving spoken communication. As expected, speech-in-babble thresholds were worse with older age.

Conclusions: Preliminary results suggest a relationship between self-reported hearing ability and early signs of dementia. They also suggest that poorer self-reported hearing ability relates to reduced participation in social activities involving spoken communication. The final analyses will examine whether participating in social activities modulates the relationship between hearing ability and dementia, and whether any relationships with social activities are specific to spoken, rather than non-spoken, communication.

SU119. Consonant Confusion Features Due to Hearing Loss

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Category: Hearing Loss: Consequences and Adaptation

Background: Individual assessment of the effects of hearing loss on speech-in-noise (SIN) recognition may suffer from performance-metric variability. SIN test efficiency could be improved by the selection of speech tokens that are known to be especially sensitive to hearing loss.

Methods: In a set of vowel-consonant-vowel (VCV) speech tokens that represented 13 consonants in three vowel contexts, speech-shaped noise was added to each token that achieved an average of 90% correct recognition in normal-hearing listeners. From this large set of speech tokens, presented at 65 dB SPL, a smaller set of ten consonants in a single vowel context was selected that had consonant recognition scores that were most affected by hearing loss. The elements of consonant confusion matrices (CCMs) provided features for multivariate logistic regressions, which were used to (1) predict clinically relevant classifications such as hearing-loss status and (2) estimate pure-tone-average (PTA) threshold. Overfitting of the data was avoided by incorporating regularization into the regressions that optimized performance across 100 random training/validation data splits.

Results: Prior to regularization, regression-based PTA estimates had a mean absolute error (MAE) of about 1 dB. After regularization, the average cross-validated MAE was about 7 dB, which is a more realistic estimate of real-world clinical performance. The average cross-validated area under an ROC curve for NH/HL classification indicates excellent discrimination.

Conclusions: These results indicate that VCV CCMs contain useful information about hearing thresholds. A ten-VCV constant-confusion test, which could be completed in a clinical setting in about 5 minutes, could indicate potential or actual hearing-aid benefit. Future studies with larger numbers of subjects could potentially derive individual hearing-aid recommendations based on VCV CCMs.

SU120. Autosomal Recessive GJB2 Mutation Carriers Have Hyperacusis-Like Hearing Sensitivity Increased

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Category: Hearing Loss: Consequences and Adaptation
Background: Autosomal recessive GJB2 mutations cause 50% of nonsyndromic hearing loss and affect as many as 3 of every 1,000 babies. The heterozygote carriers are estimated up to 10-20% of the general population. These recessive heterozygous mutation carriers typically exhibit good hearing sensitivity in the clinic. However, whether these Cx26 heterozygous mutation carriers have other auditory dysfunctions is unclear. In this study, we examined the distortion production otoacoustic emissions(DPOAEs) and auditory brainstem response(ABR) to evaluate the hearing functional changes in GJB2 heterozygote carriers.
Methods: To limit environmental factors' effects, we recruited children for this study. Children in this study were recruited from daily clinical visiting in Shenzhen Maternity and Child Healthcare Hospital, Shenzhen, China, who were previously diagnosed with GJB2 heterozygous mutations by standard genetic diagnosis with gene sequencing. Any double or multiple point-mutations in GJB2, digenic GJB2 mutations with other gene mutations, and homozygous GJB2 mutations were excluded. The control group was recruited from normally delivered, typical development, and age-matched children with normal hearing sensitivity verified by ABR screening tests. ABR and DPOAE were recorded to assess cochlear and hearing function.
Results: Fifteen of GJB2 single-point heterozygous mutation carriers (8 females and seven males; median age: 117 days) were recruited from daily-visiting in the clinics, including 13 of GJB2 c.109G>A (p.V37I) heterozygote carriers and 2 of GJB2 c.235delC heterozygote carriers. These GJB2 heterozygote carriers passed the newborn hearing screening tests after birth. The control group had 15 normally delivered, typical development, age-matched children (7 females and 8 males; median age: 101 days). GJB2 heterozygote carriers demonstrated active cochlear amplification and increased hearing sensitivity. Compared with the control group, the amplitudes of DPOAEs in GJB2 heterozygote carriers significantly increased by 4-8 dB SPL at 2-8 kHz frequency range. The amplitudes of ABR in GJB2 heterozygote carriers also appeared large. The peak I, II, III, VI, and V of ABR amplitudes were 0.070±0.020, 0.011±0.010, 0.092±0.018, 0.144±0.026, and 0.172±0.017 μV, respectively. In comparison with the peak I, II, III, VI, and V (0.026±0.004, 0.001±0.002, 0.074±0.005, 0.095±0.010, and 0.119±0.011 μV, respectively) in the control group, the peak I, VI, and V in GJB2 heterozygote carriers were significantly increased (P=0.008, 0.048, and 0.008, respectively, one-way ANOVA with a Bonferroni correction).
Conclusions: Our data indicate that recessive GJB2 heterozygous mutations are not "harmless" for hearing. The heterozygote carriers could have increased hearing sensitivity (hyperacusis), which may increase sensitivity to noise. These findings warrant further study and may have implications for the susceptibility of noise-induced hearing loss in these Cx26 heterozygous mutation carriers.

SU121. Does TRPA1 Deficiency Lead to Abnormalities in Cochlear Innervation and the Medial Olivocochlear Reflex?

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Category: Inner Ear: Anatomy and Physiology
Background: TRPA1 channels are expressed in nociceptive neurons, where they are necessary for pain-like responses. These channels are sensors of tissue damage since they are gated and/or regulated by endogenous compounds generated upon tissue stress or injury. Mice lacking TRPA1 channels (Trpa1-/-) have normal hearing thresholds but exhibit abnormal wave 1 amplitudes in auditory brainstem responses (ABR) to high sound intensities (>90 dB SPL). Our study of the cochlear innervation in Trpa1-/- mice has found abnormalities in fibers presumed to be type II spiral ganglion neurons (SGNs). These unmyelinated afferent fibers innervate the outer hair cells, respond to cochlear tissue damage, activate neurons in the cochlear nucleus following moderate to high sound intensity (>80 dB SPL) (Weisz CJC et al., J Neurosci, 2021), and

may trigger the medial olivocochlear (MOC) efferent negative feedback (Froud KE et al., Nat Commun, 2015). This MOC reflex decreases cochlear amplification in noisy environments and might be a protective mechanism against noise-induced hearing loss. Here we explored in detail the cochlear innervation abnormalities in Trpa1-/- mice and whether the MOC reflex was affected.
Methods: Abnormalities in cochlear innervation in Trpa1-/- and wild type mice were assessed via fluorescent confocal microscopy of cochlear tissue immunolabeled against neurofilament heavy chain (NF-H), CtBP2/RIBEYE, parvalbumin, and peripherin. The MOC reflex was evaluated in wild type and Trpa1-/- mice by measuring amplitude changes of distortion product otoacoustic emissions (DPOAE) after the application of contralateral noise.
Results: Ribbon synapse counts in inner hair cells were indistinguishable between wild type and Trpa1-/- mice. In wild type mice, labeling against NF-H showed that long thin fibers (presumed to be type II SGNs) normally crossed the tunnel of Corti and turned towards the cochlear base. However, in Trpa1-/- mice, we found a ten-fold increase (~21%) of fibers turning towards the cochlear apex. We are currently performing labeling against markers of afferent innervation, such as parvalbumin and peripherin, to confirm the identity of those neurons. Our preliminary results show the presence of an MOC reflex in Trpa1-/- mice, but we are further testing whether there are defects in its magnitude.
Conclusions: Our results show that TRPA1 activity is also required for the proper innervation of the cochlea. To the best of our knowledge, this is the first study linking TRPA1 channel activity to proper neuronal maturation and/or innervation.
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SU122. Three-Dimensional Distribution of Type II Spiral Ganglion Cells in Gerbil
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Category: Inner Ear: Anatomy and Physiology
Background: Spiral ganglion cells (SGCs) have dendrites that contact hair cells and axons that form the auditory nerve – terminating in the cochlear nucleus. The mammalian spiral ganglion (SG) contains two populations of SGCs (Type I and Type II). Type I contact inner hair cells (IHCs) and Type II contact outer hair cells (OHCs). Type II SGCs have often been suggested to be preferentially located at the peripheral margins of the SG, or even absent in apical regions. At last year's meeting we presented our findings on the three-dimensional (3D) organization of gerbil Type I SGCs, where we describe an 'apical bulge' similar in nature to that found in humans. Here we continue this line of inquiry into the 3D organization of gerbil SG, using light sheet fluorescent microscopy (LSFM), to study the 3D distribution of Type II SGCs.
Methods: Paraformaldehyde fixed cochleae were prepared for LSFM after immunolabeling with primary antibodies to TuJ1 (for Type I SGCs), Peripherin (for Type II SGCs), in some cases paired with Myosin 7a (for hair cells) followed by secondary antibodies conjugated to AlexaFluor dyes. Cochleae were then dehydrated, cleared, and imaged using a LaVision UntraMicroscope II. Images were viewed and analyzed using Bitplane Imaris 9.7 software.
Results: TuJ1 and Peripherin labeled separate populations of SGCs along the entire basal-to-apical extent of Rosenthal's canal. Each antibody marked cell bodies, dendrites, and axons. TuJ1-labeled SGC dendrites formed a dense terminal plexus under the base of individual IHCs. Additionally, TuJ1 marked efferent fibers (seen as tunnel crossing fibers) destined for OHCs, their large terminals were identifiable under individual OHCs. Peripherin-labeled dendrites were difficult to follow beyond their initial course through the osseous spiral lamina. The distribution of Type II SGCs was not restricted to the peripheral margins of the SG, rather they were found at all depths of the ganglion and showed no preference for location relative to the scala vestibuli or tympani sides of the ganglion. Consistent with the literature, Type II cells in the gerbil accounted for approximately 5% of total SGC population.
Conclusions: Similarities in 3D organization of the gerbil and human SG suggest the gerbil to be an ideal model for studying many features of SGC organization. This is most evident at the apex, where both gerbil (Hutson et al, 2022) and human (Li et al, 2020), have an 'apical bulge' region. The comparatively sparse distribution of Type II SGCs may present an advantage for unraveling the geometric arrangements within this apical region of the ganglion. Furthermore, we found no evidence of a preferred spatial location for Type II cells within the SG. Although the primary functional role of Type II SGCs is not yet clear, they contribute to central projections originating from each cochlear turn.

SU123. Effect of Inner Ear Malformations on Cochlear Implant Outcomes

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Category: Inner Ear: Anatomy and Physiology

Background: Inner ear malformations (IEM) are a group of congenital deformities that can impact the cochlea, vestibular system, and surrounding structures. IEMs are implicated in approximately 20% of sensorineural hearing loss (SNHL) cases worldwide. Individuals with IEM can have significant hearing loss that may require non-surgical (hearing aids) or surgical (auditory implants) intervention. Cochlear implantation has been demonstrated to pose challenges in patients with IEM. Therefore, it is essential to review surgical and audiometric outcomes of patients with IEM undergoing cochlear implantation.
Methods: In this retrospective cohort study, data from patients with IEM were collected to assess surgical complication rate and post-operative outcomes. Information including demographics, pre- and post-operative speech recognition test results, radiographic imaging results, and surgical information were collected. In total, 26 patients were included in the study and divided into children (n=16), implanted between 0-8 years old, and adults (n=10), implanted between 19-83 years old. In addition, 26 controls matched on sex, age at implantation, implant laterality, and implant type were identified. 7 validated audiometric techniques (AzBio, PedAzBio, CNC, SRT, HINT, PTA, MLNT) were utilized to examine the difference in auditory and speech outcomes amongst the groups over a 3-5 year follow up period.
Results: In our cohort, the most common IEM was enlarged vestibular aqueduct (EVA) (n=19). Incomplete partition, type 2 (IP-2) and EVA showed higher complication rates than incomplete partition, type 1 (IP-1) and cochlear hypoplasia. Patients with IEM demonstrated slower improvement of hearing when comparing speech recognition test scores post-implantation to matched controls. In addition, patients with IEM who were implanted earlier in life showed better hearing outcomes as evidenced by PTA, CNC, and AzBio scores.
Conclusions: Cochlear implantation can provide auditory rehabilitation to patients with IEM. Our study highlights both surgical complications and audiometric outcomes in patients with IEM across a wide age range at implantation. Although complication rates are higher in patients with IEM compared to the general population, most complications in the study were able to be managed intraoperatively. The information derived from this study will help hearing healthcare providers to counsel patients with IEM on postoperative surgical and audiologic outcomes. Future prospective studies using large cohort with appropriately matched control group are warranted to determine the cochlear implantation outcomes in patients with IEM especially having co-morbid conditions such as autism spectrum disorder, CHARGE syndrome, Pendred syndrome, and Waardenburg syndrome.

SU124. Automatic Cochlear Implant to Facial Nerve Proximity Assessment Tool Using Transformer-Based Segmentation Networks

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Category: Inner Ear: Anatomy and Physiology

Background: Cross-stimulation or unrestrained stimulation to the facial nerve (FN) leads to undesired facial muscle movement, nerve and muscle damage. Recent studies performed on cochlear implant (CI) patients have suggested that unintended facial nerve stimulation (FNS) possibly occurs as a result of its closeness to some of the implanted electrodes. The detection and segmentation of the FN is a complex procedure owing to the absence of contrast in computed tomography (CT) scans making the neural structures look extremely similar to other types of tissue. The aim of this study was to design a framework for automatically

determining extra-cochlear electrodes and computing distances between implanted electrodes and the labyrinthine FN section in patients undergoing CI therapy.
Methods: An automatic segmentation and detection pipeline was developed using artificial intelligence techniques to extract appropriate FN, cochlea and implant parameters. A multi-center dataset of 70 Oticon Medical EVO patients was used for training our pipeline. Firstly, we evaluated UNet, UNETR and UTNETV2 architectures with multiple cost functions to segment cochlea and FN from preoperative images whereas their corresponding electrode placements in postoperative scans were determined using a UNet-based network. Secondly, the pre- and post-operative images were rigidly registered together based on mutual information and both outputs were aligned. Lastly, using FN Maurer distance maps and the registered electrode coordinates, the nerve-electrode distances were computed.
Results: Our experiments with three different neural network architectures and multiple cost functions demonstrated that a combination of Dice and focal losses applied on UNETR style architecture achieves the best performance. The obtained Dice coefficients for cochlea (92%) and the facial nerve (61%) on test dataset suggest that our transformer-based approach outperforms the current state of the art for cochlea segmentation. The average Hausdorff distance (3D) for cochlea and FN segmentation were 0.8 and 0.7 mm respectively. Furthermore, our analysis across the dataset yielded a 0.1 – 50.0 mm range for nerve-electrode distances with electrodes 15 and 16 often being the closest to the FN.
Conclusions: In our study, we compared multiple segmentation architectures and showed that transformer-based architectures offer further improvements in performance over the well-established UNet architectures. To the best of our knowledge, this is the first tool for automatic proximity analysis of CI electrodes and FN in CT scans. The ability to follow the FN without requiring human intervention along the cochlea is a significant benefit. Even though soft tissue is difficult to segment in CT images, the created pipeline accurately characterises both structures. Our pipeline opens a window to study the distance between FN and electrodes that can be used to prevent FNS by appropriate surgical planning i.e., selecting appropriate CI and stimulation patterns.

SU125. Epiphycan, a Small Leucine Rich Proteoglycan is Required for Normal Morphogenesis of the Tectorial Membrane

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Category: Inner Ear: Anatomy and Physiology
Background: The mammalian tectorial membrane (TM) is composed of radially-oriented bundles of collagen fibrils imbedded in matrix containing a number of non-collagenous proteins including TECTA, TECTB, CEACAM16, OTOG and OTOGL. Collagen fibrils in many tissues are closely associated with members of the small leucine-rich proteoglycan (SLRP) family, but SLRPs present in the TM remain unknown. A recent study, however, has shown transcripts for the type III SLRP EPYC (Epiphycan) are enriched in supporting cells lying medial and lateral to the organ of Corti at postnatal day 8 and 7 weeks of age (Hanada et al., 2017). Epyc KO mice have a high-frequency hearing loss, and it was suggested this may be due to a change in basilar membrane elasticity.
Methods: Tectorial membranes and underlying spiral limbal tissues were collected by dissection for proteomic analysis. Crispr-Cas9 technology was used to create a mouse with a targeted deletion of exon 3 of the Epyc gene. In situ hybridisation (ISH) and antibodies were used to study, respectively, the distribution of Epyc transcripts and EPYC protein in the developing and mature cochlea. The structure of the TM was studied using confocal and electron microscopy, and ABRs were used to assess cochlear function.
Results: Proteomics detected two SLRPs in the TM, EPYC and LUM (Lumican). EPYC was the 5th most abundant protein in the TM, but enrichment relative to the limbal tissue (1.27x) was very low compared with CEACAM16 (328x), TECTA (1974x) and TECTB (1744x). LUM was detected, but at levels 2-3 orders of magnitude lower than that of EPYC. ISH revealed Epyc mRNA is expressed in the greater and lesser epithelial ridges at P2-4, regions of the developing cochlear duct producing the TM. Antibodies revealed EPYC is diffusively distributed throughout the TM, and transiently associated with the apical surface of the greater epithelial ridge in a region from which the lateral lip of the spiral limbus develops. Confocal microscopy reveals bundles of collagen fibrils are less clearly resolved in the TMs of Epyc-/- mice stained with the collagen-binding protein CNA35 relative to those in Epyc+/- mice, but ultrastructural analysis

indicates that fibril diameter (20 nm) and inter-fibril spacing (40-60 nm) within the collagen fibril bundles are unchanged. The normal, cross-sectional profile of the TM is, however, altered in Epyc-/- mice and there is evidence for a partial detachment of the TM from the lateral lip of the spiral limbus.
Conclusions: EPYC is the major SLRP of the TM and the cross-sectional profile of the TM is abnormal in Epyc-/- mice. Alterations in the structure and properties of the TM may contribute to the high-frequency hearing loss observed in Epyc-/- mice.
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SU126. Upper-Harmonic Deficits in Temporal Envelope Coding of Tone Complexes and Amplitude Modulations Differentiate Inner Hair Cell Damage From Synaptopathy

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Category: Inner Ear: Anatomy and Physiology
Background: Sensorineural hearing loss (SNHL) is an umbrella diagnosis encapsulating several profiles of hearing impairment with differential inner hair cell (IHC), outer hair cell (OHC), and cochlear synapse damage. Otoacoustic emissions have paved the way for diagnosing OHC dysfunction. However, studies of synaptopathy and subsequent diagnostic approaches may not be free from confounds of IHC damage – as the two pathologies could have similar envelope and pitch-coding deficits. Carboplatin-induced (CA) hearing loss and noise-induced temporary threshold shifts (TTS) in chinchillas can be employed to differentiate the consequences of IHC damage and synaptopathy. We identified differential consequences of IHC damage and cochlear synaptopathy present in envelope-following responses (EFRs) to modulated stimuli and place-time coding of complex tones.
Methods: Baseline ABR thresholds, dpOAEs, wideband Middle-Ear Muscle Reflex (MEMR) thresholds were collected from eight chinchillas (4 female) to assess hearing status. EFRs to sinusoidal and rectangularly amplitude modulated stimuli (Fc = 4kHz, Fm = 100 Hz) were collected. Chinchillas were assigned to either IHC damage or synaptopathy cohorts induced by CA (38 mg/kg) and TTS exposure (1-kHz center frequency, 100 dB SPL, 2hrs), respectively. Tone-complex (F0 = 103 Hz, 6 harmonics, alternating-phase) EFRs were collected on a subset of six (3 CA, 3 TTS) chinchillas to investigate the effect of temporal coding deficits on place-time pitch and compared to EFRs in three unexposed chinchillas.
Results: ABR thresholds and dpOAEs appeared unaffected by either exposure. MEMR strength post-exposure was reduced in the TTS group only. A stark reduction in the upper harmonics (3rd to 16th) in the phase-locking value (PLV) spectra of the EFRs was observed in the CA group for all modulation and pitch stimuli. This finding was quantified using R_plv: the sum of these upper harmonics, normalized by the sum of the first two. In contrast, R_plv was relatively unaltered after TTS exposure, with the exception of one stimulus condition (rectangular-envelope with 25% duty cycle, suggested as a useful stimulus for isolating synaptopathy; Vasilkov 2021). A place-coding correlate of pitch was calculated using strength of the doubling in EFR periodicity as a function of harmonic rank reflecting the transition of resolved to unresolved harmonics in alternating-phase tone complexes. This place transition in the CA and TTS conditions seemed relatively unaffected.
Conclusions: Our findings imply that IHC damage and cochlear synaptopathy result in differential modulation and pitch-related temporal coding deficits, present in the upper harmonics of the EFR. A well-accepted physiologically based model involving IHC transduction will be adapted to predict possible cellular impairments that may explain these deficits. The observed place-time pitch implications will inform our pending cross-species study of pitch in SNHL. Studies investigating electrophysiological diagnostics of SNHL and synaptopathy must appropriately consider the confounding effects of not only outer-hair-cell dysfunction, but also inner-hair-cell dysfunction.

SU127. Modeling Mechanism of Entry of SARS-CoV-2 Into the Inner Ear Microvasculature

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Category: Inner Ear: Anatomy and Physiology
Background: Sensorineural hearing loss (SNHL), tinnitus, and vertigo are neurotological manifestations of SARS-CoV-2 that are increasingly being reported. The etiopathogenesis of these audio-vestibular disorders in the context of SARS-CoV-2 infection remains unknown. SARS-CoV-2 has been postulated to be a neurotropic and vasculotropic virus. The blood-labyrinthine barrier (BLB) is analogous to the blood-brain

barrier and serves to insulate the perilymph from the contents of blood vessels and strictly control the ionic and chemical environment of the inner ear. We provided the first studies that evaluate the integrity and permeability of the human BLB. Preliminary studies in our laboratory on the mechanisms of entry of SARS-CoV-2 in the human inner ear suggest that ACE2 is expressed in the vascular endothelial cells. In the present study we investigate the presence of caveolin and clathrin mediated endocytic pathway that SARS-CoV-2 may use following spike protein interaction with ACE2 receptor using antibodies against anti-caveolin 1, 2 and 3 and clathrin in the human macula utricle.
Methods: For this purpose, we use vestibular endorgans acquired at surgery from patients diagnosed with Meniere's disease (MD) obtained from labyrinthectomy (n=5, 3 male and 2 female, ages 50 to 70). For controls, we utilize vestibular endorgans dissected from human temporal bones with normal hearing and balance, corresponding to the age, gender, and race of that MD specimens. We used primary antibodies against caveolin-1, caveolin-2, and caveolin-3, glucose transporter-1 marker for vascular endothelial cells -VEC-, and alpha-smooth muscle actin (marker for pericytes), clathrin (clathrin mediated endocytosis and GFAP (to identify supporting cells), immunofluorescence (IF) secondary antibodies and laser confocal microscopy to identify these proteins in formalin fixed 20-micron thick cryostat sections of the macula utricle.
Results: Caveolin-1 and -2 punctate IF was localized in the VECs of the microvasculature located in the stromal epithelium, caveolin-3 was found in supporting cells of the vestibular sensory epithelium. Interestingly clathrin was localized in vestibular hair cells and transitional epithelial cells and to lesser extent in VECs. The distribution of these proteins was similar in MD and normal macula utricle.
Conclusions: These results suggest that the machinery of entry for SARS-Co-2 is present in the microvasculature of the human vestibular sensory periphery. Identifying the distribution of ACE-2 receptors in the BLB, modeling the entry of SARS-CoV-2 into vascular endothelial cells, and evaluating potential mechanisms of BLB disruption is crucial to understanding the neurotologic profile of SARS-CoV-2, identifying therapeutic targets for BLB decompensation, and developing effective therapies for BLB vascular normalization.

SU128. Congenital Murine CMV Promotes Cochlear Inflammation and Focal Loss of Sensory Cells

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Category: Inner Ear: Anatomy and Physiology
Background: Congenital cytomegalovirus (CMV) is the most common cause of progressive hearing loss in childhood, and current treatments provide limited benefit for preservation or restoration of hearing. This mouse model of congenital CMV takes advantage of the atricial nature of hearing in mice in which infection at P0 results in infection at a time when auditory development is comparable to the second trimester in human development, when congenital CMV exerts deleterious effects.
Methods: Mice were injected IP within 12 hours of birth with mCMV, and the inner ears were harvested at P7, P14 and P24. Mice received ABR testing at P24. Cochleas were processed and tested using immunohistochemistry to identify cochlear inflammatory cells and sensory cells.
Results: Large numbers of inflammatory cells were observed in the inner ear after CMV infection peaking at P14. Macrophages were located in patches along the cochlear sensory epithelium. These macrophages extended processes around hair cells. Focal areas of missing hair cells were observed where macrophages were active. Phagocytosis was observed in areas where there was no evidence of CMV infection by antibody staining or by fluorescently tagged virus.
Conclusions: We postulate that the immune response to virus contributes to the pathology that leads to hearing loss in congenital CMV infection.

SU129. Contralateral Noise Enhances the Cochlear Microphonic for Frequency-Swept Tones: Evidence for the Medial Olivocochlear Reflex

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Category: Inner Ear: Anatomy and Physiology
Background: Hearing in background noise is a significant challenge for individuals with hearing loss (HL). The medial olivocochlear reflex (MOCR) is hypothesized to improve listening in noisy backgrounds by increasing the neural signal-to-noise ratio. This improvement may be diminished with age and HL and

contribute to age- and HL-related difficulties in understanding speech in noisy backgrounds. Assessment of the MOCR may reveal the extent to which a patient's speech-in-noise difficulties are explained by MOCR dysfunction. Despite this potential, established assessments of the MOCR, based on otoacoustic emissions, require individuals to have no more than a mild HL. This study assesses MOCR function using an evoked response – the cochlear microphonic (CM) – that is measurable in individuals with HL. The CM is an indirect measure of cochlear function that is sensitive to the effects of the MOCR. The amplitude of the CM increases when the MOCR is elicited by contralateral sound as a result of greater outer hair cell (OHC) conductance. This study determined which probe frequencies resulted in the largest increase in CM amplitude in young adults with normal hearing when the MOCR was elicited by contralateral noise.
Methods: The CM was measured from an active electrode placed on the tympanic membrane and a reference electrode on the ipsilateral earlobe. Broadband noise (BBN, 50 dB SPL) was presented to the contralateral ear, while an ipsilateral, 2.25 sec. tone (probe, 90 dB SPL) swept upward or downward in frequency from 100 to 6000 Hz. Experimental conditions with and without contralateral BBN were interleaved. The probe was presented in alternating polarity for 1000 sweeps (500 with BBN, 500 without BBN). Participants completed a sweep in each direction in a randomized order. A vibration motor on the leg kept the participants alert. This motor activated every 90 seconds and turned off when the participant pressed a button. Least-squares fits were performed to quantify the CM amplitude envelope for conditions with and without contralateral BBN. The fits were compared to determine the effect of contralateral BBN on CM amplitude as a function of probe frequency.
Results: CM amplitude increased for most participants in the presence of contralateral BBN, consistent with an increase in OHC conductance from eliciting the MOCR. This enhancement was greatest for frequencies between 500 and 1500 Hz, suggesting that future assessments of MOCR function based on the CM should focus on this frequency region. For some participants, an increase in CM amplitude of > 3 dB relative to baseline was observed.
Conclusions: The next step is to extend this design to include older adults with normal hearing and HL to test the hypothesis that a CM-based assessment is sensitive to the putative declines in MOCR function that result from age and HL.

SU130. Active and Passive Kinematic Gains for the Organ of Corti Mechanotransduction

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Category: Inner Ear: Anatomy and Physiology
Background: The organ of Corti (OoC) vibrates due to fluid pressures and outer hair cell (OHC) somatic motility. OoC vibrations result in hair bundle deflections, which modulate the mechanotransduction current of the hair cells. We quantified a key parameter of OoC mechanotransduction—the relationship of basilar membrane displacement or OHC length change versus stereocilia deflection.
Methods: The cochleas were acutely excised from young Mongolian gerbils (15-30 days old, both sexes). Isolated cochleas were reduced by removing the apical and the basal turns leaving the middle turn of which BF ranges 1-4 kHz. The reduced cochlear turns were placed in a custom designed microfluidic chamber filled with perilymph-like solutions. The OoC in the chamber was stimulated either mechanically or electrically. Mechanical stimulation corresponded to inducing pressure differences with little active feedback (external agitation), and electrical stimulation corresponded to transepithelial voltage change to induce OHC somatic motility (internal agitation). Resulting OoC vibrations were measured using optical coherence tomography, of which image resolution was fine enough to distinguish sub-tectorial gap and individual hair cells. The tissue vibrations were measured at two orientation angles, approximately 40 degrees apart. From reconstructed two-dimensional vibrations, hair bundle deflection (ΔHB), OHC length change (ΔOHC), and basilar membrane displacement (ΔBM) were obtained.
Results: OoC vibrated differently under external and internal agitations. When mechanically stimulated, all sub-structures of the OoC vibrated in phase with maximum amplitude of motion being in transverse direction near the arcuate-pectinate junction of the basilar membrane. Radially, the largest motion took place at the reticular lamina. When electrically stimulated, there are large variations in phase, both transversely and radially. Maximum amplitude of motion took place at the RL and neighboring Hensen's cells. Passive kinematic gain represented the ratio between stereocilia deflection and basilar membrane transverse vibration, or gpsv = ΔHB/ΔBM. Active kinematic gain represents stereocilia deflection per outer hair cell length change, or gact = ΔHB/ΔOHC. The two kinematic gains and their spectra within an octave of best

frequency were measured. gpsv was greater than gact (0.8 versus 0.4). Albeit not significant statistically, the innermost OHC had smaller gain value than that of the outermost OHC. The elongation of the OHC resulted in hair bundle deflection toward its taller edge (gact > 0).
Conclusions: We have separated OoC vibrations into two components (modes)—active and passive and measured hair bundle deflection due to either OHC deformation or basilar membrane displacement. Depending on stimulating frequency, the kinematic gains will add up or cancel. The spectra of kinematic gains will provide quantitative information of how OHC motility modulates hair cell mechanotransduction.
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SU131. High Resolution Measurements and Visualization of Relative Motion of the in situ Mammalian Cochlear Apex

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Category: Inner Ear: Cochlear Mechanics
Background: There has been considerable progress in understanding the response properties of isolated hair cells, including the ability of outer hair cells to generate motions that underlie cochlear amplification. However, there has been slower progress (and considerable debate) in characterizing how accessory structures interact to shape cochlear responses to acoustic stimulation -- in part because of the inherent difficulty of observing structures with low reflectivity (such the tectorial membrane), and cellular structures within the cochlear partition in an intact state with high resolution.
Methods: Here we characterize cochlear responses of low frequency (~300-500 Hz) apical regions of excised in situ gerbil cochleae, with mechanical piston-like stapes stimulation delivered via a piezoelectric driver. High resolution motion measurements with a subnanometer aggregate motion noise-floor, were obtained through a submillimeter optical access hole using a custom Doppler optical coherence microscopy (DOCM) system (40x water immersion objective, 0.8 numerical aperture). This DOCM system uses heterodyne interferometry at 500 kHz (100 kHz motion bandwidth) to resolve, with directional discrimination, subnanometer axial motions of accessory and cellular cochlear structures throughout the cochlear partition. Results are visualized with motion magnified high resolution video.
Results: Results include characterizations of spatial modes of absolute and relative motion of all visible structures in radial cross-sections of the organ of Corti, including the basilar membrane arcuate and pectinate zones, pillar cells, rotation of the tunnel of Corti, inner and outer hair cells, tectorial membrane, and Reissner's membrane. Although structures throughout the radial cross-section are roughly in phase, relative motions between the structures show significant differences that are important to understanding the signal processing paths.
Conclusions: Motion measurements of all visible structures allow cycle-by-cycle characterization of absolute and relative motions of key features in physiologically important frames of reference, such as tectorial membrane motion relative to basilar membrane motion and hair cell motion relative to tectorial membrane motion.

SU132. Basilar Membrane and Organ of Corti Vibration in Guinea Pigs Measured With OCT During Bone Conduction Stimulation

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Category: Inner Ear: Cochlear Mechanics
Background: The vibration pattern of the structures inside the living ear when stimulation is by bone conduction (BC) has not been presented in detail. The differences and similarities of the vibrations between air conduction (AC) and BC stimulation is therefore unknown. This is here investigated in sedated guinea pigs.
Methods: The inner ear vibrations are measured with an optical coherence tomography system (OCT). The bulla of the guinea pig is opened enabling optical access to the inner ear through the round window. First, hearing thresholds at frequencies between 2 and 12 kHz are obtained in the guinea pig with AC and BC stimulation using measurements of the compound action potential. This facilitates comparison of AC and BC stimulation at the same hearing level. The vibration measurements with the OCT are conducted at 30 and 40 dB HL for both AC and BC stimulation. The measurements are done both across and along the basilar membrane.

Results: With AC stimulation, the maximum vibration is seen at the organ of Corti with only small vibration levels visible at the spiral lamina. With BC stimulation, the entire cochlea vibrates but the maximum vibration is found at the organ of Corti. When the BC vibration is analyzed in relation to bony vibrations at the cochlear boundary, the vibration at the organ of Corti is enhanced. However, the vibration of the spiral lamina is significantly greater than with AC stimulation, especially at lower frequencies. At higher frequencies, the vibration pattern of the inner ear structures become more complex with BC stimulation.
Conclusions: The OCT system is well suited for inner ear vibration measurement with both AC and BC stimulation. The threshold estimation enabled AC-BC comparisons at the same stimulation levels inside the cochlea. Overall, AC and BC stimulation seem to generate the same relative vibration at the organ of Corti when stimulated at the same hearing level, but BC stimulation tend to result in greater vibration levels of the spiral lamina and spiral ligament compared to AC stimulation.

SU133. Radial Motion Within the Organ of Corti of the Gerbil Mid-Frequency Region Using High-Resolution Optical Coherence Tomography (OCT) Vibrometry

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Category: Inner Ear: Cochlear Mechanics
Background: Low- and mid-frequency hearing below 5 kHz is important for human speech and music, yet most knowledge about cochlear micro-mechanics has been primarily from the more-accessible, basal, high-frequency region measured through the round-window membrane (e.g., He et al., 2018; Cooper et al., 2018; Fallah et al., 2019). There are few motion measurements from the low and mid best frequency (BF) regions. Using a radial approach to the gerbil ~3 kHz best-frequency (BF) region, we report radial displacement measurements from (1) the gerbil tectorial membrane (TM), (2) the outer-hair-cell (OHC) tops at the reticular lamina (RL) and (3) the OHC bottoms at the OHC-Deiter-cell junction (OHC-DCJ). Using our high-resolution OCT system, we separately measured motions from the tops and bottoms of each of the three OHC rows, e.g. at RL1, RL2, and RL3.
Methods: Cross-sectional imaging and vibrometry measurements were made using a Spectral-Domain Optical Coherence Tomography (OCT) system (GAN620C1, Thorlabs, Germany; Cho and Puria, 2022). The stimuli were sequences of pure tones (0.2–4.5 kHz) at ear-canal sound levels from 30 to 86 dB SPL. The noise floor varied among structures but was generally ~0.8 nm below 0.5 kHz and <0.5 nm 0.5 to 4.5 kHz. Measurements were rejected at frequencies where the displacement magnitude was within 6 dB of the noise floor. Gain was calculated as the ratio of displacement to ear-canal sound pressure.
Results: The TM, RL and OHC-DCJ all showed compression in their gain magnitudes from the lowest frequency to BF, and phases that decreased by up to 4 cycles, indicating a traveling wave. However, the TM showed less compression than RL and OHC-DCJ. In the BF region (1.3–3 kHz) gain was higher than in the sub-BF (0.2-1.3 kHz) region for levels less than about 60 dB SPL. Postmortem, the gain decreased by up to 30 dB near the BF, for all three locations. The pattern of RL3 gain relative to RL1 gain was not consistent. Some animals had RL3 radial motion that was greater than RL1 radial motion in the sub-BF region with smaller differences near BF. Other animals showed different patterns.
Conclusions: Our measurements of TM and OHC-DCJ motions are generally comparable to those reported by Meenderink et al (2022). Because of our improved spatial resolution, we could measure radial motion near the tops and bottoms of individual OHCs. Our present results differ from our previous results from the gerbil high-frequency basal region where differences between RL3 and RL1 transverse motions were observed near BF but not sub-BF (Cho and Puria, 2022, biorXiv). [Work supported in part by grant R01DC07910 from the NIDCD of NIH to SP.]

SU134. Magnitude and Phase Patterns of the Cochlear Partition Vibration on the Cross Section of the Basal Turn in Gerbil Cochleae

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Category: Inner Ear: Cochlear Mechanics
Background: Recent low-coherence heterodyne interferometry and optical coherence tomography revealed differences between the reticular lamina and basilar membrane vibration in the living cochleae.

Measurements in the basal cochlear turns in gerbils showed that the reticular lamina vibration is more robust and delayed than the basilar membrane vibration. The phase relationship between the reticular lamina and basilar membrane vibration changes with frequency. However, these results provide limited information on complex motions of different structures within the organ of Corti. To extend these findings, we measured the vibration patterns and the structural images on the cross section of the cochlear partition in the basal turn of living gerbil cochleae.
Methods: Young Mongolian gerbils of either sex with normal hearing were used in this experiment. The bulla on the left side was opened through a ventrolateral surgical approach, and the stapedial artery was partially removed from the bony surface of the cochlea. A small opening was made in the lateral wall of the scala tympani of the basal turn, and the cochlear partition was positioned approximately in the horizontal plane. The magnitude and phase patterns of tone-induced vibrations were measured from the cross section of the cochlear partition using a scanning heterodyne low-coherence interferometer. The structural images were constructed based on the carrier signal and confirmed by optical coherence tomography.
Results: The magnitude patterns show that the low-level tone-induced vibration in the reticular lamina-outer hair cell region is significantly larger than the basilar membrane vibration. The vibration spreads from the peak region to other structures as the sound level increases. The phase patterns reveal in-phase vibrations between the reticular lamina and basilar membrane at the best frequency and anti-phase vibrations at low frequencies. The data also show relative motions among the different structures within the cochlear partition, such as the tunnel of Corti and the outer tunnel.
Conclusions: The present results suggest sound-induced fluid movements within the tunnel of Corti, outer tunnel, and other extracellular fluid spaces within the organ of Corti in the longitudinal direction, which may play an essential role in cochlear amplification.

SU135. Characterizing Nonlinear Wave Propagation in the Cochlea

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Category: Inner Ear: Cochlear Mechanics
Background: Much remains unknown about the active, nonlinear physics regulating the mechanical responses of the mammalian cochlea. Theoretical efforts to deduce cochlear physics—whether directly using ”inverse” methods or indirectly via anatomically constrained mechanical models—universally rely on linear models that aim to explain the data in linear regimes (e.g., at low sound pressure levels or post-mortem). Although physics-based models are often made nonlinear (by inclusion of strategic nonlinear terms) in an attempt to reproduce the level and stimulus dependencies seen in the experiments, nonlinearity is arguably underutilized to deduce cochlear function from the experimental data.
Methods: In this work, we derive an idealized physics-based model of cochlear active nonlinear signal processing from the experimental data. We focus on characterizing how nonlinear cochlear amplification modifies the complex wavelength of the basilar-membrane (BM) traveling wave. This is accomplished by comparing BM responses to exponential sweeps measured under a variety of experimental conditions.
Results: Comparison of BM responses measured at moderate sound levels post-mortem and in vivo reveals that the effects of cochlear amplification are well captured by a simple mathematical framework. Comparison of BM responses to probe sweeps measured both in the presence and absence of a suppressor sweep allows one to separate the “cause” (excitation at the suppressor frequency) and the “effect” (reduced amplification at the probe frequency) of cochlear nonlinearity and thereby deduce a set of approximate relations that characterize cochlear nonlinear signal processing.
Conclusions: Comparison of BM responses obtained under different experimental conditions allows to deduce useful constraints for physics-based nonlinear models of the mammalian cochlea.

SU136. Responses in the Hook Region of the Gerbil Cochlea Before and After Disruption of the Endocochlear Potential With IV Furomsemide

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Category: Inner Ear: Cochlear Mechanics
Background: Optical coherence tomography (OCT) vibrometry of the cochlea has revealed a region, extending from the outer hair cells (OHCs)/Deiters’ cell junction to close to the reticular lamina (RL), which moves with higher amplitudes than the basilar membrane (BM), exhibits a broad-band sub-best frequency

(BF) nonlinearity that is more pronounced when the stimuli are wide-band, and shows hypercompression at high sound levels.
Experiments at the 25 kHz location observed that the amplitude and tuning were reduced following disruption of the endocochlear potential (EP) by furosemide, while the broad-band nonlinearity persisted. Recovery of tuning occurred over hours. Due to the viewing angle required to access the 25 kHz location, the measured motion includes significant longitudinal components and place effects. Less is known about mechanical responses at the most basal locations in the cochlea. Here, we have extended our experiments, including furosemide, to the hook region of the gerbil cochlea, with BFs 40 to 50 kHz.
Methods: Organ of Corti (OoC) vibrations were measured in the hook region through the round window with a Thorlabs Telesto III OCT. At this location, it is possible to align the OCT beam nearly parallel to the OoC's transverse axis, minimizing the contribution from longitudinal motion. Uniaxial measurements were taken near the intersection of the arcuate and pectinate zones, including the RL, BM and OHC-region. Areal vibration maps were constructed from sequential uniaxial measurements taken in 10 micron steps. Furosemide was administered intravenously following baseline measurements. Distortion product otoacoustic emissions (DPOAEs) were recorded in response to swept two-tone stimuli at two levels and served as a surrogate for general cochlear condition and to assess the efficacy of drug delivery.
Results: DPOAEs and baseline vibrometry measurements showed that, following opening the bulla, the hook locations exhibited similar, but reduced, sensitivity compared to the more apical ~25 kHz location. At both locations, baseline vibrations showed: (1) Tuning and nonlinearity close to the BF at the BM and RL, and (2) higher amplitudes and a wide-band nonlinearity in the OHC-region. In contrast to the 25 kHz place, hook region responses showed: (1) BF peak frequencies that were independent of sound pressure level, (2) troughs before the BF-region in the response at the highest levels, instead of hypercompression, and (3) more abrupt changes in phase delay around the BF. (4) Post-furosemide, OoC structures moved in synchrony; the responses were largely linearized and failed to recover.
Conclusions: The 40-50 kHz hook location in the gerbil cochlea shows both similarities and differences compared to the 25 kHz place. Following furosemide, responses were more dramatically reduced in the hook location and did not recover.

SU137. Estimating Corti Fluid Motion From Organ of Corti Vibrations

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Category: Inner Ear: Cochlear Mechanics
Background: The organ of Corti (OoC) consists of hair cells and their supporting cells. Besides these cells, the OoC includes substantial extracellular fluid spaces such as the tunnel of Corti, the Nuel's space and the outer tunnel. These extracellular fluid spaces, collectively called the Corti fluid, form a fluid-filled tube along the cochlear length. Outer hair cells contract or elongate due to changes in their transmembrane potential. Resulting deformation of the OoC could drive the Corti fluid longitudinally. We have measured the areal displacement of the Corti fluid within an OoC section.
Methods: From young Mongolian gerbils (15-30 days old, both sexes), cochleas were isolated. After removing the apical and the basal turns, the middle turn (BF 1-4 kHz) of the cochlea was placed in a custom-fabricated chamber. The OoC in the chamber was agitated either mechanically or electrically, which represents the insensitive (passive) or sensitive (active) cochleas, respectively. Resulting vibrations were recorded by optical coherence tomography, of which image resolution was fine enough to distinguish individual cells and fluid spaces in the OoC. The vibrations measured at two orientation angles were used to reconstruct two-dimensional vibrations. Using a continuum mechanics approach, we obtained local areal strains of an OoC section from its displacement field.
Results: When mechanically stimulated, the OoC vibrated like a block sitting on the basilar membrane. The OoC sub-structures vibrated in phase. In contrast, when electrically stimulated, the OoC structures vibrated out of phase. The phase gradient was greater in the radial direction as compared to the transverse direction. That is, outer hair cell length change was transformed into lateral deformation of the OoC. While the areal strain due to mechanical stimulation was spread over the entire OoC section, the areal strain due electrical stimulation was greatest over the outer hair cells. For quantification, the areal strain was normalized by the peak basilar membrane displacement for the passive case, by outer hair cell length change for the active case. The values were 0.01 um^-1, 0.2 um^-1 for the passive and active cases, respectively (three cochleas

each). The fluid motion in the active OoC vibrations was decomposed into two components—in-plane (radial) motion and out-of-plane (longitudinal) motion.
Conclusions: Outer hair cell electromotility induces out of phase motion across the outer hair cell region, resulting in cross-sectional area change. Considering the incompressibility of fluid, the cross-sectional area change must result in fluid motion within the radial section and out of the radial section. Our observations suggest that outer hair cell electromotility can create longitudinal fluid motion, reminiscent of peristaltic action.
This study was supported by NIH NIDCD R01 DC014685.

SU138. Modeling the Effect of Changes in Endocochlear Potential and Hair Bundle Resting Probability on the Micromechanical and Electrical Responses of the Cochlea

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Category: Inner Ear: Cochlear Mechanics
Background: In the cochlea, the nonlinear mechanoelectrical transduction (MET) channels provide a current pathway between the scala media (SM), which has a positive resting voltage (the endocochlear potential – EP) and the inside of the outer hair cells (OHCs). The resulting potential difference between the OHCs and the scala tympani (ST) drives OHC force generation, with nonlinearity manifesting in micromechanical responses. Experiments have shown that nonlinearity extends sub-Best Frequency (sub-BF) in the ST voltage and reticular lamina (RL), indicating that OHC feedback is not limited to the narrower region of BM nonlinearity. Further experiments have demonstrated the key role of the EP in BF nonlinearity of the BM response and sub-BF nonlinearity of the OHC region response and suggest that a changing resting probability of the MET channels (P_0^s) can compensate for a reduction in the EP to partially restore near-BF nonlinearity.
Methods: Our computational model of the gerbil cochlea consists of a 3D fluid domain of the cochlear ducts that are coupled to the basilar membrane (BM), which is coupled to other organ of Corti (OoC) structures. The OoC mechanical model is coupled to an electrical model which includes OHC intracellular potential and potentials in the scala vestibuli (SV), SM, and ST, which are coupled with electrical longitudinal cables. The MET channels and the OHC basolateral membrane are represented with an associated resistance and capacitance for each. While the electrical parameters of the previous model were calibrated based on ST electrical measurements only, the present model is also calibrated based on SM electrical measurements. The calibrated model is used to study the effect of varying the EP and P_0^s on the nonlinear micromechanical and electrical responses to a pure tone.
Results: We first assess the model's predictive ability by comparing experimental measurements and model predictions in the micromechanics (BM and RL displacement) and electrical responses (SM and ST voltage). This provides a good starting point to predict the effect of the EP and P_0^s. The link between EP and P_0^s and the nonlinearity of the BM near-BF and to the RL near-BF and sub-BF will be compared to available measurements (Strimbu et al., Biophys. J, vol. 119, pp. 2087-2101, 2020.). Model outputs that are inaccessible to experimental studies, such as OHC force and BM resistance to assess power delivery to the BM and RL, will be used to further gain some insight into how changes in endocochlear potential affect OHC electrical outputs and cochlear micromechanics.
Conclusions: This work is an important step in understanding key questions regarding the role of the various potentials in the cochlea, and how their tuning relates to mechanical tuning via intermediation of the OHCs. Overall, we aim to increase insight on nonlinear electromechanical interplay in the cochlea.

SU139. Effects of Whirlin Haploinsufficiency on Progressive Sensorineural Hearing Loss

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Category: Inner Ear: Damage and Protection
Background: Whirlin is a protein localized on stereocilia in hair cells and involved in cilia organization and lengthening. Mutations in gene of whrlin, Whrn, causes Usher syndrome type 2 (USH2) which is characterized by hearing loss and retinitis pigmentosa. Mutations of USH2 genes have been known as autosomal recessive, although some of homologous genes showed haploinsufficiency effect in previous studies. Previous evidence suggests the haploinsufficiency effect may be detected slowly, such in age-

related hearing impairment. Given the hypothesis we studied the haploinsufficiency effect of Whrn in mouse model.
Methods: Using Whrn heterozygous mouse line, hearing ability was examined by measuring auditory brainstem responses (ABRs) and Distortion Product Otoacoustic Emissions (DPOAE). In addition, Immunohistochemistry methods were used to examine hair cell loss and reduction of synaptic ribbon number changes.
Results: The results revealed that Whrn haploinsufficiency mice showed earlier onset of hearing loss than age matched control by showing higher ABR amplitude, and DPOAE levels. Consistent with hearing ability results, the Whrn+/- showed hair cell loss and reduction of synaptic ribbon number while those of control remained intact.
Conclusions: Collectively, Whrn showed haploinsufficiency effect on hearing impairment, and the upstream of the effect was related to hair cell loss and disturbance of communication between hair cell and afferent neurons. This results suggest that Whrn carriers should be treated more carefully, since the carriers are possibly exposed to higher risk of hearing impairment.

SU140. Estrogen Receptor 2 Agonists Ameliorate Noise-Induced Hidden Hearing Loss in Gonadally Intact Female Mice

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Category: Inner Ear: Damage and Protection
Background: Noise Induced Hidden Hearing Loss (NIHHL) or cochlear synaptopathy is characterized by a temporary threshold shift (TTS) with concomitant damage to, or loss of, synaptic connections between inner hair cells and afferent spiral ganglion neurons. Currently there is no FDA approved treatment for NIHHL. Our laboratory previously published data demonstrating that 17β-estradiol (E2) protects female mice against NIHHL. Our laboratory established that E2-replacement in ovariectomized female mice ameliorated the TTS and cochlear synaptopathy after a noise exposure that induced NIHHL. Additional reports suggest that the protective effects of E2 may be mediated through estrogen receptor 2 (ESR2). The goal of this study is to determine if augmentation of ESR2-mediated signaling via treatment with DPN (diarylpropionitrile, an ESR2-specific agonist) or E2 can protect against NIHHL in gonadally intact female mice.
Methods: B6CBAF1/J mice were obtained at 7 weeks of age. At 8 weeks of age, 21-day, slow-release pellets containing placebo, E2, or DPN were subcutaneously implanted in the mice. At 9 weeks of age, baseline auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) thresholds were established. At 10 weeks of age, mice were noise exposed (97 dB SPL, 8-16 kHz, 2 h). ABR and DPOAE thresholds were quantified 24 hours, 1 week, and 6 weeks post-noise exposure. At each of these timepoints, cochlear tissue was collected for histological analysis of outer hair cell (OHC) loss and cochlear synaptopathy.
Results: Treatment with E2 or DPN reduced threshold shifts 1-week post-exposure. Furthermore, treatment with E2 or DPN prevented a reduction in ABR wave-1 amplitude 1-week post exposure. In contrast, placebo-treated mice displayed reduced ABR wave-1 amplitudes at all frequencies examined. Treatment with E2 or DPN also reduced DPOAE threshold shifts 1-week and 6-weeks post-exposure. Histological analysis of cochlear tissue revealed loss of paired synapses at 24 and 32 kHz, however, we did not observe any differences in the synapse reduction between treatments.
Conclusions: Our data demonstrate that augmentation of ESR2-mediated signaling protects against NIHHL in gonadally intact female mice. These data are translationally significant as new ESR2-specific agonists, which do not induce feminizing effects, are now available. These drugs could be considered for pre-clinical studies for hearing preservation or otoprotection in females.

SU141. Small-Molecule Kv7.4 Activator ACOU085 Protects from Cisplatin-Induced Hearing Loss and Outer Hair Cell Death in a Guinea Pig Model

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Category: Inner Ear: Damage and Protection
Background: Cisplatin is a widely used chemotherapeutic agent in cancer treatment causing ototoxic hearing loss in up to more than 70% of cases (Cheung et al., 2022). A recent metanalysis estimated a global burden of ototoxic, platinum-based chemotherapy-induced hearing loss of almost half a million annual cases (Dillard et al., 2022), making prevention of cisplatin-related ototoxicity an important medical need. Kv7.4 is a voltage-gated potassium channel expressed in outer hair cells (OHCs) mediating potassium efflux and maintaining OHC resting potential. Reduced Kv7.4 surface expression or activity has been associated with age- and noise-related forms of acquired hearing loss. The novel small-molecule Kv7.4 activator ACOU085 (Bös, 2018) recently demonstrated protection against age-related hearing loss in the senescence-accelerated mouse prone 8 (SAMP8) mouse model (Pinheiro et al., 2022). Here we demonstrate functional and morphological otoprotection of ACOU085 in a guinea pig model of cisplatin-induced hearing loss.
Methods: Guinea pigs received local applications of cisplatin (1 mg/mL) into the middle ear for 15 min. Cisplatin was removed and ears received either no treatment or ACOU085 locally perfused using an osmotic minipump (Alzet 2001; 1 µL/h) for 7 days onto the round window membrane at five different dose levels (0.1/1/3/10/100 µM) with a vehicle control (0.1 M PBS w. 10% PEG 400). Following baseline recording at day 0, auditory function was assessed by toneburst-evoked compound action potential (CAP) responses at day 7 and 14 after cisplatin. Cochleae extracted at day 14 were analyzed in cytocochleograms using immunolabelled whole-mount preparations of the organ of Corti.
Results: Local cisplatin administration induced a moderate functional hearing loss with 20–50 dB threshold shifts and corresponding OHC loss. Functional protection was assessed comparing CAP thresholds at 7 and 14 day for respectively ACOU085 doses and vehicle to the untreated condition. Vehicle treatment showed no functional protection whereas ACOU085 treatment demonstrated a dose dependent protection starting at 3 µM and with the strongest threshold improvement of 49 dB at a dose of 100 µM ACOU085 at 14 days after Cisplatin application ($p<0.05$ at all frequencies tested). Morphologic cytocochleogram analysis revealed a statistically significant reduction of OHC loss in ACOU085 treated cochleae for all doses ($p<0.05$) including the lowest 0.1 µM dose. IHC hair cells were protected starting at a dose of 3 µM ($p<0.05$).
Conclusions: In summary, local treatment with the novel small-molecule Kv7.4 agonist ACOU085 dose-dependently protects from cisplatin-induced hearing loss with a clinically relevant effect size and protects from cisplatin-induced inner and outer hair cell loss. The drug candidate ACOU085 is currently under clinical development in a Phase Ib clinical study sponsored by Acousia Therapeutics. A phase 2 clinical trial for assessment of otoprotection in cisplatin-treated cancer patients is planned for 2023.

SU142. Electrocochleography-Guided, Robotically Controlled Ci Electrode Array Insertions in Sheep
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Category: Inner Ear: Damage and Protection
Background: Preservation of cochlear structure and function is critical for optimal cochlear implant (CI) outcomes. Advances in CI surgery have established electrocochleography (ECochG) as an effective intraoperative method to monitor and potentially mitigate insertion-related intracochlear trauma. Current systems provide the surgeon an audible or visual indication that trauma may be occurring. However, given the limits of human responsiveness, especially during manual insertions in which every millimeter is critical, trauma may have already occurred by the time the surgeon can react to an ECochG change. To address this issue, a robotically controlled insertion device guided by ECochG feedback was developed and evaluated.
Methods: CI electrode arrays were implanted in 12 sheep using the robotics-assist device in one cochlea and manually (by-hand) contralaterally. Insertions were halted by the device when real-time ECochG recordings determined a likely trauma event occurrence, whereas manual insertions were performed per standard practice. Surgical events were noted and ECochG recordings of all insertions were evaluated.
Results: Intraoperative ECochG measures were able to detect surgical events in real time. Significant drops in ECochG signal (>20% drop) were detected in multiple sheep, in both robotic and manual insertions. The robotic device was able to halt insertion in response to particular drop detections.
Conclusions: The future of CI surgery will feature novel tools designed to assist the surgeon in reducing intracochlear trauma. In this study, robotically controlled electrode array insertions and intraoperative ECochG were used in tandem to detect and rapidly respond to likely trauma events. Future development and

use of these technologies will enable better hearing preservation, improved outcomes, and increase adoption rates for CIs.

SU143. Detection and Quantification of Gentamicin in the Inner Ear Using Different Liquid Chromatography Methods

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Category: Inner Ear: Damage and Protection

Background: Quantifying drugs in the inner ear is challenging due to its relative inaccessibility. The ototoxic drug, gentamicin, used to treat vestibular disorders like Meniere's disease, consists of 4 major C-subtypes - C1, C1a, C2, and C2a. Simultaneous detection of these subtypes is difficult because gentamicin lacks UV-absorbing chromophores. Liquid chromatography coupled to mass spectrometry (LC-MS) can quantify gentamicin due to its high sensitivity and separation of components. Liquid chromatography with UV or fluorescence can also quantify derivatized gentamicin. We present new LC-MS/MS and HPLC-UV-fluorescence protocols to simultaneously detect multiple gentamicin subtypes.

Methods: Gentamicin was fractionated on an Acquity UPLC BEH C18 column (Waters, 2.1 x 50 mm) using a solvent gradient of 0-100% acetonitrile in water containing nonafluoropentanoic acid (100 mM) at 0.3 mL/min. Gentamicin standards (0.1-50 µg/mL) were prepared, with amikacin as an internal standard. MS/MS analysis was performed where gentamicin and amikacin were ionized positively using electrospray ionization and fragmented with 20 eV collision energy. This method was validated by determining parameters such as specificity, linearity, accuracy, and precision where a general relative standard deviation (RSD) of ≤15% is accepted per the United States Pharmacopeia guidelines.

To develop HPLC-UV-fluorescence methods, gentamicin was derivatized with ortho-phthaldehyde (OPA) and isocratically eluted from an ACE 5 C-18 column (250 x 4.6 mm), using a mobile phase of 0.02 M sodium heptanesulfonate monohydrate in methanol:water:acetic acid (80:18:2 (v:v:v)) at 1 mL/min. To derivatize gentamicin, 50 µL of gentamicin standards were combined with 700 µL HPLC-grade water and 250 µL of reagent (5 mL of a solution of 256 mg OPA dissolved in 50 mL methanol, 20 mL 0.4 M boric acid, pH 10.4, 50 µL mercaptopropionic acid), and 50 µL of this mixture was loaded onto the column. Gentamicin derivatives were detected at 330 nm for UV detection or 340 nm-418 nm (excitation-emission wavelengths respectively) for fluorescence detection.

Results: Gentamicin could be detected at 100 ng/mL, and the standard curve obtained after plotting concentrations of gentamicin vs ratios of the area under UHPLC-MS chromatogram peaks specific to gentamicin was linear with $R2 > 0.99$. Fragment ions for each major component had retention times of 2.85 - 2.90 min for gentamicin, or 2.12 min for amikacin. The RSD of accuracy and precision for intra- and inter-day repetitions were <15%. For HPLC analysis, multiple OPA-gentamicin derivatives were observed. The retention times for the four major components of gentamicin were between 5.5-7.5 min.

Conclusions: A new LC-MS/MS protocol and HPLC-UV-fluorescence method were developed to detect and quantify low levels of gentamicin in vitro, and these will next be tested in inner ear samples.

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SU144. Metformin Protects Male but Not Female Mice Against Noise-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection

Background: Our laboratory recently published an atlas of the cell type-specific transcriptional changes in the mouse cochlea following permanent threshold shift (PTS)-inducing noise exposure. By intersecting the list of dysregulated genes with the DrugCentral database, we identified candidate therapeutics to prevent noise-induced hearing loss (NIHL). The top-ranking candidate was metformin, an FDA-approved, antidiabetic drug. To date, there exists no comprehensive evaluation of the effects of metformin treatment on protection from PTS-inducing noise in animals of both sexes. Here, we examine the effect of metformin treatment on the physiological and histological outcomes of a PTS-inducing noise exposure in mice of both sexes and in an ovariectomy model of menopause.

Methods: Male and female B6CBAF1/J mice were obtained at 7-8-weeks of age. A subset of female mice underwent bilateral ovariectomy at 8-weeks of age. At 9-weeks of age, baseline auditory brainstem response (ABR) thresholds were established. Mice were then administered metformin (200 mg/kg) or a saline control in their drinking water for the remainder of the study. At 10-weeks of age, mice were exposed to a PTS-inducing noise (102.5-105 dB SPL, 8-16 kHz, 2h). ABR threshold shifts were quantified 24-hours (compound threshold shift, or CTS) and 1-week post-exposure (permanent threshold shift, or PTS). Following the 1-week ABR, the mice were euthanized to collect cochlear tissue for histological analysis of outer hair cell (OHC) loss and cochlear synaptopathy.
Results: Our data demonstrate that metformin treatment reduced the CTS and PTS in male mice. Additionally, metformin treatment in intact male mice reduced high-frequency OHC loss 1-week post-exposure. In contrast, metformin treatment did not reduce the CTS or PTS in the gonadally intact female mice. To determine if the protective effects of endogenous estrogens influenced the outcome of metformin treatment in gonadally intact female mice, we utilized an ovariectomy model of menopause. Nevertheless, metformin treatment in ovariectomized female mice did not ameliorate hearing loss. Furthermore, histological studies revealed no protective effect of metformin treatment on OHC loss or synaptopathy in ovariectomized female mice.
Conclusions: Metformin exhibits sex-dependent efficacy as a therapeutic for noise-induced hearing loss. These results provide evidence of Metformin's potential utility as an NIHL therapeutic in males and compel continued investigation into its otoprotective effects. Importantly, these data also demonstrate the critical importance of evaluating therapeutic efficacy of drugs in subjects of both sexes, separately.

SU145. Efficacy of AC102 for the Preservation of Residual Hearing Following Cochlear Implantation

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Category: Inner Ear: Damage and Protection
Background: Cochlear implantation (CI) currently is the only established method to restore auditory function in profoundly hearing-impaired and deaf patients. Individuals with residual hearing eligible for CI benefit from a combined electric-acoustic stimulation (EAS) resulting in better speech discrimination, speech understanding in noise, and an overall better quality of life. Early and late onset of hearing loss is a common adverse effect in EAS-CI. Insertion of the electrode array causes direct mechanical injury to the cochlea, while subsequent loss has been attributed to an inflammatory response driven by pro-inflammatory cytokines, reactive oxygen species, and apoptosis. AC102 is a small lipophilic pyridoindole. Its precursor 9-methyl pyridoindole has been shown to exert neuroprotective, anti-apoptotic, and anti-inflammatory effects. This study aimed to evaluate the efficacy of AC102 regarding the preservation of residual hearing following CI.
Methods: 20 normal-hearing Mongolian gerbils randomized into two groups were unilaterally implanted with a 4mm custom-made cochlear implant. 24 hours prior to implantation, either an AC102-loaded or control hydrogel was transtympanically delivered to the round window niche. Compound action potentials were measured over the course of 28 days. Implanted and contralateral cochleae were subsequently prepared as whole-mounts and histologically assessed by immunofluorescence staining. Inner and outer hair cells (IHC, OHC), auditory nerve fibers (ANF), and IHC synapses were quantified over the whole cochlear length. To address AC102's mode of action in CI, an established in vitro model of electrode insertion trauma (EIT) was utilized and mRNA expression of proinflammatory cytokines was examined by RT-PCR. Cochleae of P5-C57BL/6 mice were extracted and cultured in serum-free media. EIT was mimicked by insertion of a 3-0 monofilament suture through a small cochleostomy. After EIT, AC102 was applied for 24 hours and compared to an EIT-only and untreated control group.
Results: Pretreatment with an AC102-loaded hydrogel resulted in significantly higher recovery of hearing thresholds across the entire frequency range, with the greatest effect immediately apical to the maximum insertion depth. Histologically, treatment with AC102 resulted in significantly higher survival of OHC and IHC as well as nearly complete preservation of hair cells apical to the maximum insertion depth. Furthermore, AC102 provided almost absolute protection of IHC synapses and significant preservation of

type II ANFs. Quantification of mRNA expression revealed an attenuating effect of AC102 on pro-inflammatory cytokine release after EIT in vitro.
Conclusions: Overall, AC102 significantly improved residual hearing following CI over the course of 28 days. Histologically, its effect is attributed to the preservation of OHCs, IHCs, and their respective synaptic connections. Its mode of action is thought to be anti-inflammatory by decreasing the expression of pro-inflammatory cytokines and enzymes. AC102 appears to be a promising compound for the attenuation of EIT and warrants further investigation regarding the long-term outcome in cochlear implantation.

SU146. Safety and Efficacy of Intratympanic Alpha-Lipoic Acid Injection in a Mouse Model of Noise-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection
Background: Alpha-lipoic acid (ALA) is an antioxidant with oto-protective effects. In the present study, the safety and effectiveness of ALA therapy after noise-induced hearing loss was confirmed based on the administration method.
Methods: The safety of intratympanic ALA (IT-ALA) was evaluated with oto-endoscopy and middle ear mucosa morphologic study. Perilymph ALA concentrations according to the administration routes were compared, and the efficacy of ALA was investigated through hearing tests and cochlear histological studies.
Results: The middle ear mucosa was swollen 1 week after IT-ALA but completely recovered within 3 weeks. ALA concentration in the perilymph was significantly higher in the IT-ALA group. Recovery of organ of Corti morphology and hearing levels were predominant in the IT-ALA group compared with the intraperitoneal injection group (IP-ALA) and showed similar rescue effects in the IT-dexamethasone group (IT-DEX). Interleukin-1 beta and nuclear factor-kappa B expression was significantly downregulated in the IT-ALA group.
Conclusions: IT-ALA showed better cochlear recovery from acoustic trauma with higher inner ear penetration rate than IP-ALA. The rescue effect of IT-ALA after noise-induced hearing loss was similar to IT-DEX; however, the ALA and DEX mechanisms are different. IT-ALA appears to be another safe and effective treatment modality after acoustic trauma and comparable to IT-DEX.

SU147. Occluded Insertion Loss From Intracochlear Pressure Measurements During Acoustic Shock Wave Exposure

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Category: Inner Ear: Damage and Protection
Background: Auditory injuries are a common result of high intensity noise exposure, and hearing protective devices (HPDs) can mitigate this injury. Current evaluation methods use acoustic manikins to measure the sound pressure level (SPL) in the ear canal, but cannot assess the impact of alternate sound conduction pathways. We have previously reported intracochlear pressures in cadaveric human specimens to high-level impulse noise, revealing a substantial bone conducted component. Here, we estimate insertion loss during HPD use from intracochlear pressures in those same specimens, and provide an update on new measurements collected in additional cadaveric specimens, with different classes of HPDs, including passive and active earplugs and earmuffs.
Methods: Cadaveric specimens were exposed to shock waves with peak overpressures of 7-83 kPa. Fiber optic pressure sensors were placed in the external, middle, and inner ears, and responses were measured with ears unoccluded, and with four HPDs. Spectral insertion loss was calculated for each exposure level in the frequency domain from the ear canal (EAC) and intracochlear pressure sensors.
Results: Insertion losses calculated from EAC pressures were comparable across SPLs, consistent with results from acoustic manikins. In contrast, insertion loss calculated from intracochlear pressures were

generally lower in magnitude, but increased with exposure level, likely due to substantial contributions of secondary transmission pathways. Differences in insertion losses calculated from EAC and intracochlear pressure sensors are assessed to determine the relative contribution of non-air-conducted sound transmission to the inner ear, and measurements here and in manikins demonstrate cross-device differences. Unfortunately, variability in intracochlear pressures limit insertion loss estimate utility, but averaging multiple exposures increased signal-to-noise considerably, similar noise reduction strategies should be utilized in future studies.
Conclusions: The long-term goal of this effort is to develop and standardize novel acoustic performance metrics for HPDs that will support application-specific selection of existing HPDs and inform the design of new HPDs. Acoustic and behavioral measurements and refinements of predictive metrics are ongoing.

SU148. Investigation Into the Role of AMPA, NMDA and GABA in Mediation of Synaptopathy in the Zebrafish Lateral Line

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Category: Inner Ear: Damage and Protection
Background: Sensorineural hearing loss (SNHL) can result from pathology of the inner ear, auditory nerve fibers, or the central nervous system. Hidden hearing loss is a subtype of SNHL whose mechanisms are not fully understood. This type of deficit does not register during traditional audiometric testing in quiet but manifests as difficulty comprehending speech in the presence of background noise. One proposed mechanism of hidden hearing loss is synaptopathy, i.e., damage to synapses that connect inner hair cells to type one spiral ganglion neurons. Prior research shows that excess calcium influx through postsynaptic AMPA receptors, specifically those lacking the calcium-filtering GluA2 subunit, causes swelling and degeneration of synaptic elements. However, the time course of damage is not fully known, nor how inhibitory neurotransmission may alter synaptic morphology. We investigated the relative contribution of ionotropic glutamate and GABA receptors in mediating synaptopathy using the zebrafish lateral line model.
Methods: We used five-day-old Tg (myo6b: ribeye a-GFP) transgenic zebrafish, which express green fluorescent protein in their pre-synaptic ribbons. Antibody labeling was used to identify the postsynaptic density. The fish were exposed to 0-500 μM of AMPA and NMDA or the inhibitory neurotransmitter GABA. Fish were then allowed to recover for up to 72 hours. We quantified hair cell survival, the number of intact and orphaned synapses, and ribbon area.
Results: Our preliminary work shows that the concentration of AMPA does not significantly affect hair cell number. However, AMPA overexposure significantly affects colocalization of pre- and post- synaptic elements per hair cell in a dose- and time-dependent manner, with two hours of AMPA exposure facilitating delayed damage that occurred 72 hrs after AMPA removal.
Conclusions: Prior research shows that AMPA treatment can cause immediate synaptic dysfunction. Our research demonstrates that high concentrations of AMPA can cause delayed synaptopathy, likely via separate mechanisms. Future work will examine the downstream mediators of synaptopathy to identify targets for therapeutic intervention.

SU149. A Cochlear Hair Cell Loss Model by a Single Infusion of Aminoglycoside into the Posterior Semicircular Canal in the Adult Mouse

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Category: Inner Ear: Damage and Protection
Background: Aminoglycoside antibiotics are responsible for hair cell death and permanent hearing loss in patients. Hair cell loss can also occur because of genetic predisposition, acoustic trauma, or aging. Understanding the steps involved in the processes of hair cell death and the response of the surrounding supporting cells is important to develop novel hearing loss treatments.

A single-dose aminoglycoside hair cell damage model able to induce acutely and synchronously hair cell loss is missing in the adult mouse cochlea.
Methods: We infused a single dose of sisomicin, a highly ototoxic aminoglycoside, into the posterior semicircular canal of one-month-old FVB mice. Cochlear immunohistochemistry was conducted at different time points post-infusion. Apoptotic cells were identified with TUNEL assay. Cell type-specific antibodies were used to identify hair cells and supporting cells. Auditory Brainstem Response (ABR) and Distortion Product of Otoacoustic Emission (DPOAE) were recorded to reveal functional deficits associated with sisomicin-induced hair cell loss.
Results: We observed robust outer hair cell loss 10h after sisomicin infusion. The outer hair cells died through apoptosis, which peaked between 5-7h post sisomicin infusion. Outer hair cell loss was complete and consistent except for the cochlea's most apical region. Loss of inner hair cells depended on the infused sisomicin concentration, and a dose-damage relationship was established, allowing for a potential fine-tuning of the extent of hair cell loss. Supporting cells were not affected by the injection. Consistently, we observed a maximal ABR threshold shift and a loss of DPOAE in all frequencies as early as 6 hours after injection. The induced hearing loss was permanent because no thresholds were detected in the damaged ears seven days post-infusion.
Conclusions: We present an adult cochlear hair cell damage mouse model without affecting supporting cells. The sisomicin-induced hair cell loss paradigm will provide the framework for collecting transcriptomic and epigenetic data and for modulating pathways in short- and long-term changes in gene expression and regenerative approaches in the adult mammalian cochlea.

SU150. Effect of Elevated HDL on Hearing Loss in Mice

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Category: Inner Ear: Damage and Protection
Background: We hypothesized that elevated high-density lipoprotein (HDL) is protective against cisplatin ototoxicity related to removal of cytotoxic peroxidated lipids. To test this, we compared hearing loss by auditory brainstem response (ABR) analysis in TgAPOA1 mice overexpressing APOA1, the major protein component of HDL, to that in wildtype controls.
Methods: Mice underwent pre-treatment ABR at ~1 month. Mice received cisplatin (200 mg/kg furosemide IP and 1 mg/kg cisplatin IP 1 hour later daily for 3 days) or kanamycin (180 mg/kg furosemide IP and 1000 mg/kg kanamycin subcutaneously 40 minutes later). ABR was repeated at 2 weeks and at 1-month post-treatment. Wave I thresholds, amplitudes and latencies were measured. Effects of genotype and sex on hearing functional measures were tested by repeated measures ANOVA. After ABR, cochleae were collected for single nucleus RNA-seq and for immunofluorescence analysis of hair cell loss, neuron density, and strial atrophy.
Results: TgAPOA1 mice have higher HDL levels than control mice (175 mg/dl vs. 78 mg/dl, respectively, p=0.003). Male TgAPOA1 mice have lower mean hearing threshold shifts in response to cisplatin than wildtype controls (e.g., +13 dB vs. +39 dB, respectively, at 24 kHz, p=0.003). In contrast to cisplatin ototoxicity, threshold shifts in kanamycin-treated TgAPOA1 mice were not reduced. Also, threshold shifts in aging TgAPOA1 male mice were greater than wildtype males at high frequencies (e.g., +1.9 dB per month at 30 kHz, p=0.043), while TgAPOA1 females were unaffected. Single nucleus RNA-seq analysis revealed differential expression related to HDL and lipid trafficking, cisplatin uptake, and cellular survival in TgAPOA1 mice versus wildtype controls.
Conclusions: These findings suggest that HDL/APOA1-based therapeutics may reduce hearing loss after cisplatin chemotherapy in males.

SU151. Valproic Acid Delays Progressive Hereditary Hearing Loss in KCNQ4 Variant Model Through HDAC1 Suppression

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Category: Inner Ear: Damage and Protection
Background: Genetic cause contributes to approximately 50% of congenital or childhood hearing loss (HL). Onset time and speed of the hearing deterioration is important since there is a chance for intervention

before the HL reaches its advanced stage. The earlier and faster, the narrower treatment time window will be for the HL. In the current study, we evaluated the feasibility of valproic acid (VPA) for slowing down the hearing deterioration in a KCNQ4 variant progressive HL model.
Methods: KCNQ4 p.W276S point variant murine model was used. VPA (200mg/kg) was delivered intraperitoneally from age 3 to 6-weeks. Auditory brainstem response (3RZ6 TdT) was done every week to evaluate the hearing level. At age 6-week, mice were euthanized, cochleae were collected for immunohistochemistry to check hair cell damage. In vitro study using HEK293T cell was performed to see the effect of VPA on HDAC1, KCNQ4, and HSP90 β. Overexpression was done using pcDNA3.1-KCNQ4-EGFP-8xHIs, pcDNA3-HA-HSP90β, pCs2+-3myc-HDAC1 transfection. Immunoprecipitation assay was done to see the interaction between KCNQ4 and HSP90β.
Results: VPA activated its known downstream target, survival motor neuron (SMN) gene within the cochlea. Histone H4 acetylation was also increased by VPA in the cochlea showing direct effect of VPA on the cochlea. In KCNQ4 p.W276S homozygote, HL started at 4-weeks age, reaching up to near total deaf already at 6-weeks age. VPA treatment from 3 to 6-weeks age, HL was significantly attenuated (83±1.72dB) compared to the PBS-treated control group (92.6±1.94 dB). Immunohistochemistry revealed that VPA preserved outer hair cell damage compared to the control in all cochlear turns. When HDAC1 was over-expressed via transfection in HEK293T cells, both KCNQ4 and HSP90β expression were reduced. This demonstrates that KCNQ4 protein expression is regulated by HDAC1 activation. VPA treatment counteracted this effect of HDAC1 and increased HSP90β and KCNQ4 expression. VPA also stimulated HSP90β-KCNQ4 interaction.
Conclusions: VPA inhibited HL progression in KCNQ4 point variant model. VPA suppressed HDAC1, leading to upregulation of KCNQ4, HSP90β expression, and interaction between KCNQ4-HSP90β. VPA is a candidate drug to slow down HL progression to increase the time window for definite treatment of KCNQ4 genetic HL.

SU152. Changes in the Vestibular Organs of the COVID-19 Hamster Model

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Category: Inner Ear: Damage and Protection
Background: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the virus responsible for the ongoing COVID-19 pandemic. In human autopsy studies, SARS-CoV-2 was detected in inner ears after COVID-19 infection. However, there are no animal model studies that investigated the inner ear, or confirmed the presence of SARS-CoV-2 in the inner ear. Hamster models of COVID-19 share many symptoms with the human disease and have been used in many studies. In this study, we conducted a detailed histological analysis of the cochlear and vestibular organs in SARS-CoV-2 infected hamsters derived from a previous study on anosmia. We looked for the presence of SARS-CoV-2 in the inner ear and its correlation with the behavioral timeline characteristics of anosmia.
Methods: Three-week-old female hamsters (n=36) were intranasally infected with SARS-CoV-2 virus alpha strain. Temporal bones were dissected from the hamsters on days post infection (dpi) 2 (n=4), 3 (n=4), 5 (n=4), 8 (n=4), 12 (n=4), 21 (n=4), 35 (n=4), and 42 (n=4), and were fixed in 10% buffered formalin for 2 days. After decalcification, the temporal bones were embedded in paraffin, thin sectioned and processed for further histology studies. The specimen was stained with either H and E or labeled with SARS-CoV-2 nucleocapsid antibody and visualized with DAB and counterstained with hematoxylin. The slides were observed under transmission light microscopy. Vestibular structures (utricle, saccule, horizontal crista ampullaris, anterior crista ampullaris, posterior crista ampullaris, and superior and inferior vestibular nerves) were evaluated for a) hemorrhage, b) lymphocytic infiltration, and c) structural damage. The slides were scored by three independent observers and averaged across parameters. The scores were assigned as 0= no change, 1= minimal change, 2= significant change.
Results: Structural damage was the most observed (hemorrhage and lymphocytic infiltrate were negligible). The difference in damage scores, e.g. the higher average scores in the infected hamsters compared to the mock hamsters, was statistically significant ($p<0.05$, paired two tailed T-test). Based on the H and E score, the most significant structural damage was observed at 12 dpi. Furthermore, the largest difference in the score was observed in the superior vestibular nerve and saccule at 12 dpi. We also found an overall

statistical significance for the presence of SARS-CoV-2 in the vestibular system, with a strong peak damage at 12 dpi and the superior vestibular nerve exhibiting the most amount of COVID-19 antibody damage.
Conclusions: In the hamster COVID-19 model, changes in the vestibular organs were seen at later time points at 2 weeks post infection compared to the peak of anosmia at 2-5 days. Thus, we speculate that the vestibular organs were affected indirectly, and most likely not a primary target for viral invasion. The presence of SARS-CoV-2 virus antigen in the perilymph and perineural structures suggest the viral entry mechanism via traffic from CSF.

SU153. Traumatic-Noise-Induced Hair Cell Death and Hearing Loss is Mediated by Activation of CaMKKβ

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Category: Inner Ear: Damage and Protection
Background: The Ca2+/calmodulin-dependent protein kinase kinases (CaMKKs) are serine/threonine-directed protein kinases that are activated following increases in intracellular calcium, playing a critical role in neuronal signaling. Inner-ear-trauma-induced calcium overload in sensory hair cells has been well-documented in the pathogenesis of noise-induced hair cell death and hearing loss, but there are no established pharmaceutical therapies available due to a lack of specific therapeutic targets. In this study, we investigated the activation of CaMKKβ in the inner ear after traumatic noise exposure and assessed prevention of noise-induced hearing loss (NIHL) with RNA silencing.
Methods: Both CBA/J and FVB/NJ mouse strains were exposed to traumatic noise that induces losses of inner hair cell ribbon synapses and outer hair cells. Knockdown of CaMKKβ by short hairpin RNA (shRNA) via adeno-associated virus vector transduction and small interfering RNA (siRNA) via intra-tympanic delivery were used to evaluate hearing function. Losses of inner hair cell ribbon synapses and OHCs were counted from whole mount surface preparations along the entire cochlear spiral. Auditory function was assessed by auditory brainstem responses (ABRs) and distortion product of auto-acoustic emissions (DPOAEs). Immunolabeling and Western-blotting were used to detect associated molecular changes.
Results: Short hairpin RNA of CaMKKβ (shCaMKKβ) via adeno-associated virus transduction significantly knocked down CaMKKβ expression in the inner ear. Knockdown of CaMKKβ significantly attenuated noise-induced hair cell loss and NIHL. Additionally, pretreatment with naked CaMKKβ small interfering RNA (siCaMKKβ) attenuated noise-induced losses of inner hair cell synapses and OHCs and NIHL. Furthermore, traumatic noise exposure activates CaMKKβ in OHCs as demonstrated by immunolabeling for p-CaMKI. CaMKKβ mRNA assessed by fluorescence in-situ hybridization (FISH) and immunolabeling for CaMKKβ in OHCs also increased after the exposure. Lastly, pretreatment with siCaMKKβ diminished noise-induced activation of AMPKα in OHCs.
Conclusions: These findings demonstrate that traumatic-noise-induced OHC loss and hearing loss occur primarily via activation of CaMKKβ. Targeting CaMKKβ is a key strategy for prevention of noise-induced hearing loss. Furthermore, our data suggest that noise-induced activation of AMPKα in OHCs occurs via the CaMKKβ pathway.
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SU154. Nox3-Derived Superoxide in Cochleae Induces Acquired Sensorineural Hearing Loss

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Category: Inner Ear: Damage and Protection
Background: Sensorineural hearing loss (SNHL) is one of the most common sensory impairments in humans. However, treatment options mostly rely on medical instruments, with no reliable pharmacological interventions. Reactive oxygen species (ROS) produced by NADPH oxidases (Nox) contribute to the development of different types of acquired SNHL, such as age-related HL (ARHL), drug-induced HL (DIHL) and noise-induced HL (NIHL). Although the essential role of Nox3 in otoconia biosynthesis and its possible involvement in hearing function have been reported in rodents, immunohistological methods

targeted at detecting Nox3 expression in inner ears reveal ambiguous results. Therefore, the mechanism underlying Nox3-dependent acquired SNHL remains unclear and warrants further investigation.
Methods: We generated Nox3-Cre knock-in (KI) mice, in which Nox3 was replaced with Cre recombinase (Cre). Using Nox3-Cre;tdTomato mice, in which tdTomato is expressed under the control of the Nox3 promoter, we examined Nox3-expressing regions and cell types in cochlear cryostat sections. Furthermore, to examine the correlation between Nox3 expression and acquired SNHL, we established models of acquired SNHL (ARHL, DIHL and NIHL). Using these models, we observed how Nox3 expression changes in cochlear whole-mount and surface preparations of the organ of Corti, and evaluated hearing function by auditory brainstem response.
Results: We identified that Nox3-expressing cells in cochleae included various types of supporting cells (SCs), outer hair cells (OHCs), inner HCs, and spiral ganglion neurons. We also found that Nox3 expression increased with age, cisplatin, and noise insults in specific cell types in cochleae, and resulted in OHC loss by apoptosis. Moreover, increased Nox3 expression in SCs and OHCs, especially at the basal turn of cochleae, played essential roles in OHC loss of all three types of acquired SNHLs.
Conclusions: In the present study, we identified Nox3-expressing regions and cell types in inner ears using Nox3-Cre;tdTomato mice, which were particularly useful as no reliable Nox3 antibodies are available. We also found that increased Nox3 expression in SCs and OHCs, especially at the basal turn, is directly involved in developing mechanisms of acquired SNHL. We propose that Nox3 inhibition in cochleae is a promising approach to prevent acquired SNHL.

SU155. Natural Product-Based Therapies for Protection Against Aminoglycosides-Induced Hearing Loss
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Category: Inner Ear: Damage and Protection
Background: Hearing loss is a major health concern in our society, affecting over 360 million people worldwide (World Health Organization, 2017). Aminoglycoside therapy causes permanent hearing loss in 40-60% of treated patients (i.e., cystic fibrosis patients). To date, no drugs have been approved by the US Food and Drug Administration (FDA) for protection from aminoglycoside-induced hearing loss (AGIHL). Most candidate compounds currently in pre-clinical and clinical trials are related to antioxidants, vitamins, and glutathione metabolism. We have conducted a high-throughput screening of bioactive natural compounds employing zebrafish as our platform for aminoglycoside ototoxicity, and identified piperlongumine, an alkaloid extracted from the long pepper Piper Longum L., as an important therapeutic molecule for aminoglycoside-induced hair cell death. The goal of the current study is to assess piperlongumine protection in a mouse model for aminoglycoside ototoxicity.
Methods: C57BL6 animals (carrying the corrected Cdh23 gene) received kanamycin (600 mg/kg b.w., S.Q., twice a day) for 14 consecutive days in combination with vehicle (corn oil) or piperlongumine (40 mg/kg b.w., IP) for 17 days. Piperlongumine administration was initiated one day before the kanamycin regimen and continued for 2 more days post-kanamycin. Two additional groups (vehicle alone and piperlongumine alone) were also included in our studies. Auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) were measured before (baseline) and four weeks after the finalization of the treatments. We also assessed the lateral wall function by endocochlear potential (EP) assessments. At the end of the experiments, cochlear tissue was collected for immunohistochemistry analysis.
Results: Piperlongumine was protective against aminoglycoside ototoxicity. ABRs from animals co-treated with piperlongumine and kanamycin showed significantly lower thresholds at all the frequencies (4-64 kHz), compared to animals that received kanamycin alone. Furthermore, piperlongumine alone was not ototoxic. Similarly, when assessing outer hair cell function, piperlongumine cotreatment resulted in lower DPOAEs thresholds compared to animals treated with kanamycin alone. EPs remained unchanged suggesting that the lateral wall is not affected by kanamycin ototoxicity. We performed the quantification of outer hair cells and pre-synaptic boutons at 5 different cochlear frequency regions (8, 16, 22.6, 32 and 64 kHz) and found that piperlongumine helped to preserve the numbers of both structures. Finally, we confirmed that piperlongumine did not interfere with aminoglycoside bacteria-killing activity by a diffusion disc assay.

Conclusions: The present work demonstrates the beneficial effect of a natural product against aminoglycoside-induced hearing loss and set the bases for future studies aiming to address piperlongumine's molecular target(s) as well as its potential as an otoprotectant for additional forms of acquired hearing loss.
Fundings: LB692-289325 and 5R01DC015444-04 (JZ), 1R43DC019065-01 (MZ).

SU156. RIPOR2-Mediated Autophagy Dysfunction is Critical for Aminoglycoside-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection
Background: Aminoglycosides play their antibacterial roles by inhibiting the synthesis of proteins in bacteria. Long-term clinical studies have found that these antibiotics have strong side effects. The use of aminoglycoside antibiotics in clinical practice is restricted because there is no way to inhibit the serious side effects. However, aminoglycoside antibiotics are still in great clinical demand due to their broad spectrum of antibacterial effects, especially their effectiveness against severe infections caused by drug-resistant Gram-negative bacteria. Therefore, it is of great significance to explore ways to reduce the side effects of these antibiotics.
Over the years, significant insights have been made in understanding the mechanisms by which aminoglycoside antibiotics cause hearing impairment. Early studies have found that mitochondria and ROS play crucial roles in the ototoxicity of aminoglycoside antibiotics. In addition, autophagy may also be involved. Autophagy is thought to protect cells by removing unwanted molecules and damaged subcellular organelles, but abnormal or improperly triggered autophagy can also lead to cell damage and death. At present, the function of autophagy in deafness induced by aminoglycoside antibiotics is controversial.
Methods: Cochlear explant culture and immunostaining
Cochlear explants were dissected and cultured in DMEM/F12 media at 37 °C. Then, explants were treated with 1 mM AGs. Samples were fixed with 4% PFA, blocked with HBSS containing 5% BSA and 0.5% Triton X-100, and then incubated overnight at 4 °C with primary antibodies in HBSS containing 1% BSA. Tissues were washed in HBSS and incubated with secondary antibodies for 2 hours at room temperature. Then, images were captured by a DM6 FS automated deconvolution microscope (Leica).
Kanamycin administration
KAN was dissolved in 0.9% NaCl and then adjusted to a concentration of 80 mg/ml. Subcutaneous injections were administered twice daily for 14 days. The administered dose of KAN was adjusted based on the body weight of animals, which was monitored before injection.
Results: In the current study, we found that AGs bind to RIPOR2/Fam65b and trigger rapid subcellular translocation of RIPOR2 from the base of stereocilia to the region of the pericuticular necklace in hair cells. RIPOR2 then interacts with GABARAP. Strikingly, reducing RIPOR2 or GABARAP expression completely prevents hair cell death and subsequent hearing loss caused by AG when systemically administered. Additionally, we found that disrupting the mitochondrial autophagy pathway by abolishing the expression of PINK1 or Parkin protects hair cells from AG ototoxicity.
Conclusions: Our findings delineate molecular components of a pathophysiological response pathway induced by AGs in hair cells that ultimately affect the autophagy pathway, thus causing hair cell dysfunction and death. Our findings also identify that the autophagy pathway is a highly promising therapeutic target for preventing hearing loss caused by AGs and possibly other insults that affect the inner ear.

SU157. Pyroptosis Accelerates Spiral Ganglion Neuronal Degeneration Induced by Aminoglycosides in Murine Cochlea

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Category: Inner Ear: Damage and Protection
Background: Ototoxic drug-induced hearing loss is one of the main types of hearing impairment, affecting millions of people worldwide. For patients with profound hearing loss, cochlear implant is a promising treatment but highly depending on the survival and function of spiral ganglion neurons (SGNs). In aminoglycoside-induced hearing loss, SGNs damage aggravates gradually after the acute outer hair cell death, accompanied by macrophage infiltration and cytokine release. The mechanisms underlying SGNs loss are yet to be clarified. Pyroptosis is an inflammatory programmed cell death initiated by inflammasomes,

and it plays a critical role in neurodegenerative diseases. Here, we explored the potential role of pyroptosis in SGN degeneration induced by aminoglycoside.
Methods: C57BL/6J mice were randomly divided into kanamycin with furosemide treated group and saline control group. Auditory functions were evaluated by auditory brainstem response conducted before treatment and at 1, 5, 15, and 30 days after treatment. HCs and SGNs were assessed for morphological alterations. SGNs were subjected to RNA sequencing, and mRNA and protein were collected for analyses of NLRP3 inflammasome-related molecules. Macrophage activation was evaluated based on morphological and mRNA alterations. The effect of NLRP3 inhibition on SGN survival after kanamycin treatment was evaluated in organ explant cultures treated with Mcc950, a specific inhibitor of the NLRP3 inflammasome.
Results: Kanamycin and furosemide co-administration led to irreversible deterioration of the auditory brainstem response, accompanied by acute loss of outer hair cells and gradually progressive loss of inner hair cells. The number of SGNs decreased progressively after aminoglycoside treatment, as well as swelling cytoplasm and ruptured membrane existing in the surviving cells. RNA sequencing of SGNs indicated that inflammation and immune-related responses were significantly upregulated, as was the expression of the inflammasome-related gene NLRP3. During 30 days of kanamycin exposure, the canonical pyroptosis pathway was constantly activated in SGNs. Activation and infiltration of microglia-like cells/macrophages, and increased production of cytokines, hallmarks of neuroinflammation, were also observed. Mcc950 significantly ameliorated SGNs degeneration by inhibiting NLRP3 expression and promoting release of interleukins 1β and 18.
Conclusions: SGNs undergo cell death via upregulated pyroptosis pathway in aminoglycoside-induced degeneration. Activation of the NLRP3 inflammasome leads to a cascade of inflammatory events. Inhibition of the NLRP3 inflammasome alleviates SGNs damage, suggesting that it could serve as a new molecular target for the treatment of aminoglycoside-induced SGNs degeneration, providing a possible treatment to prevent ototoxicity-related neuronal loss.

SU158. Fate Mapping Reveals Heterogeneity in Cochlear Macrophages and Blood Circulating CCR2-Expressing Recruited Macrophages Promote Hair Cell and Neuron Survival After Acoustic Trauma
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Category: Inner Ear: Damage and Protection
Background: Cochlear injury results in activation of resident macrophages and recruitment of monocytes from circulation, which may differentiate into macrophages. The precise roles of resident and recruited macrophages in hearing loss and cochlear injury are unclear. We have reported that macrophages promote the survival of spiral ganglion neurons (SGNs) via fractalkine (CX3CL1-CX3CR1) signaling after cochlear injury (Kaur et al., 2015, 2018, 2019). However, it remains unclear if CX3CR1-expressing resident and recruited macrophages are distinct and differentially promote SGN survival. Here, we used a robust fate mapping technique wherein CX3CR1-expressing resident and recruited macrophages are endogenously labeled with different fluorescent reporters in order to define heterogeneity in cochlear macrophages in terms of origin, spatiotemporal distribution, morphology, fate, phenotype, and function after acoustic trauma.
Methods: Tamoxifen inducible CX3CR1YFP−CreER/YFP−CreER mouse line was crossed with Rosa-lsl-tdTomato (R26RFP) reporter mouse line. The progeny CX3CR1YFP−CreER/wt:R26RFP were injected with tamoxifen or vehicle and euthanized at various time points post injection to determine Cre recombination efficiency and turnover rate of resident cochlear macrophages. To determine the origin, spatiotemporal distribution and fate of resident and recruited macrophages in the injured cochlea, a cohort of tamoxifen-injected CX3CR1YFP−CreER/wt:R26RFP mice were allowed to recover for 60 days (“wash out”) followed by acoustic trauma for 2 hours at a noise level of 112 dB SPL at 8-16 kHz. CCR2 wild type and knockout mice were exposed to acoustic trauma to determine the role of CCR2-expressing circulating recruited macrophages in hearing and sensory cell survival. Following ABRs and DPOAEs measurements, mice were euthanized at different days post acoustic trauma and tissue was analyzed by flow cytometry and confocal microscopy.
Results: By 60 days post tamoxifen administration, CX3CR1-expressing resident cochlear macrophages (98 ± 1.7% recombination efficiency) and blood circulating CX3CR1 lineage (2.5 ± 1.1% recombination

efficiency) displayed distinct YFP+ RFP+ and YFP+ RFP− phenotype, respectively. Examination of resident macrophages for one year indicate that their turnover rate is considerably slower than circulating monocytes/macrophages (1-3 days). Acoustic trauma in "washed out" mice showed the presence of proliferating resident and recruited monocyte-derived macrophages of both pro- and anti-inflammatory phenotypes and distinct temporal dynamics in the spiral ganglion and laminaris, whereas sham exposed mice only contain naïve resident macrophages. Morphometric analysis indicate that morphology is not a good indicator to distinguish CX3CR1-expressing resident and recruited macrophages. Recruited macrophages expressed chemokine receptor, CCR2, absence of which led to diminished survival of hair cells and SGNs and increased macrophage numbers when compared to mice with intact CCR2 after acoustic trauma.
Conclusions: These data establish the use of genetic fate mapping to distinguish resident macrophages from recruited macrophages in normal and injured cochlea and imply that recruited macrophages may promote hair cell and neuron survival after acoustic trauma.

SU159. Immune Cell Infiltration in the Cochlea During Development and After Deafening-Induced Spiral Ganglion Neuron Death

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Category: Inner Ear: Damage and Protection
Background: It remains unclear how the population of resident immune cells in the cochlea changes during periods of active neuron death. Rueda et al. has previously reported a ~22% developmental decrease in spiral ganglion neuron (SGN) number between postnatal day 5 (P5) and P6 in rats. SGNs die, albeit at a much slower rate, after neonatal aminoglycoside deafening of rats, resulting in the death of >80% of all SGNs over the course of ~14 weeks. Our lab has previously shown an increase in immune cell density in deafened rats by P70, possibly causal to neuronal death. To better understand the role of immune cells in SGN death, we established a comprehensive time course of neuron, macrophage, and lymphocyte density along the tonotopic axis in the spiral ganglia of neonatally deafened and normal hearing rats from P5 to P70. We describe the temporal relationship between neuronal death and immune cell appearance during normal development and in adults post-deafening and suggest different roles of immune cells in these two circumstances of SGN death.
Methods: Sprague-Dawley rats were intraperitoneally injected 1x/day with kanamycin from P8 to P16. Cohorts of deafened and hearing control rats were euthanized at various ages and cochlea were sectioned for immunohistochemistry to detect hair cells, neurons, macrophages, and lymphocytes.
Results: Our data corroborate previous findings from Rueda et al. and we report a similar development related decrease in SGN density in basal, middle, and apical regions of the cochlea from P5 to P8. Macrophage density significantly increases during this period, reaching a developmental peak at P12 that subsequently decreases with maturation. Kanamycin deafened rats undergo the same developmental changes in neuron density; however, the resulting immune response differs. In deafened rats, there is a significant increase in macrophage density in mid to apical regions beginning at P16 and in mid to basal regions by P32. In addition, lymphocyte density is significantly increased after deafening by P39 across the cochlea, except for the basal-most region. In this study, we found that post-deafening neuron death is first significant at P39.
Conclusions: Based on our observations on the timing of macrophage infiltration, it appears that during developmental programmed cell death, macrophage number increases in response to neuron death, presumably to phagocytize debris from apoptotic neurons. In deafened rats, macrophage number increases in the ganglion prior to the start of post-deafening neurodegeneration. These data suggest that macrophages are not infiltrating solely in response to an increased need for phagocytosis and may have a neurotoxic or neuroprotective role in the ganglion. Lymphocyte density in deafened rats increases by P39, indicating that the elicited immune response after deafening is a mix of innate and adaptive immune response related cells.

SU160. The Drug Discovery and Delivery Core in the Translational Hearing Center at Creighton University

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Category: Inner Ear: Drug Delivery
Background: There is a significant unmet medical need to develop pharmacotherapeutics for auditory disorders. Yet, few academic centers in the world are dedicated to these efforts. The Drug Discovery and Delivery Core (DDDC) at Creighton Translational Hearing Center (the Center) aims to establish a state-of-the-art drug development pipeline to facilitate individual research projects both within and outside the Center. Specifically, the DDDC will: 1) develop in silico, in vitro and in vivo high-throughput screens to discover and validate novel therapeutics for auditory disorders; 2) establish a medicinal chemistry pipeline to optimize chemical entities; 3) establish drug delivery and pharmacokinetic/pharmacodynamic (PK/PD) methodologies; 4) develop a sustainability plan for DDDC services. With strong commitment from Creighton University and neighboring institutes (Boys Town National Research Hospital and University of Nebraska Medical Center), the DDDC has already established nearly all necessary equipment and expertise.
Methods: We have developed primary screening assays to validate candidate ototherapeutics from small molecules to polypeptides. These include: (i) screening various cell lines using apoptosis or transcriptional reporters, (ii) screening drugs using in silico Connectivity Map (LINC databases), (iii) designing and synthesizing novel small molecules and polypeptides, (iv) screening and validating hits using zebrafish lateral line neuromasts and mouse cochlear explants, and (v) validate lead drug candidates in rodent models (mice and guinea pigs) in vivo. Modern, innovative and robust chemical synthesis and computational chemistry methodologies are used to systematically modify chemical structures to optimize their efficacy and bioavailability, while minimizing toxicity. The DDDC also maintains highly efficient and accurate analytical services for providing complete confidence in the structural integrity, purity, and stability of molecules to customers. Lead compounds are tested in dose ranges, via local or systemic delivery routes, for in vivo pharmacokinetic and efficacy studies to yield better-characterized therapeutic candidates poised for further preclinical and clinical testing.
Results: The DDDC has successfully completed multiple projects, including synthesizing compounds not commercially available, reference compounds (e.g., Compound 18 for hair cell regeneration), novel drug candidates (e.g., Piperlongumine derivatives), as well as providing cost-effective solutions to the high-cost commercial compounds (e.g., Cisplatin-Texas-Red) for Center investigators. In addition, we have performed molecular docking of a CDK2-specific degrader (PROTAC-8) for hearing protection (Hati et al, 2021) and ADMETox analysis of two novel indole-2-carboxamindes highly potent against nontuberculous mycobacteria (Agrawal, et al., AAPS 2022).
Conclusions: The long-term goal of the DDDC is to provide an innovative scientific proving ground for the Center to become a premier ototherapeutic research center. The DDDC also mentors and trains the next generation of hearing researchers to become familiar with this fast-moving field of ototherapeutics.

SU161. Development of a Growth Factor-Based Implant-Associated Drug Delivery System for Cochlear Implants

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Category: Inner Ear: Drug Delivery
Background: One critical issue of the cochlear implant is the loss of signal transmission which is a consequence of the relatively large gap between the electrode and the remaining spiral ganglion neurons (SGNs). For a better acoustical experience, an improvement of the electrode-nerve contact is necessary. For this purpose, a growth factor-based drug delivery system is developed which will be established on the cochlear electrode. This system is based on nanoporous silica nanoparticles (NPSNPs). On these particles the growth factors neurotrophin-3 (NT 3) and brain-derived neurotrophic factor (BDNF) are attached. These proteins should support the survival rate of the neurons and guide them among a concentration gradient towards the electrode.
The installation of a drug delivery system on cochlear electrodes is generally challenging due to the inert materials used (platinum/silicone). Therefore, a novel procedure to attach the NPSNP to silicone is also being developed using a coating of covalently bonded polymers.
Methods: In the first step, NPSNPs were synthesized and afterwards modified with (3-aminopropyl)triethoxysilane.
The growth factors were attached to the particle surface via incubation. For testing the release behavior, the particles were stored at 37 °C.

To attach NPSNPs to the silicone, a polymer coating is created. The methyl groups of the polydimethylsiloxane (PDMS) form radicals with the help of a radical initiator and UV light. These radicals start the polymerization with acrylamide monomers in the irradiated solution. If the NPSNPs are modified with an acrylic group they can be integrated into the polyacrylamide coating.
Results: After modification thermogravimetric measurements show a higher mass loss for the modified particles compared to the non-modified NPSNPs which is attributed to the organic functionalization on the surface. Furthermore, the BET-surface area decreased, possibly due to pore partial blocking. Additionally, there is a change in zeta-potential.
Quantification via ELISA confirmed that the immobilization of BDNF and NT-3 was successful and reversible. Cell culture investigations showed that the released amounts of growth factors have a positive effect on SGNs.
The contact angles of pure silicone and polymer coatings were analyzed to characterize the hydrophobicity. It decreased from 117° for pure silicone up to 62° for the polymer coating. Additionally, X-ray photoelectron spectroscopy showed a successful coating with polyacrylamide. Electron microscope investigations showed that the particles were bound to the polymer coating.
Conclusions: NPSNPs were successfully modified with amino groups. Moreover, these particles were able to release amounts of NT-3 and BDNF that are high enough to have a positive effect on SGNs in cell culture investigations.
In the future, a combined release of both growth factors is aimed.
Furthermore, NPSNPs were covalently bound to the polymer surface of the silicone. Which creates a good base for an implant-associated drug delivery system on the cochlear electrode.

SU162. Transport Studies of Super-Paramagnetic Iron Oxide Nanoparticles Across Guinea Pig Round Window Membrane

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Category: Inner Ear: Drug Delivery
Background: Super-paramagnetic Iron Oxide Nanoparticles (SPIONs) have the potential to be effective drug delivery vehicles to the inner ear that can be administered via intratympanic injection. To guide their rational design for cochlear delivery and future translational development, an understanding of how their physical-chemical properties relate to transport kinetics through the round window membrane (RWM) is required. In this study, we use a 3D-printed benchtop continuous-flow model to define the transport kinetics of SPIONs across guinea pig RWM with and without an external magnetic field gradient.
Methods: Mono-core SPIONs were synthesized in the organic phase and coated with polyethylene glycol (PEG)-diacid of chain length= 61; Mr 3000 (NP-PEG3000). Characterization of synthesized NPs was performed using Transmission Electron Microscopy (TEM) and Zetasizer. A 3-D printed dual-chambered flow cell device was constructed to study the transport kinetics of synthesized SPIONs across 2 distinct membrane types: acellular porcine submucosa small intestine (SIS) membrane, and freshly explanted guinea pig RWM. The external magnetic field gradient was applied using Neodymium N52 grade- 1.48 Tesla permanent magnet held orthogonal to the tissue insert. A flow rate of 3 µl/min for NPs was utilized. SPIONs conc. was quantified using the Ferene-s assay. The histologic basis of nanoparticle transport through RWM was investigated using confocal and electron microscopy.
Results: Characterization of synthesized NPs shows mono dispersion in PBS, core diameter of 7nm, the hydrodynamic diameter of 144 ± 15 nm, polydispersity index of 0.25 ±0.1, and zeta potential of -30.1 mV. The mean rate of SPION transport for NP-PEG3000 without and with magnet for SIS was found to be 1.839 (± 0.125) and 3.184 (± 0.087) ug Fe/hr, respectively, and for RWM 3.667 (± 0.126), 5.669 (± 0.106) ug Fe/hr, respectively. Nanoparticle flux was increased by 173% in SIS and 154% times in RWM with the application of magnetic field gradient. The permeability of guinea pig RWM to NP-PEG3000 was found to be 3.2405 * 10-4 cm/sec and, 5.01 * 10-4 cm/sec in the absence and presence of a magnetic field gradient, respectively. The histologic basis for the transport of SPIONs is presented with EM and confocal images respectively for the localization of NPs within the RWM.
Conclusions: We present a benchtop continuous-flow model to define the transport kinetics and effective permeability of the guinea pig RWM to SPIONs. These results quantify the extent to which an applied magnetic field augments nanoparticle kinetics and membrane permeability, and can be further translated to

the rational design of SPION conjugated therapeutics for cochlear drug delivery. This study will help design the use of SPIONs in conjunction with therapeutics for drug delivery into the cochlea.

SU163. Porcine Model to the Rescue! Addressing Hearing Loss Treatment Challenges

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Category: Inner Ear: Drug Delivery

Background: Nearly 0.5 billion people suffer from debilitating hearing impairments. Although there are promising studies to restore hearing in rodent models, there are two major translational problems: 1) The size, ontogeny, genetics, and frequency range of hearing of most rodents' cochlea do not match that of humans. 2) Delivery of therapeutics into the inner ear is problematic. Local delivery is more efficient, but invasive surgeries (e.g., cochleostomy), can cause damage to delicate structures in the inner ear or endolymphatic hydrops. Furthermore, these techniques are relatively easy to perform in rodents but are challenging and unreliable in bigger animal models with different anatomy. The alternative safe intratympanic method is clinically practiced but has low efficiency since drugs must permeate through the round window membrane (RWM). In this respect, rodent models also have low translational value for optimizing translational delivery techniques due to their smaller size.
Thus, our specific aims are: i) Establish a big animal model that could bridge the gap between rodents and humans for hearing loss studies. We studied the porcine cochlea since it shares many anatomical, physiological, and genetic similarities with its human counterpart. ii) Develop a clinically relevant ex vivo model system to improve intratympanic delivery.
Methods: To address the first problem and validate a more translational animal model, we imaged the porcine inner ear in 3D using tissue-clearing and custom light-sheet microscopy. This method maintained, with high fidelity, the cochlea's 3D structure which is important to its proper function.
To address the second major hurdle, we developed an ex-vivo system for efficient testing of therapeutic delivery. We immobilized porcine RWMs, with a similar thickness to that of humans, across a 2-cavity chamber mimicking the middle and inner ear.
Results: Using 3D imaging, we measured never-reported porcine cochlear characteristics e.g., total hair cell count and basilar membrane length. The images also revealed milestones in the ontogeny of porcine cochlear development such as cochlear turn, Reissner membrane, and Stria vascularis formations and hair cells and supporting cell organization that are remarkably like those in humans.
We validated the viability of porcine RWM explants in the ex-vivo chamber for testing drug passage. We also verified the functionality of the chamber for high and low permeability substances and tested techniques to improve the permeability of RWM.
Conclusions: Together with past porcine anatomical and auditory-brainstem-response studies, this work establishes the pig as an excellent large animal model for understanding hearing impairment, mapping cochlear development, and exploring regenerative medicine therapies before translation into humans. An animal model matching the human organ's size could also guide novel treatment plans concerning therapeutic dosage, diffusion, targetability, and efficiency. The developed ex-vivo chamber is biologically functional and translational and can be used to test novel approaches for enhancing intratympanic delivery.

SU164. An Agonistic TrkB Antibody Provides Robust Neuroprotective and Neuritogenic Effects in Vitro and is Released off Alginate as Drug Delivery Matrix

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Category: Inner Ear: Drug Delivery

Background: For optimal performance of the cochlear implant (CI), a high number of vital spiral ganglion neurons (SGN) and a short distance to the stimulating electrodes are beneficial. Both aspects may be achieved by treatment with drugs supporting neuronal survival and neurite outgrowth for bridging the nerve-electrode gap. One promising candidate in this regard is brain-derived neurotrophic factor (BDNF), whose action is mediated by the TrkB-receptor and the subsequent signaling pathway. An agonistic TrkB antibody (TrkB-AB) may have the same or even superior effects than BDNF while having better drug-features. For use as local inner ear therapeutic, a novel drug delivery approach is required. We tested the capacity of the agonistic TrkB-AB for SGN support and used alginate as delivery matrix, investigating the TrkB-AB release.
Methods: The TrkB-AB was tested in different concentrations for its neuroprotective (10fM to 7.35µM) and neuritogenic (1pM to 100nM) effect on dissociated SGN of early postnatal rats. The effect was compared with a negative control (NC, no growth factors) and a positive control (PC, including 50ng/ml BDNF). Cell culture was performed for 48 hours and cells were subsequently stained for neurofilament to assess neuronal survival and neurite length. The in vitro release of TrkB-AB and BDNF from alginate was tested using 10µl beads including three different concentrations of TrkB-AB and BDNF. Alginate was mixed with different amounts of drug solutions and beads were formed by BaCl2-crosslinking with subsequent incubation at 37°C in artificial perilymph for three weeks. Supernatants were collected after 1h and daily at day 1 to 7, 14, and 21 to test for the released amount of drugs. Pure cross-linked alginate served as NC.
Results: Compared to NC, TrkB-AB had a significant neuroprotective effect in a dose dependent manner at medium concentrations (10pM to 100nM) with a maximum at 1nM. This neuroprotection was comparable to that of BDNF. In addition, the neurite outgrowth was partly positively influenced. TrkB-AB and BDNF solutions were mixable with alginate and stable beads could be formed. Beads stayed intact during cultivation in artificial perilymph and released both drugs over the observed period.
Conclusions: The tested TrkB-AB has a neuroprotective and neuritogenic effect on SGN, which is comparable to BDNF making it a promising candidate for treatment of auditory neurons. In addition, we were able to show that TrkB-AB and BDNF were efficiently released from alginate, indicating it as suitable matrix for delivery of these drugs to the inner ear.

SU165. Development of a Novel Intracochlear Delivery Method Using Biodegradable Microneedles
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Category: Inner Ear: Drug Delivery
Background: For the treatment of chronic sensorineural hearing loss, hair cell regeneration is required. However, efficient delivery of agents into the inner ear for therapeutic purpose still remains a challenge. The purpose of this study is to develop a biodegradable microneedle that can effectively and safely deliver genes or growth factors into the cochlea for hair cell regeneration.
Methods: Biodegradable microneedle was produced by hyaluronic acid (HA). Toxicity of hyaluronic acid was evaluated by HEI-OC1 cell in vitro. Considering the size of the round window membrane and the length of scala tympani of rat, microneedle was designed in a diameter of 300 µm and a length of 3mm. Insertion stylet tool was used for the handling of microneedle. Fluorescein isothiocyanate (FITC) was loaded in the microneedle, and inserted to the round window membrane by postauricular approach. Dispersion of the FITC in the cochlea was observed over time. Finally, in order to evaluate the in vivo safety, the hearing of the animal and histological damage of cochlear tissue was evaluated 2 weeks after the microneedle insertion.
Results: Hyaluronic acid had no cytotoxicity up to 5mg/ml of concentration in HEI-OC1 cell line. FITC was well dispersed from the basal turn to the apex of cochlea after 2 hours of microneedle injection. 4 hours after the microneedle injection, fluorescein faded out in perilymph space. Auditory brainstem response was used to examine hearing preservation of animals after 2 weeks. Two of six rats which had perilymph leakage during RWM injection had hearing impairment. Other four had no hearing damage, and also no histological damage was observed in the basal turn of cochlea.
Conclusions: Hyaluronic acid microneedles can create safe and precise RWM perforation, and its soft and degradable characteristics allow relatively easy handling and hearing preservation. HA microneedle have the

potential to provide safe and effective intracochlear access, especially for the large molecules such as genes or growth factors that are hard to permeate RWM.

SU166. Microvilli Compartmentalization of the Protease, TMPRSSs, Regulates Alpha-Tectorin (TECTA) Release, Collagen Attachment, and the Organization of the Tectorial Membrane

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Category: Inner Ear: Membranes and Fluids
Background: The tectorial membrane (TM) is an apical extracellular matrix (ECM) that plays a critical role in auditory transduction. The TM exhibits a highly organized architecture along its radial axis. Specifically, there is a domain-specific pattern of organization with alpha-tectorin (TECTA) forming dense non-collagenous fibers in the limbal domain and short crosslinking fibers associated with parallel collagen fibrils in the body domain. Previously, we showed that both surface-tethering and the release of TECTA play critical roles in TM morphogenesis by preventing the diffusion of secreted TM components and mediating layer release and matrix elongation, respectively. We identified transmembrane serine proteases (TMPRSSs) and GPI-anchor lipase (GDE3) as the enzymes responsible for the proteolytic and GPI-anchor cleavage of TECTA in-vitro, respectively. Cochlear supporting cells (SC) are the primary cell types producing TM components. Multiple cell types of SCs are characterized by their unique shapes and gene expression profiles. Interestingly, the columnar cells (Co) that generate the body domain of the TM display densely arrayed microvilli on their apical surface that have bundles of collagen fibrils connected to their distal tips. Noting that TMPRSSs are the main releasing enzyme expressed in both the limbal and body domains, we hypothesized that the compartmentalization of TMPRSSs through the distinct shape of the SCs and the microvilli regulates the release of TECTA and mediates the domain-specific organization of the TM.
Methods: To identify the role of surface shape in the compartmentalization of the sheddases, we generated animal models in which each release mode of TECTA was specifically blocked and studied how these mutations impact the organization of the TM by immunohistochemistry and Transmission electron microscopy (TEM).
Results: TEM of R2061S mice that specifically block the TMPRSS cleavage showed an irregular organization pattern for the collagen bundles. Instead of being attached to the microvilli tip membrane, they are attached to the lateral and base membrane and fit vertically into the extracellular space between the microvilli of the Co cells. Furthermore, we observed a significant increase in the microvilli length and extracellular vesicle density around them.
Conclusions: Our findings indicate that the distinct surface shape of SCs regulates the surface release of the TECTA and consequently restrains the collagen attachment site and mediates the domain-specific organization of the TM.

SU167. Effects of Kainic-Acid-Induced Auditory-Nerve Loss on Pure-tone Responses in the Budgerigar Inferior Colliculus

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Category: Midbrain: Structure and Function
Background: Loss of auditory-nerve (AN) fibers is a common pathology in humans. Studies in humans and multiple animal models suggest that audiometric sensitivity is preserved after selective AN injury, possibly due to increasing “central gain” following diminishment of AN input. The budgerigar is a parakeet species that can behaviorally discriminate many simple and complex sounds with similar sensitivity to humans. Budgerigars with moderate-to-severe selective AN injury due to kainic acid (KA) exposure also show preserved tone-detection thresholds. Here we examined the effects of AN loss on pure-tone responses in the budgerigar inferior colliculus (IC), to better understand the neural bases of robust behavioral tone detection following AN injury. We expected mild elevation of IC tone thresholds immediately after KA exposure followed by complete threshold recovery over several days.
Methods: Extracellular neural responses to pure tones (100 ms duration, 0.25–8 kHz, 15–75 dB SPL) in awake budgerigars were recorded via 16-channel microelectrode arrays (Cambridge Neurotech) implanted within the tonotopic region of the IC. After 15-30 days of baseline data collection, excitotoxic AN injury was induced with KA infusions (1-2 mM, 1.2-2.5 µL). Auditory brainstem responses (ABRs) and otoacoustic emissions were measured at multiple time points before and after KA exposure to confirm

selective AN injury without damage to cochlear hair cells. Electrodes remained at the same location during the full recording period (~60 days). Daily neural recording sessions were resumed ~2 days after the infusion surgery.
Results: The characteristic frequencies (CFs) of units sampled with the same recording electrode spanned a range of 1.5-2 octaves. Recording sites had consistent pure-tone responses during the baseline recording period. IC excitatory tuning to tones was sharply V-shaped, with thresholds at CF ranging from 10-30 dB SPL. Units with lower CFs (< 1k Hz) had lower tuning quality (10-dB bandwidth divided by CF) and were slightly less sensitive than higher CF units. Inhibition was often observed above CF at moderate-to-high sound levels (>50 dB SPL). Preliminary results in one animal with unilateral AN damage showed that ~60% loss of contralateral AN input, estimated based on ABR wave I, produced no notable changes in excitatory and inhibitory tone responses in the time period following exposure (i.e., as little as 2 days after infusions).
Conclusions: These experiments quantify the detailed time course of changes in IC tone responses following AN injury. New neurophysiological results will provide valuable insight into the neural bases of preserved audiometric sensitivity with this common cochlear pathology.

SU168. Widespread Projections From VIP-Expressing Glutamatergic Stellate Neurons in the Inferior Colliculus

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Category: Midbrain: Structure and Function
Background: We found previously that the neurons labeled in vasoactive intestinal peptide (VIP)-IRES-Cre x Ai14 mice represent a distinct subtype of neuron in the inferior colliculus (IC) (Goyer et al., 2019). VIP cells form a class of stellate cells that receive inputs from the contralateral IC and the dorsal cochlear nucleus and participate in multiple output pathways of the IC. They are not immunoreactive for markers of GABAergic cells, implying they are glutamatergic. Here, we use in situ hybridization to confirm that IC VIP cells are glutamatergic and express VIP mRNA. Additionally, we use Cre-driven fluorescent protein expression to examine in detail the projection patterns of the VIP subtype of IC neurons.
Methods: VIP-IRES-Cre mice (Viptm1(cre)Zjh/J, Jackson Laboratory, stock #010908; Taniguchi et al., 2011) were crossed with Ai14 reporter mice to yield F1 offspring that expressed tdTomato in VIP neurons (VIP-IRES-Cre x Ai14 mice). Because mice on the C57BL/6J background undergo early onset age-related hearing loss, experiments were restricted to an age range (younger than P70) where hearing loss should be minimal (Zheng et al., 1999). Three mice were used for in situ hybridization (RNAscope) with probes for tdTomato, VIP and Vglut2 (Slc17a6). Eight additional mice underwent intracranial AAV injection (AAV1.CAG.FLEX.eGFP.WPRE.bGH; Addgene #51502) into one IC to examine the projections of VIP cells.
Results: In situ hybridization confirmed that tdTomato expression colocalized with VIP mRNA. In addition, the VIP+ neurons were co-labeled with Vglut2, confirming their identity as glutamatergic. Cre-dependent tracing of VIP axons revealed a surprisingly wide range of projections. We confirmed dense projections throughout the ipsilateral and contralateral IC and to the auditory thalamus, superior colliculus, nucleus of the brachium of the IC and sagulum. In addition, we observed projections to many lower auditory centers (e.g., nuclei of the lateral lemniscus, superior olive), periaqueductal gray, several regions of the reticular formation (cuneiform nucleus, ventrolateral tegmental nu., lateral paragigantocellular nu.) and regions associated with classical neuromodulators (e.g., subparafascicular nu., associated with dopaminergic cells).
Conclusions: The results suggest that VIP+ IC neurons are glutamatergic. The excitatory projections from these stellate cells are surprisingly widespread, suggesting that VIPergic IC neurons play a role in many of the functions attributed to the IC, such as sound localization, speech processing, mediation of defensive behaviors, and modulation of incoming auditory information.
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SU169. CD68 Confirmation of Phagocytosed Multisensory Inputs by Microglia in the Lateral Cortex of the Inferior Colliculus

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Category: Midbrain: Structure and Function
Background: The lateral cortex of the inferior colliculus (LCIC) receives multisensory input arrays that preferentially target its compartmental organization. Inputs of somatosensory origin terminate within modular zones, while projections arising from auditory areas end throughout its encompassing matrix. This discrete mapping emerges during an early postnatal critical period via a process of segregation. Similar to other primitive brain maps, it appears an initial excess of connections is later pruned through a refinement process. Microglial cells (MGCs) have been implicated in a variety of systems in the selective removal and subsequent degradation of engulfed material. Aberrations in map plasticity during early critical periods have been linked to certain neurodevelopmental conditions, including autism spectrum disorders (ASD). Despite mounting evidence linking early multisensory integration deficits with cognitive and behavioral disturbances associated with ASD, the mechanisms that govern multimodal network modifications remain poorly understood. Thus, the present study combines double-labeling tract tracing and immunocytochemical approaches in CX3CR1-GFP mice to determine: (1) whether MGCs actively phagocytose multisensory inputs in the nascent LCIC, (2) if fractalkine signaling (CX3CL1-CX3CR1) influences MGC engulfment behaviors, and (3) whether consumed product is degraded via the MGC's lysosomal pathway.
Methods: Auditory (10,000MW dextran AlexaFluor 647 direct conjugate) and somatosensory (biocytin, DyLight 549 streptavidin) LCIC inputs were labeled simultaneously as previously described (Lamb-Echegaray et al., 2019, doi: 10.1007/s00429-019-01979-6; Weakley et al., 2022, doi:10.3389/fncir.2022.882485) in early postnatal (P0, P4, P8, P12, and P20) CX3CR1-GFP tissue. Immunocytochemistry for CD68, a microglial-specific lysosomal marker, facilitated organelle visualization relative to engulfed material. ImageJ and Imaris software packages were used to perform 3-D surface renderings of MGCs, engulfed contacts, and lysosomes from confocal z-stack acquisitions.
Results: Multimodal inputs to the LCIC at birth are sparse and intermingle as LCIC compartments have yet to emerge. Engulfment of both auditory and somatosensory terminals by individual MGCs is readily apparent throughout the peak period of projection shaping (P4-P12), as compared with that observed after critical period closure (P20). Analysis of 3-D renderings show clear co-localization of engulfed contacts with CD68 labeling. Engulfment assays comparing fractalkine signaling mutants with that of age-matched wild-type and heterozygous mice reveal no significant differences.
Conclusions: These findings implicate MGCs in the selective pruning of emerging multisensory maps in the LCIC, that phagocytosed material is degraded through its lysosomal pathway, and that the mechanisms governing these processes appear to be fractalkine signaling independent.

SU170. Measurement of Three-Dimensional Vibration of the Human Tympanic Membrane Using a Scanning Laser Doppler Vibrometer

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Category: Middle and External Ear
Background: Vibration of the tympanic membrane (TM) converts sound waves in air into mechanical ossicular
chain vibration. As different vibrational patterns of the TM result in different ossicular chain vibration, it is of great importance to thoroughly measure TM vibration for a better understanding of middle ear sound transmission. While previous studies measured one-dimensional vibration of the TM (i.e., perpendicular to the annular ring plane), data on vibrational components tangential to the TM surface are almost completely non-existent.
This study aims to close this gap by developing a technique to measure three-dimensional vibration of the human TM and thus providing useful data to reveal contribution of the TM to middle-ear mechanics.
Methods: TMs with attached malleus and surrounding annular bone were isolated from fresh-frozen temporal bones and laterally connected to an artificial ear canal, where acoustic stimuli were provided and recorded. Vibrational motion and coordinates of approximately 200 evenly spaced points on the medial surface of the TM and four reference points were measured using a scanning laser Doppler vibrometer (SLDV) mounted to a robotic arm. In total, 5 measurements with different angles between the annular ring plane and SLDV axis were performed for

each stimulation frequency and TM specimen.
Based on the documented angles and coordinates of the four reference points, data of all five measurements were registered into a single measurement frame, thus allowing for calculation of velocity components normal and tangential to the annular ring plane. Using micro-CT imaging, morphometric data of each specimen were obtained and registered into the measurement frame as well, allowing for additional calculation of velocity components normal and tangential to the conical-shaped TM surface.
Results: Preliminary data show simple, in-phase motion at low frequencies and complex motion patterns at higher frequencies. At low frequencies, out-of-plane motion is dominant compared to in-plane motion.
Conclusions: A technique to measure the three-dimensional vibrational motion of the TM using a SLDV has been
established. Further work will explore the velocity components at higher frequencies and TMs with altered morphometry after middle-ear reconstructions

SU171. Effects of Cigarette Smoke on Haemophilus Influenzae-Induced Otitis Media in a Rat Model

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Category: Middle and External Ear
Background: Exposure to cigarette smoke (CS) is a factor that could delay or worsen the recovery of otitis media (OM) by causing inflammatory swelling of the Eustachian tube (ET). However, despite the suggested relationship, little is known about the association between OM and CS. Therefore, we aimed to evaluate the effects of CS on the development, progression, and recovery of OM, as well as the histological and molecular changes caused by CS exposure, by using a rat model of OM infected with non-typeable Haemophilus influenzae (NTHi).
Methods: Eighty Sprague-Dawley rats with normal middle ears (MEs) were divided into four groups (n = 20 rats/group): control, CS, OM, and CS+OM. The CS and CS+OM groups were exposed to CS for 2 weeks. The control and experimental groups were further subdivided into sets of 5 rats (10 ears), one for each of the four time points (0, 2, 7, and 10 days after inoculation, at which point the rats were sacrificed). A bacterial suspension of 1 × 105 NTHi cells/mL was used in the experiment. The rats in the CS and CS+OM groups were placed within the chamber on an acrylic plate above the cigarette. One exposure in the chamber was defined as an exposure to one regular-sized filtered cigarette (tar, 12 mg; nicotine, 1.0 mg; Marlboro-Red, Philip Morris International, Neuchâtel, Swiss) every 12 min for a total of 1 h (i.e. 5 cigarettes) and 5 days/week.
Results: All ears in the OM and CS+OM groups showed OM on day 2, and 60% of ears in the CS+OM group still had OM on day 10. The OM group showed the peak thickness on day 2 but showed a steep decrease thereafter; however, the CS+OM group showed a gradual decrease until day 10. In the OM group, cytokine expression peaked on day 2 and decreased steeply thereafter. In the CS+OM group, cytokine expression increased and decreased gradually until day 10. Goblet cell proliferation and mucus secretion in the ET were more significant in the CS and CS+OM groups than in the other groups.
Conclusions: The inflammatory reaction to NTHi was more intense and lasted longer in the CS+OM group than in the other groups. Goblet cell proliferation and mucus secretion in the ET were more significant in the CS and CS+OM groups than in the other groups. These findings suggested that because CS directly affects the ET and ME mucosa, bacterial OM can become more severe and may resolve more slowly in the presence of CS exposure rather than in its absence.

SU172. Near-Infrared Light Emitting-Diode Irradiation Ameliorates LPS-Induced Otitis Media in a Rat Model

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Category: Middle and External Ear
Background: Otitis media (OM) is an infectious and inflammatory disease occurring at the middle ear (ME) that often recurs and requires long-term antibiotic treatment. Light emitting diode (LED)-based devices have shown therapeutic efficacy in reducing inflammation. This study aimed to investigate the anti-inflammatory

effects of LED irradiation on lipopolysaccharides (LPS)-induced OM in rats, human middle ear epithelial cells (HMEEC), and murine macrophage cells (RAW 264.7).
Methods: The animal models were established by LPS injection (2.0 mg/ml) into the ME of rats via the tympanic membrane. The rats and the cells were irradiated with LED wavelengths at 655 nm and 842 nm, intensity: 102 mW/m2, time: 30 min/day for 3 days and 653 nm and 842 nm, intensity: 49.4 mW/m2, time: 3 hrs, respectively, after LPS injection. Hematoxylin and eosin staining was performed to see the pathomorphological changes of the tympanic cavity in the ME of rats. Enzyme-linked immunosorbent assay, immunoblotting and RT-qPCR analyses were used to determine the mRNA and protein expression of interleukin-1β (IL-1β), IL-6 and tumor necrosis factor-α (TNF-α), respectively. The mitogen-activated protein kinases (MAPKs) signaling were examined to see molecular mechanism by which the production of LPS-induced pro-inflammatory cytokines is reduced by LED irradiation.
Results: The animal models were established by LPS injection (2.0 mg/ml) into the ME of rats via the tympanic membrane. The rats and the cells were irradiated with LED wavelengths at 655 nm and 842 nm, intensity: 102 mW/m2, time: 30 min/day for 3 days and 653 nm and 842 nm, intensity: 49.4 mW/m2, time: 3 hrs, respectively, after LPS injection. Hematoxylin and eosin staining was performed to see the pathomorphological changes of the tympanic cavity in the ME of rats. Enzyme-linked immunosorbent assay, immunoblotting and RT-qPCR analyses were used to determine the mRNA and protein expression of interleukin-1β (IL-1β), IL-6 and tumor necrosis factor-α (TNF-α), respectively. The mitogen-activated protein kinases (MAPKs) signaling were examined to see molecular mechanism by which the production of LPS-induced pro-inflammatory cytokines is reduced by LED irradiation.
Conclusions: This study demonstrates that LED irradiation effectively suppressed inflammation caused by OM. Moreover, LED irradiation reduced pro-inflammatory cytokines production in HMEEC and RAW264.7 cells through blockade of MAPKs signaling.

SU173. Specific Input Ear Impedance and Eardrum Energy Reflectance: Comparison Between Pressure Measurements and Intensimetric Measurements

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Category: Middle and External Ear
Background: The evaluation of the middle ear reflectance is a crucial issue in the diagnosis of hearing damage due to clinical pathologies or ototoxic agents (e.g., noise, vibration, chemicals, etc.). Useful indicators are the specific input ear impedance (Z) and the eardrum energy reflectance (R), as shown by, e.g., Allen et al. 2005 (JRRD 42, 63-78), Withnell et al. 2009 (Hear. J. 62, 36-39), Ibraheem 2014 (AAAA 01, 87-96). Z is the ratio of acoustic pressure (P) to the acoustic field velocity (V). R is the squared magnitude of the ratio of backward to forward propagating acoustic pressure. The measurement of Z is a critical issue when only P is measured with a standard microphone equipped with loudspeakers (P-probe). The Thevenin parameters of the P-probe (pressure PTh and specific impedance ZTh) are preliminarily measured using a set of cavities of known acoustic impedance (Keefe 1984, JASA 75, 58-62; Keefe et al. 1992, JASA 91, 470-485), and Z is computed by measuring the response of the P-probe inserted in the ear canal. In this study, we propose the direct measurement of Z obtained measuring both P and V using a P-V detector equipped with loudspeakers (PV-probe).
Methods: The two techniques are compared by measuring Z and R in eight normal-hearing ears. In the P-probe case, an Etymotic ER-10B+ microphone equipped with two ER-2 loudspeakers was used. PTh and ZTh were estimated using five tubes of known impedance. In the PV-probe case, a Microflown PU-Match p-v detector equipped with two Etymotic ER-2 loudspeakers was used. Z and R high-resolution complex spectra obtained with the two probes in the 1000-6000 Hz range were averaged over all tested ears. Differences in the probe insertion depth between the two probes requested introducing in the analysis an arbitrary depth shift, which is an additional uncertainty source.
Results: The two techniques yielded comparable results for Z and R in the range 2000-5000 Hz, with trends similar to those reported by, e.g., Withnell and Gowdy 2013 (JARO 14, 611-622), Voss and Allen 1994 (JASA 95, 372-384), Allen et al. 2005 (JRRD 42, 63-78). However, the P-probe method introduces larger fluctuations, mainly due to the phase-sensitive calculations involved in the estimate of PTh and ZTh, and of Z from P, PTh and ZTh.
Conclusions: The PV-probe provides a more direct measure of Z, yielding values of R that are not affected by the phase-sensitivity of the numerical procedures of the P-probe method. On the other hand, the currently

available velocity probes are delicate devices, sensitive to dust, and affected by relatively higher noise levels, with respect to the best available microphones. Further investigations are planned to improve both techniques, particularly in the frequency ranges below 2000 Hz and above 5000 Hz.

SU174. Comparison Between IVUS and OCT for Investigation of the Eustachian Tube

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Category: Middle and External Ear

Background: Eustachian Tube dysfunction (ETD is one of the most frequent diseases in otolaryngology and results in inadequate equilibration of the pressure in the middle ear. The underlying mechanism of ETD remains poorly understood, except for obvious causes like nasopharyngeal tumors or cleft palate. With the current imaging methods such as computed tomography, magnet resonance imaging or endoscopy, a detailed analysis of the Eustachian tube (ET) and its epithelium is hardly possible. Optical coherence tomography (OCT) and intravascular ultrasonography (IVUS) are two lumen-oriented methods that potentially could be used to investigate the ET in more detail.

Methods: Experiments were conducted in a fresh frozen human cadaver head after thawing for more than 24 hours. IVUS was performed using an Eagle Eye Platinum catheter and a retraction speed of 0.5 mm per second. To allow OCT imaging without the risk of damaging the middle ear, 30 mm of the tip of the 1.3 mm Dragon fly OCT catheter were cut off. Then the catheter was inserted into the ET through a translucent BUSTER dog catheter with an outer diameter of 1.5 mm without contact with the surrounding tissue. Additional scans were performed after injection of hyaluronic acid (HA) in the tissue adjacent to the ET. Positions of the catheters were additionally documented by cone beam CT.

Results: OCT and IVUS catheters could be inserted into the ET without visible damage. Due to the larger diameter of the protecting outer catheter and the small diameter of the isthmus of the human ET, insertion depth of the OCT fiber could be limited in some cases to the cartilaginous part of the ET. Quality of the scans was reasonable in all cases with the OCT providing a higher resolution in surface near regions whereas the IVUS enabled a larger penetration of the tissue.

Conclusions: Application of both methods appears feasible for investigation of the ET. Modifying the tip of the commercial OCT catheter and use of a translucent outer catheter to protect the tissue from damage enables application of OCT to investigate the human ET.

SU175. Shaping Spectral and Threshold Properties in the Auditory Cortex With Bimodal Stimulation in Guinea Pigs

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Category: Multisensory Processing/Interactions

Background: Bimodal stimulation is a method of applying two stimuli from different modalities or locations simultaneously and has been shown to alter activity in the brain. This method has been used to treat conditions such as essential tremor and tinnitus. Bimodal stimulation with acoustic and electrical stimuli has been shown to induce auditory plasticity, characterized as changes to frequency selectivity and thresholds. This plasticity can potentially be applied to enhance hearing, in individuals with or without hearing loss, by improving frequency selectivity or thresholds in compromised or targeted hearing regions. Multiple variables may affect the outcomes of this bimodal stimulation including stimuli amplitude, duration, latency and location; acoustic frequency; and electrical pulse pattern. This study aims to characterize the types and magnitude of the effects of these variables on plasticity in the auditory cortex.

Methods: The right cortex of ketamine-anesthetized female Hartley guinea pigs is exposed. A 4-shank, 32-site electrode array is inserted into the right primary auditory cortex (A1), with sites centered at layer IV. Bimodal stimulation consisting of acoustic and electrical stimulation is delivered using a closed-field speaker to the left ear and subdermal electrodes to both the left and right ear. Future experiments will stimulate other body regions. Tuning curves for A1 are collected before and after bimodal stimulation. Best frequencies, Q10 and Q30 values, spiking activity, and thresholds are analyzed for each site to characterize plasticity before and after bimodal stimulation.

Results: Initial data shows changes to tuning curves including best frequencies, Q10 and Q30 values, and thresholds similar to previous studies. However, changes were not as large as in previous studies, which used awake animals.

Conclusions: Changes were not as large possibly because of the use of anesthesia in this study. Previous studies also did not use electrical stimulation of the ear; more effective regions of the ear may need to be identified, such as the region near the vagus nerve, which has been shown to induce plasticity in other regions of the brain. A broader range of stimuli may also need to be used to induce larger changes. These initial experiments can lead to the use of bimodal stimulation to shape spectral and threshold properties in A1 and a noninvasive and accessible device for enhancing hearing.

SU176. Visual Bias for Speech Perception in Age-Related Hearing Loss: An Eye-Tracking Study

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Category: Multisensory Processing/Interactions

Background: Adults with hearing loss appear to show a bias toward visual processing during audiovisual perception, as shown by more frequent fusions of McGurk stimuli (Rosemann and Thiel 2018, Neuroimage 175:425-37) and stronger susceptibility to visual distractors during an auditory task compared to visual tasks with auditory distractors (Puschmann et al. 2014, Hear Res 316: 28-36.). Visual bias could result from cross-modal reorganization where auditory neurons are repurposed to support visual perception, or from a chronic reliance on the visual modality to resolve auditory signals degraded by hearing loss. Here we test how visual bias in hearing loss is affected by environmental background noise, and how visual attention plays a role. Participants completed a task requiring them to identify if a visual sentence preceding an auditory sentence (or the reverse of this order) matched or mismatched, and eye fixations were recorded for visual sentences. We predicted that adults with age-related hearing loss (ARHL) will benefit more when presented with visual speech before auditory speech. Second, we predict that participants with greater hearing loss will focus more on the mouth of the speaker when presented with auditory speech then visual speech. Lastly, we predict that participants who spend more time looking at the mouth of the speaker will perform better when presented with visual speech before auditory speech.

Methods: Participants aged 40 to 80 with untreated hearing loss or typical hearing participated in the study. All participants underwent pure-tone audiometry to 8 kHz, and QuickSIN measured speech-in-noise (SIN) listening. Participants were presented with audio and visual IEEE sentences across two manipulations. First, sentences were presented with audio-only presentations preceding visual-only presentations, or in reverse of this order. Second, sentences were presented in either quiet or −5 and −10 dB SNR (babble noise). For each trial, participants reported if the audio and visual sentences were the same or different. Half of the trials had mismatched audio and visual sentences, and in half of the trials the sentences were matched. During the task, eye-tracking measured visual attention to facial features (e.g., mouth, eyes) for visual sentences.

Results: Data collection is ongoing.

Conclusions: Results consistent with our hypotheses may suggest that visual speech creates stronger perceptual priors in ARHL that could compensate for auditory signals degraded by HL, especially for individuals directing attention to orofacial cues and listening in the presence of background noise.

SU177. Functional Implications of a Patch/Matrix-Like Compartmental Organization in the Mouse Inferior Colliculus

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Category: Multisensory Processing/Interactions

Background: The inferior colliculus (IC) is an obligatory relay station and massive convergence center for auditory information. In addition to its role in sound processing, the IC receives inputs from diverse multisensory and neuromodulatory structures and is implicated in acoustico-motor behavior. The lateral cortex of the IC, a multisensory-recipient region, contains a network of neurochemical modules, similar to the patch/matrix system of the basal ganglia, that subsect this structure into discrete processing regions. Somatosensory inputs to the IC target these modules, which stain heavily for markers of inhibition, plasticity, and metabolic processing, while auditory inputs target complementary extramodular zones. While these auditory inputs have been shown to mediate diverse functions, including predictive processing and flight behavior, the role of somatosensory inputs to the IC is unknown. Previous studies have shown that inputs from the somatosensory cortex target inhibitory colliculo-thalamic projection neurons in the neurochemical modules, leading to suppression of auditory responses in the auditory thalamus. These results

suggest that modular regions of the IC may serve as somatosensory-driven gating regions for auditory information.
Methods: To test this hypothesis, we trained mice to perform a go/no-go task in which they lick for a water reward after presentation of a noise target, with the goal of then selectively activating somatosensory-inputs to the IC on a subset of behavioral trials to determine how this affects target detection. We also performed anterograde trans-synaptic labeling of somatosensory-recipient neurons in the IC. In addition to assessing the functional role of somatosensory inputs to the IC, we used two-photon imaging to determine the sound response properties of neurons in modular and extramodular regions of the IC.
Results: Preliminary data suggest that mice can learn the go/no-go task paradigm with high accuracy following ~3 weeks of training. Axon fibers from trans-synaptically labeled somatosensory-recipient IC neurons were found in known targets of the lateral cortex, including the medial geniculate body and the superior colliculus. Two-photon imaging of IC responses to pure tones, FM sweeps, noise, and vocalizations was successfully performed.
Conclusions: The results of the experiments will determine what effect somatosensory input to the IC has on sound processing and target detection and will show whether modular and extramodular regions of the IC have distinct sound processing features.

SU178. A New Signal-Detection Theory Based Method for Quantifying Audiovisual Speech Perception
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Category: Multisensory Processing/Interactions
Background: The comprehension of speech is often supplemented by watching a talker's facial movements. We present a new method which will aid our understanding of how we integrate auditory and visual information, focussing on whether audiovisual performance depends only on the unimodal-information or whether the integration process itself varies across individuals and listening situations. Our goal is to develop tools which enable visual cues to speech intelligibility to be taken into account when treating hearing impairment.
Methods: We developed an analysis based on signal detection theory (SDT), to quantify how auditory and visual information are integrated.. According to SDT, the benefits of multiple modalities depends on whether internal noise occurs in unimodal processing, or in later processing after multisensory integration (Micheyl and Oxenham, 2012; J Acoust Soc Am. 131:3970). Both sources of internal noise may be present (Stacey et al. 2016; Hear Res. 336:17). Our novel analysis accounts for both, and is implemented within a Bayesian statistical framework (brms/Stan/R), allowing quantification and identification of where differences in multisensory processing arise. To evaluate the potential of the model for quantifying individual differences, audio-visual, audio-only and visual-only speech perception (IEEE sentences) in noise (3 SNRs) was measured via on-line testing in 37 normal-hearing participants who were presented with 148 sentences, including 50 sentences where there was no sound (visual-only speech).
Results: We sought to quantify individual differences in audio only, visual only and audiovisual speech perception. Analysis with the SDT model revealed robust individual performance differences in audio-only and visual-only speech perception. Individual audiovisual performance could be explained as a function of an individual's unisensory scores via the SDT model. The model required to explain the data suggested that performance was limited by a mix of unimodal and post-integration noise. However, individuals did not differ markedly in the proportions of these noise sources. In other words, these individuals differ in their ability to use the individual senses, but integrate across the senses in a similar way.
Conclusions: This method offers a theoretically principled and practical way to quantify relationships between audio-visual speech perception and audio-only and visual-only speech perception. It can identify and quantify where specific, even individual, differences lie. The method is flexible, and easily extended to include any desired predictor variables. Such models have clinical importance; they have the potential to predict the visual speech benefits for people with hearing loss, and thus allow these benefits to be effectively accounted for when treating hearing impairments.

SU179. Neural Mechanisms of Audio-Tactile Speech Integration
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Category: Multisensory Processing/Interactions

Background: Speech is a complex signal that contains a hierarchical structure, spanning from phonemes to syllables, words and sentences. Therefore, for the brain to process it, it is necessary to first segment the continuous speech stream into smaller subunits. This process presumably relies on neural oscillations in the delta and theta frequency ranges (1-4 Hz and 4-8 Hz) in the auditory cortex. In particular, the theta-oscillations appear to track incoming speech at the rhythm of syllables. The activity in this frequency band can be modulated through the somatosensory system and could potentially affect speech processing.
Methods: Here we used vibrotactile pulses at the syllable rate to investigate the effect on speech-in-noise comprehension. The pulses were aligned to the centre of syllables and a shift in time was introduced to study the effect of different lags. Speech comprehension was assessed using semantically unpredictable sentences. Moreover, we studied the neural encoding of speech and pulses through electroencephalographic recordings (EEG) while subjects were listening to continuous speech.
Results: We found that vibrotactile feedback can modulate speech-in-noise comprehension following oscillations at the syllable rate. Moreover, speech comprehension could be enhanced through the tactile stimulation as compared to a sham condition or the audio-only signal This enhancement was maximal when the auditory and tactile streams were synchronized. We similarly observed that the audio-tactile stimulation modulated the neural responses to both speech and tactile pulses, thus reflecting our behavioural findings. This multisensory activity was detected early on in the auditory cortex. Finally, we demonstrated that the comfort of subjects in response to these stimuli could be predicted from the electrophysiological markers of multisensory integration.
Conclusions: Our results therefore evidence of a role of cortical oscillations and somatosensory information in speech processing and opens avenues for applications in auditory prosthesis

SU180. Visual Plasticity and Functional Communication in Hearing Aid Users

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Category: Multisensory Processing/Interactions
Background: Functional communication outcomes for hearing aid users are dependent on both speech recognition and satisfaction. Though numerous studies have examined the impact that different intrinsic (e.g. age, degree of hearing loss) and extrinsic (e.g. technology, cost) factors have on hearing aid satisfaction, findings have not always been consistent. One factor that could account for this inconsistency is differences in the way the brain processes sensory signals. There is evidence for changes in sensory processing following deafness, but little is known about how these changes impact functional communication outcomes for hearing aid users. This exploratory research aims to investigate the role of neural plasticity in the variability of functional communication outcomes for hearing aid users.
Methods: A prospective study was completed with nine bilateral hearing aid users with symmetric sensorineural hearing loss (planned N = 30). A visual temporal order judgement task, a McGurk illusion task, and an auditory, visual and audiovisual speech in noise task were used to characterize performance, unisensory weighting, and integration of auditory and visual stimuli. Since behavioral similarities may mask differences in underlying neural differences, functional near infrared spectroscopy (fNIRS) was also used to assess neural activation in response to visual and audiovisual stimuli. These behavioral and objective measures were then compared to standardized measures of patient satisfaction with hearing aids.
Results: Behavioral data suggest that there is not only a large degree of variability in functional communication outcomes for hearing aid users, but also a large degree of variability in visual temporal thresholds (39 – 415 milliseconds) and audiovisual integration (audiovisual gain = 31-83%). Early findings indicate that higher levels of audiovisual integration are significantly correlated with less satisfaction with communication outcomes ($r = -.496$, $p = .018$). Multivariate analyses including the fNIRS data is ongoing and will be discussed once data collection is complete.
Conclusions: Differences in neural plasticity may contribute to the variability in functional communication outcomes for hearing aid users. In turn, this understanding could influence clinical decision making in determining both hearing aid and cochlear implant candidacy.

SU181. The Human OPA1delTTAG Mutation Induces Adult Onset and Progressive Auditory Neuropathy in Mice

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Category: Other, Auditory neuropathy
Background: Autosomal dominant optic atrophy (DOA) is one of the most frequent forms of hereditary optic neuropathies, and is mainly caused by heterozygous variants in the OPA1 gene encoding a mitochondrial dynamin-related large GTPase. In the last decade, the clinical spectrum of DOA has been extended to a wide variety of syndromes, including deafness, called dominant optic atrophy plus (DOAplus). To date, the mechanisms underlying the deafness in DOA remain unknown.
Methods: To gain insights into the pathophysiological mechanisms, we have used a transgenic mouse model carrying a recurrent Opa1delTTAG mutation recapitulating the DOAplus syndrome. Hearing function was assessed with auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs). Cochlear cell morphology was analyzed using transmission (TEM) and scanning (SEM) electron microscopy. Molecular dissection of the OHC lateral wall components, mitochondrial functions and oxidative stress were assessed using immunolabeling methods, qPCR, Western blot and spectrophotometric method.
Results: Our results reveal that the Opa1 delTTAG het mice displayed an adult onset and progressive hearing loss, as attested by the ABR threshold shift over time. However, the mutant mice harbored larger otoacoustic emissions in comparison to WT. Finally, the endocochlear potential, which is a proxy for the functional state of the stria vascularis, was comparable between mutant and WT mice. Ultrastructural examination revealed a selective loss of sensory inner hair cells, together with a progressive degeneration of axons and their myelin sheaths of the afferent terminals of the spiral ganglion neurons in mutant mice. These results are therefore in favor of an auditory neuropathy spectrum disorder. Molecular assessment demonstrated increased age-related depletion of MtDNA, oxidative stress, autophagy and mitophagy with impaired autophagic flux.
Conclusions: These results support a new role for the OPA1 in contributing to the maintenance of inner hair cells and auditory neural structures and opens new perspectives for the exploration and the treatment of OPA-linked deafness.

SU182. The Effect of Nature Versus Nurture on Implicit Auditory Learning: Evidence From Children With Cochlear Implants and Normal-Hearing Children From Low and High Socio-Economic Status

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Category: Other, Auditory prosthesis and auditory cognition
Background: Spoken language development has been shown to depend on auditory processing mechanisms that extract regularities in the auditory input. To date, the few studies that attempted to assess the extraction of sequential regularities in cochlear implant (CI) users have done so in the visual modality despite the fact that the primary mode for speech and language development is via hearing. Therefore, such learning should be assessed in the auditory modality. Moreover, it is not clear whether CI provides sufficient auditory information to facilitate such learning. The goals of the present study were: (1) to assess implicit auditory sequence learning (IASL) in CI children (CIC) users using linguistic and environmental stimuli (LS and ES); (2) to compare CI performance to that of normal hearing peers from high (NH-H) and low (NH-L) socioeconomic status in order to tease apart auditory from linguistic deprivation; and (3) to associate outcomes of IASL with speech or language performance.
Methods: A total of 78 children participated in the study: 16 CIC (6-15 years old), 25 NH-H and 25 NH-L (6.5 -8.8 years old) and 12 NH-H (13-15 years old). A serial reaction time (SRT) task was applied with two sets of natural auditory stimuli: ES (dog barking, bird singing, door knocking and bell ringing) and LS (/sa/, /ta/, /si/ and /ti/). For each set of stimuli, children listened to 5 blocks (each with 108 sounds), 4 of which had a repetitive order of sounds (12 sounds in a sequence repeated 9 times) and one block (the 4th) with a different order. After each stimulus, participants were required to key what they heard from four possible alternatives. Measures included reaction time (RT) to correct responses. The underlying assumption was that if learning of the stimuli pattern occurred, a decrease in RT will be observed during the first 3 blocks, then an increase in the 4th block after which RT will continue to decrease in the last block.
Results: (1) Clear learning patterns were observed for all groups; (2) CIC and NH-H had similar RT, whereas NH-L had significantly longer (poorer) RTs for LS compared to CIC and NH-H; (3) Learning from

blocks 1 to 3 was similar and significant for CIC and NH-H; (4) RT on first block was associated with speech perception for CIC and language skills in NH.
Conclusions: (1) The CI device provides sufficient auditory information for implicit auditory learning; (2) Insufficient exposure and interaction with spoken language negatively impacts the development of auditory learning; (3) Duration of auditory processing (RT) is the main factor associated with speech perception and language skills. These outcomes provide important insights to the development of auditory cognition with clinical implications to auditory and language intervention.

SU183. Thin Film Zwitterionic Hydrogel Coating on Inner Surfaces of Tympanostomy Tubes Facilitates Mucus Discharge

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Category: Other, Biomaterial Research
Background: The most common childhood surgery in the United States is placement of tympanostomy tubes with over 667,000 cases per year. Tubes are placed for children with recurrent cases of OM, allowing ventilation and discharge of the infected middle ear to compensate for the poor function of developing eustachian tubes. Despite their frequent use, a major complication is obstruction of the lumen of the tympanostomy. Obstruction can result from mucus stasis, dehydrated conditions that dries mucus, and/or deposition of granulation tissue within the tube. Plugged tympanostomy tubes carry the risk of chronic OM, hearing loss, and further surgical intervention. A potential solution to this problem comes in the form of thin film zwitterionic hydrogels. Our lab has demonstrated that these hydrogels lead to a layer of hydration that is extremely versatile. The hydrogel has been shown to increase lubricity and reduce friction, which may prevent mucus stasis. The intensely hydrophilic nature of the hydrogel can prevent desiccating conditions, thus keeping mucus hydrated. Additionally, it has been previously demonstrated that the thin films exhibit anti-fouling properties that prevent immune tissue formation and deposition. The purpose of this study was to determine how thin film zwitterionic hydrogel coatings on the inner surface of tympanostomy tubes would affect the dynamics of mucus discharge and obstruction.
Methods: Thin films were first photografted onto PDMS sheets using sulfobetaine methacrylate (SBMA), carboxylbetaine methacrylate (CBMA), or polyethylene methacrylate (PEGMA) as the principal monomer. Using mucus harvested from banana slugs, the sheets were used to test mucus contact angles, force required for mucus flow, and the effects of cross-link density. The thin films were photografted onto the lumen of PDMS tubes. The tubes were used to test the amount of force required to discharge mucus from a tube. Each variable was compared to uncoated PDMS.
Results: Zwitterionic thin films were shown to dramatically reduce the mucus contact angle and the force required to initiate mucus flow. All hydrogel types dramatically reduced the contact angle, though CBMA saw the largest reduction. Each hydrogel type was shown to reduce the force required to initiate flow on sheets, with no significant difference between hydrogel types. It was determined that mucus flowed most optimally between 10-25% cross-linker percentages. When testing SBMA-coated PDMS tubes, there was a significant reduction in force required to discharge the mucus through the tube.
Conclusions: The increase in mucus flow from the addition of thin film zwitterionic hydrogel coatings is likely attributed to its hydrophilic properties. As mucus is made predominately of water, it spreads itself to associate with the hydrogel, preventing large and static blockages of the tube. Coating of tympanostomy tubes with the hydrogel has a high potential to improve mucus flow and prevent stasis that would lead to plugging.

SU184. Phase Locking of the Gamma Brain Wave When Listening to News Program and Music Whose Background Sound are Amplitude-Modulated at 40 Hz

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Category: Other, Brain
Background: Improvement of the cognitive function by presenting sound stimuli with a repetition period of 40 Hz is expected. In previous studies, however, only the stimulus of pulse train or simple sinusoidal tones

were used. Therefore, we investigated the synchronization of the brain wave (electroencephalograms) when presenting more natural sounds that we listen to on our daily lives.
Methods: The recordings of a news program and a music program of Japanese TV were used. From each program, four parts were selected: opening section, economy, entertainment, and weather forecast from the news program, and songs by two female and one male solo vocalists and one male vocalist group from the music program. Each sound source was separated into voice/vocal part and the other background part by using a commercial software SpectraLayers 7 (Steinberg, Germany). As stimuli, the original sound with 100-% amplitude modulation by sinusoidal and inverse-sawtooth wave, the sound whose background parts were 100-% modulated by 40-Hz sinusoidal and inverse-sawtooth wave, and the sound without modulation were used. In addition, 1-kHz sinusoidal wave, 1-kHz sound modulated by 40-Hz sinusoidal and inverse-sawtooth, and a pulse train with a period of 1/40 s (each pulse contains 1 cycle of 1-kHz sinusoidal wave) which was used in a literature (Martorell, 2019) were used.
Each stimulus with a duration of 15 s were presented randomly four times at an A-weighted equivalent sound level of 72 dB (dBA) via earphones. In this report, we present the result of an active electrode at Cz channel. The phase locking indices (PLI) of the 40 Hz components of the derived signals were calculated. 17 young participants with normal hearing were employed. This study was performed in accordance with protocols approved by the ethical review committee.
Results: As a result, clear increases of the PLI were seen in all stimuli with amplitude modulation compared to the non-modulated stimuli, whose PLIs were less than 0.03. First, the PLIs of the pulse train and the inverse-sawtooth modulation of 1 kHz wave were 0.40 and 0.35, respectively, which were quite high. Next, the 100-% modulated news program and music showed high synchronization with the PLIs of around 0.20 to 0.25. In addition, the sound only whose background sound were modulated also showed clear synchronization with the PLI of around 0.10 to 0.15, telling us that the modulated sound stimuli without reducing the intelligibility of the narrations or vocal still can synchronize the brain wave at its modulation frequency.
Conclusions: Synchronization of the electroencephalograms when presenting sound stimuli whose background sounds were amplitude-modulated was confirmed. This implies the possibility of improving cognitive function by processing the sounds we hear on our daily lives without reducing their intelligibilities showing the possibility of clinical application.

SU185. Two-Talker Babbles, But Not Steady-State Noise, Impair Cognitive Function
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Category: Other, Cognition and speech perception
Background: Speech perception depends on not only peripheral factors such as hearing thresholds but also cognitive factors such as selective attention and working memory. The cognitive factors possibly perform a more significant role than the peripheral factors in noisy listening environments (e.g., the cocktail party effect). Unlike speech perception, which can be evaluated in both quiet and noise listening conditions, cognitive function has been overwhelmingly evaluated in quiet. Thus, it remains unclear whether noise directly impairs cognitive function, and if so, how much the impaired cognitive function contributes to the speech-in-noise difficulty. The present study addresses these two questions by measuring the effects of two types of noise on cognitive function and speech perception in the same group of human adults under the same conditions.
Methods: Cognitive function included measurements of auditory selective attention, auditory or visual forward and backward digit span in three conditions: (1) quiet, (2) steady-state noise, (3) two-talker babbles. In the selective attention task, subjects identified two keywords signaled by a specific call sign presented with a distractor sentence. In the digit span task, subjects repeated back as many digits (0-9) as they could, either presented auditorily or visually on a laptop screen, in the original or reversed order. The signal was always presented to the right ear in auditory tasks, and noise to the left ear in both auditory and visual tasks. Speech reception thresholds were measured using the HINT sentences in the same steady-state noise and two-talker babbles, but presented diotically. All auditory stimuli were presented at 80 dB SPL through Sennheiser-280D headphones for all conditions. Normal-hearing, normal-vision and cognitively functioning young adults performed all tasks in a double-walled sound booth.
Results: Preliminary data from 20 subjects showed that compared with performance in quiet, two-talker babbles significantly impaired all cognitive tasks (all $p < 0.05$) but the visual backward digit span, whereas

the steady-state noise did not produce any effects. Cognitive function affected speech reception thresholds differently: better speech in noise recognition was correlated with higher selective attention in both types of noise (r = -0.48; r=-0.53), auditory forward and backward digit span in the two-talker babbles only (r = -0.56; r=-0.54), but not with the two visual digit span measures.
Conclusions: The most interesting finding was that cognition is impaired by the two-talker babbles but not the steady-state noise, suggesting that the intelligible babble contains information requiring auditory selective attention and working memory. However, the impaired auditory selective attention contributed more than working memory to speech in noise recognition. This conclusion may be limited to the high-context sentences used in measuring speech reception thresholds. The present study suggests that future research should consider including cognitive measures in relevant noise to quantify the cognitive role in speech recognition.

SU186. Symmetric Stimulation of the Distal Branch of a Transected Facial Nerve

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Category: Other, Facial Nerve Reanimation
Background: Our goal is to demonstrate, using a rat model, that it is possible to stimulate a facial nerve that has sustained injury without repair to produce movement that is spatially and temporally symmetric to a movement on the contralateral uninjured side.
Methods: All procedures were approved by an Animal Care and Use Committee and conducted in accordance with the United States Public Health Service's Policy on Humane Care and Use of Laboratory Animals. Urethane was used for anesthesia and temperature was maintained with a thermal light. Using careful dissection, the facial nerve was exposed and seen emanating from the stylomastoid foramen. Upon completion of exposure of the facial nerves bilaterally, the rat was transferred to the nerve stimulation area upon which the rat received kainic acid (5mg/kg) to induce seizure activity.
Both left and right facial nerves were recorded from with and without stimulus to demonstrate normal nerve activity. The nerve on the right was transected to elicit facial nerve damage. The damaged nerve was recorded from with and without stimulus. The filtered nerve signal was passed through a custom window discriminator that generated a trigger pulse for each action potential that fell within the specified amplitude window set on the discriminator. Trigger pulses drove constant current stimuli from the digital pulse generator. The threshold for the amplitude was determined by looking for rhythmic (vibrissae) whisker movement while adjusting the stimulus.
Nerve stimulation was performed using a digital pulse generator with stimulus isolation from ground. Pulse widths of 0.001 ms to 1 ms with stimulus current amplitudes from 0.01 microAmps to 5 microAmps was tested to identify the optimal threshold (T) stimulus conditions. Whisker tracking was achieved through video based quantitative analysis.
Results: Episodes of kainic-acid-induced whisking activity with regular amplitude and ~10-12 Hz frequency were visible on the intact side, as was more irregular whisking on the bisected side resulting from our electrical stimulation of the distal nerve segment triggered by nerve activity recorded on the intact side. Deflections were smaller under anesthesia combined with kainic-acid treatment than in awake animals. Video-based quantitative analysis demonstrated synchronized whisker movement on the damaged nerve with nerve stimulation.
Conclusions: This study has demonstrated successful symmetric stimulation of the distal branch of a transected facial nerve driven by monitoring of the healthy facial nerve, thus demonstrating that stimulation recording methods work. Trigger pulses using constant current stimuli from the digital pulse generator produced whisker movement on the damaged (transected nerve) side. However, these experiments were performed on a freshly transected facial nerve wherein the axons have not undergone Wallerian degeneration or undergone a period of recovery and/or regrowth. Future studies will extend this system to the stimulation of facial nerves after repair from injury and regeneration.

SU187. Hyperacusis is Associated With Reduced Gray Matter Volume in the Right Supplementary Motor Area

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Category: Other, Hyperacusis
Background: Hyperacusis occurs in about 9% of the adult population (Baguley et al. 2003). There is a strong association between hyperacusis and tinnitus, with up to 90% of individuals with hyperacusis reporting concurrent tinnitus, and 80% of individuals with severe tinnitus reporting hyperacusis (Cederroth et al. 2020). Functional imaging studies in humans have found that hyperacusis is associated with increased sound-evoked responses in cortical and subcortical nodes of the auditory pathway (Gu et al. 2010; Koops et al. 2021). However, nothing is known about the structural changes that may be associated with hyperacusis. In this retrospective study, we used whole-brain voxel-based morphometry to investigate gray matter volume (GMV) changes associated with hyperacusis.
Methods: Sixty-six participants with tinnitus and hearing loss were included from two previous MRI studies. Hyperacusis was defined by a cut-off score ≥22 on the Hyperacusis Questionnaire (Aazh et al. 2017), resulting in 25 subjects with hyperacusis (59±8 y.-o., 10 females) and 41 subjects without hyperacusis (58±11 y.-o., 9 females). For every participant, pure tone audiometry was performed at octave frequencies from 0.25 to 8 kHz, and additional clinical characteristics were assessed using self-report questionnaires about handedness (EHI), anxiety and depression (HADS), and tinnitus burden (THI). T1-weighted whole-brain images were acquired using a Philips Itera 3T MRI scanner with voxel size of 1x1x1mm3. Pre-processing of the data included spatial normalization, modulation, segmentation, data quality check, and smoothing (12mm FWHM) using the CAT12 toolbox. Whole-brain two-sample t-tests were performed using age, handedness, hearing thresholds, and TIV as covariates to investigate GMV changes associated with hyperacusis. Statistical maps were thresholded at $p \leq 0.05$ with FWE correction at the voxel-level.
Results: Participants with hyperacusis had significantly lower GMV in the right supplementary motor area (SMA) compared to those without hyperacusis. Furthermore, a receiver operating characteristic curve showed that right SMA GMV could classify participants with hyperacusis and controls well above chance (AUC=0.82, $p \leq 0.001$). There was no significant correlation between right SMA GMV and the levels of anxiety, depression or tinnitus handicap (HADS and THI scores, $p > 0.05$).
Conclusions: This study suggests that hyperacusis is associated with decreased GMV in the right SMA. The SMA is involved in action preparation (Nachev et al. 2008; Lima et al. 2016). The SMA neurons fire before a movement is initiated and can trigger a behavioral response to an external (auditory) cue. From this perspective, altered SMA GMV might reflect the tendency to react to sounds perceived as too loud. In support of this hypothesis, animal studies found that hyperacusis is associated with shorter response latencies to sounds as a function of intensity (Auerbach et al. 2019). Future work should investigate the role of the SMA in relation to hyperacusis.

SU188. A Chinchilla Mini-Eeg Cap Improves Cross-Species Translation for Cortical and Subcortical Evoked Potentials

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Category: Other, Methodologies in Electrophysiology
Background: The translation of auditory-evoked-potential research hinges on careful stimulus design, acquisition of electrophysiology, and post-processing of data collected at the bench in preclinical models of hearing loss. Therefore, there is exceptional utility in methods that reduce the differences in experimental design between measurements of evoked responses in animal versus human subjects. We adapted a 32-channel rodent EEG mini-cap (Cortech Solutions' ActiveRat) to bolster the translatability of our cross-species work on synaptopathy, age-related hearing loss, and pitch. Here, we evaluate the feasibility of using the mini-cap to collect chinchilla Auditory Brainstem Responses (ABRs), Frequency-Following Responses (FFRs), Mismatch Negativity (MMN), Acoustic Change Complex (ACC), and temporal coherence (COH) measures in the same manner as collected in humans.
Methods: Subcortical (ABR, FFR) and cortical (MMN, ACC, COH) responses were collected from 5 normal-hearing chinchillas. Stimulus presentation was conducted using Tucker-Davis Technologies hardware and custom MATLAB software. Potentials measured by all electrodes were recorded using the BioSemi ActiveTwo system. To study the effects of our anesthesia protocol, these measures were collected with and without ketamine/xylazine. Three subdermal electrodes (mastoid, vertex, ground) were positioned prior to mini-cap placement to validate and compare mini-cap responses with our gold-standard paradigm.

All data was analyzed using the same framework (Python, MNE) we have previously used to process these measures in humans.
Results: The mini-cap captures subcortical responses with waveform morphology and amplitudes comparable to those collected with subdermal electrodes. Preliminary data suggest that the response SNR from individual channels is slightly lower than subdermal electrodes, though this can be ameliorated by averaging data across neighboring channels or increasing the number of repetitions. Spatial visualization of channels revealed a marginally higher amplitude of subcortical responses in posterior channels rather than fronto-central channels typically used in human EEG measures, perhaps reflecting anatomic rostrocaudal axis differences between species. MMN, ACC, and COH elicited robust cortical onset responses even under anesthesia. This is particularly informative as there is a dearth of protocols detailing cortical responses in chinchillas, despite their high potential as a model of human hearing. Additionally, anesthesia decreases the number of trials needed to reach an exploitable SNR due to the reduction of noise and motion artifacts.
Conclusions: The EEG mini-cap is a valuable tool that has improved and streamlined our methods for collecting evoked potentials across species. The mini-cap enhances the translatability of our animal work by facilitating the use of the open-source, widely-accepted MNE framework used in human neuroimaging. Furthermore, the multi-channel data collected by the mini-cap permits a precise investigation of the spatial distribution of cortical and subcortical evoked potentials in chinchillas. Interestingly, cortical measures of temporal coherence and pitch discrimination may be viably collected under ketamine/xylazine, given that the observed onset responses appear unimpaired by this anesthesia protocol.

SU189. Neuropathy of the Inner Ear Due to Plasma Membrane Ca2+-ATPase Mutations

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Category: Other, Pathogenesis
Background: It remains unclear which mechanism underlies the onset and development of human age-dependent hearing loss or presbycusis. It also controversial whether a rodent model of age-dependent hearing loss can be applied to human. Using a forward genetics procedure, we isolated four Pmca2 mutant mouse lines, each of which had a different missense mutation in Pmca2 and a distinct phenotype. We found large differences in the age-dependent ascending rate of ABR thresholds and period of hair cell/SG cell disappearance between the mutant mice. Age-dependent phenotypic progression in different mouse allelic mutants [1,2,3] and a variety of diagnostics for patients with the de-novo mutations in human paralogues have been reported [4]. These observations suggest that intracellular Ca2+ status plays a latent role in deafness progression.
[1] Spiden SL, PLOS Gen. (2008)
[2] Bortolozzi M, JBC. (2010)
[3] Watson CJ, Hear Res. (2013)
[4] Smits JJ, Hum. Gen. (2019)
Methods: We screened mice for startle responses evoked by a click box and then measured auditory brainstem responses (ABR). The isolated mutants were further subjected to comprehensive screening to confirm phenotypically that they did not have other traits, which is a typical characteristic of human diagnostic-type non-syndromic deafness. Hearing function and morphological analysis were performed successively by performing DPOAE measurements and histological analysis, including immunofluorescence microscopy. To detect possible defects in hair cell calcium-ion exporting activity, Ca decay assays of cells expressing the mutant product were performed.
Results: Functional and histological analyses showed that the early-phase of impairment could be distinguishable from the late-phase, during which hair cells and SG cells are impaired and completely obliterated. The clear differences in phenotype between the four mutants appear to be due to differences in Ca2+-pump activities of P-type Ca2+-ATPase, the product of the Pmca2 gene.
Conclusions: Assuming that the biomolecular pathway leading to age-dependent emergence of hearing loss phenotypes can be divided into two axes (functional impairment and cell death process), clarifying these axes may facilitate the development of an appropriate model of progressive hearing loss in human. Ca ion is generally thought to act as a second messenger in multiple signaling pathways over very short time periods; however, it might also act via an as-yet-unknown pathway over life-long periods. The status of intracellular

Ca-mobilization could act as a timer. To investigate these axes in vitro, we have established a cell culture system in which the expression of each mutated protein is controlled so that its amount and localization is the same between the mutants. Molecular analysis of this system is expected to reveal the latent basis of the long term effects in the mutants.
Acknowledgements; We acknowledge Dr.Toshihiko Shiroishi and Dr.Tetsuo Noda for establishing and providing RIKEN-mouse forward genetics screening platform and for valuable discussions.

SU190. Speech Recognition in Noise, Self-Reported Hearing Difficulty, Mobility and Risk of Falling in Adult Population

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Category: Other, Speech Perception in Noise and Balance
Background: Using an ecological approach by utilizing functional hearing and balance tests/questionnaires to determine the effect of changes in suprathreshold speech on postural control was recommended by Carpenter and Campos (2020). This study examined the association between speech recognition in noise, and mobility and risk of falling in adults using hearing in noise and mobility tests and self- reporting questionnaires.
Methods: 295 community dwelling adults (age: M=58.5; SD ± 6.1), participated in this study. All subjects had preserved cognitive ability according to Montreal Cognitive Assessment (MoCA) and their hearing thresholds were measured at the range of 0.5-4kHz. Their speech perception in noise performance was measured using the Hebrew version of Words In Noise Test (HWIN) and the Amsterdam Inventory for Auditory Disability and Handicap (H-AIADH). The HWIN test consists of two lists of 35 common CVC words mixed with 6 talkers babble noise, at 7 SNRs from 24- to 0-dB SNR in 4-dB decrements. Each subject listened to two lists, one per ear, for open-set identification. The HWIN results were quantified in terms of the 50% point calculated with the Spearman–Kärber equation. Mobility and balance were assessed using the Timed Up and Go (TUG) Test and the Activities-Specific Balance Confidence Scale (ABC) questionnaire.
Results: Results: HWIN performance was correlated with Pure Tone Average of 500, 1000 and 2000 Hz hearing thresholds (PTA). TUG was correlated with HWIN and with H-AIADH total score, and localization and discrimination subscales scores. However, TUG was not correlated with Amsterdam quiet and noise subscales. ABC score was only correlated with Pure Tone Average of 500, 1000 and 2000 Hz.
Conclusions: Increasing the awareness to the interaction between hearing and balance is important for early detection of balance/hearing impairments. Clinicians should consider referring patients with hearing disorders to balance evaluation and patients with balance disorders to hearing evaluation. Further examination of these interactions is needed and should be considered in early detection of populations at high risk of falling.

SU191. RadioEar: Comparison of a Commercial Distortion Product Otoacoustic Emissions (DPOAE) Test to a Novel Low-Cost, Wireless Prototype Probe

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Category: Otoacoustic Emissions
Background: Few affordable audiology screening tools exist for underserved populations such as farmers, individuals with intellectual disability, and infants in the developing world. Yet, moderate to profound hearing loss is prevalent among these groups. Evidence suggests monitoring Distortion Product Otoacoustic Emissions (DPOAEs) is beneficial in identifying noise-induced hearing loss (NIHL). Using DPOAEs to screen infants for deafness has already been widely adopted across the United States because they are fast, objective, and do not require a cooperative patient. These traits would also be helpful in reaching underserved populations, however, DPOAE screeners cost significantly more than screening audiometers. A low-cost, wireless, and noise-tolerant DPOAE prototype has been developed. This study seeks to determine whether this new probe will present comparable data to a known commercial DPOAE device, allowing it to be used in underserved communities as a screening tool for hearing loss.
Methods: 30 participants (22 male, 18 female; mean age=30.4+/-16.7) were included in this study. Normal tympanograms were defined as peak pressure +35 to -125 decaPascals, static admittance between .20 and

1.50 mmhos, and tympanometric width less than 25. Exclusion criteria included excessive cerumen, abnormal tympanograms, fluctuating hearing loss, conductive hearing loss, and Meniere's disease. Tympanometry was performed and ear canal volume in both ears was recorded for each subject. DPOAEs were measured using both the newly developed device and a commercial OAE device for each ear. The presence of DPOAEs was determined as an SNR >6 in 5/6 frequency tests. DPOAE amplitudes from both devices at 2, 3, and 4 kHz were compared using Bland-Altman analysis. The presence or absence of DPOAEs was also recorded.
Results: Of 60 ear exams eligible for this study, 1 left and 4 right ear canals were excluded for excessive cerumen (n=55). Correlations of the DPOAE amplitudes at 2,3,and 4 Hz between the commercial device and new probe were r=0.69, 0.31, and 0.75 respectively. For all frequencies combined the Bland-Altman analysis showed an offset of -7.2 dB SPL (the new device read lower on average). The repeatability coefficient was 13 dB SPL. The new device correctly detected the presence of 50 out of 51 DPOAEs and absence of 3 of 4 using the commercial device as a control measurement.
Conclusions: The newly developed probe prototype and commercial device provide similar DPOAE results. Future directions will be to revise the probe to detect SNRs with increased accuracy through adjustment of the noise floor compared to the commercial device.

SU192. Dynamics of Synchronized Spontaneous OAE in Humans Within and Across Click Intervals
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Category: Otoacoustic Emissions
Background: The synchronized spontaneous (SS) otoacoustic emission (OAE) refers to the oscillation evoked by clicks after transient-evoked OAEs (TEOAEs) attenuate. SSOAEs are present at isolated frequencies; some components decay with time on the order of several hundred milliseconds while other components sustain. It is unclear how different components of SSOAE change within hundreds of milliseconds, and whether the dynamics exhibit any drifting effects when the click stimulus is repeated.
Methods: 30 normal-hearing subjects were recruited and SSOAEs were measured by presenting clicks at a fixed interval of 156 ms. The response was analyzed 30 ms after the click to observe SSOAEs. A singular value decomposition-based denoising technique called optimal shrinkage (OS) was used to identify oscillation modes from raw acoustic data (Liu et al., 2021 JASA). To study the short-term dynamics within 150 ms, spectral peaks were identified, and single-frequency and two-frequency components were extracted by band-pass filters. Further, the components were analyzed within three time windows (W1 = 30-90, W2 = 60-120 and W3 = 90-150 ms). To study the long-term dynamics of SSOAEs, the left and right singular vectors produced by OS were examined. The left singular vectors represent orthogonal mode functions and the corresponding right singular vectors show variation of the mode across repetitions of the clicks.
Results: For short-term dynamics, characteristics of SSOAE components were compared across different time windows. For the single-frequency components, 42% of them vanished within 150 ms. The amplitude of 10% of the components increased from one window to the next. For the two-frequency components, 54% of the dual tones both lasted over 150 ms. In other cases, one of two tones disappeared before the end of the click interval, and the tone that sustained was always the stronger one. For long-term dynamics, we observed drifting phenomena. Apparent changes in the level of the first two mode functions could be seen over eight minutes. For instance, one mode might have a tendency of growing with time while the other mode exhibited a sudden drop of level or change of polarity.
Conclusions: By applying OS, SSOAE components could be estimated in a noise-robust manner. For the short-term dynamics, some single-frequency components and two-frequency components exhibited small amplitude growth with time. The dual tones in two-frequency components sometimes competed with each other, and the stronger tone tended to dominate by the end of the click interval. For long-term dynamics, OS is a novel tool for its capability to reveal drifting phenomena in SSOAE. Whether the first 30 ms of responses, namely the TEOAEs, exhibit such drifting might be worth analyzing and comparing.

SU193. Cochlear Delays Measured With Otoacoustic Emissions Evoked by Amplitude- Modulated Low-Frequency Tone
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Category: Otoacoustic Emissions
Background: Otoacoustic emission (OAE) tests have gained widespread attention in clinical settings for objectively evaluating inner ear function. However, they have not been employed in the low-frequency range because the detection rate of OAE is reduced by the presence of low-frequency noises, such as biological noise. This study aimed to establish an efficient method for evaluating the health of outer hair cells in the low-frequency range. Although the cochlear basilar membrane oscillation shows phase nonlinearity in addition to amplitude nonlinearity due to the active mechanism of outer hair cells, the phase information has not yet been effectively utilized to evaluate OAE. Sisto and Moleti previously showed that cochlear delay with transient-evoked OAE and cochlear tuning decreases with increasing stimulus levels [Sisto R and Moleti A., 2007, J Acoust Soc Am.], indicating that the nonlinear response of basilar membrane oscillation to the stimulus level can also be measured from the phase information of OAEs. This study extended the previous study to examine whether the cochlear delay measured using OAE in the low-frequency region reflects nonlinearity. The method used to measure cochlear delay was the amplitude-modulated-evoked OAE (AMEOAE) [Goodman et al., 2004, Hearing Research], to ensure direct measurement in the time domain from low-frequency OAE.
Methods: AMEOAEs were measured at below 300 Hz in 10 adults with normal hearing, and cochlear delays were calculated at each stimulus level. The carrier frequency of the stimulus was selected in the range of 200–300 Hz (10 Hz increments) with the best signal-to-noise ratio (SNR) of stimulus-frequency OAE. The amplitude was modulated at 25 Hz, and modulation was 50%, while the stimulus level was varied between 45- and 80-dB sound pressure level (SPL) in 5 dB steps. AMEOAEs were extracted with only nonlinear components using the double-evoked method [Keefe et al., 1998, J. Acoust. Soc. Am.], and cochlear delays were calculated from the group delays of AMEOAE with an SNR above 3 dB.
Results: The cochlear delays tended to be longer at stimulus levels below 60 dB SPL and shorter at stimulus levels above 65 dB SPL. The cochlear delays measured in the acoustic cavity were short with uniformity, regardless of the stimulus level, suggesting that the above observations reflected biological processes rather than experimental artifacts.
Conclusions: By evaluating the cochlear delays obtained from AMEOAEs as a function of stimulus level, it may be possible to evaluate the nonlinearity of basilar membrane oscillation with respect to stimulus level in the low-frequency region below 300 Hz, as well as in the mid- to high-frequency regions.

SU194. Click-Evoked Otoacoustic Emissions and Stimulus-Frequency Otoacoustic Emissions Derived From a Nonlinear Cochlear Model

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Category: Otoacoustic Emissions
Background: Otoacoustic emissions (OAEs) evoked with a click (CEOAEs) and OAEs evoked with a single pure tone (stimulus-frequency OAEs, SFOAEs) are assumed to be generated by reflection of forward-going traveling waves from mechanical irregularities in the organ of Corti. Amplitudes of these OAEs have qualitatively similar fine structure and their phases have approximately the same slope.
Methods: The model approximates the basilar-membrane (BM) response with an array of fluid coupled oscillators. Gaussian distributed mechanical irregularities are introduced into the shape function controlling the amount of amplification. CEOAEs are derived from the model for a single click (sampling frequency 600 kHz) with intensities ranging between 30 dB peSPL (peak-equivalent sound pressure level) and 130 dB peSPL, and a step of 5 dB. SFOAEs are evoked with a single pure tone with intensity varying between 20 dB SPL and 60 dB SPL. The frequency of the pure tone ranges from 1 kHz to 4 kHz. The signal containing only the evoked OAEs is calculated as a difference between the responses derived from the model with irregularities and a smooth cochlear model (without irregularities).
Results: At the lowest stimulus levels, the CEOAE and SFOAE amplitudes have qualitatively similar fine structure and also the CEOAE and SFOAE phases are approximately the same. As the level increases, the CEOAE and SFOAE amplitudes grow linearly. As the click level grows further, the CEOAE amplitude saturates but the fine structure maintains its shape and the CEOAE phase is nearly level invariant. In contrast, the SFOAE fine structure not only saturates but also changes. The slope of the SFOAE phase response decreases with increasing level.

An analytical solution derived for SFOAEs reveals that the qualitative change in the fine structure of the SFOAE amplitude is due to an additional SFOAE contribution emanating from perturbation of the nonlinear electromechanical force. This analytical solution, being derived for a stationary model response, is not applicable to CEOAEs. Differences between the BM displacements from the model with irregularities and from the smooth model revealed that a significant part of the CEOAE energy is generated after the click response on the BM has almost decayed. In the BM segment with characteristic frequency of 800 Hz, the impulse response strongly decays 10 ms after the click onset, but the difference signal containing the contribution from irregularities is relatively strong 20 ms after the click onset. These later components saturate as the level increases above about 100 dB peSPL.
Conclusions: SFOAEs and CEOAEs simulated in a 2D, nonlinear cochlear model are qualitatively similar at the lowest intensities. At high stimulus intensities, the SFOAEs and CEOAEs are not equivalent.

SU195. Robust Frontal Spatial Representations Appear in the Auditory Cortex of Awake Mice

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Category: Primary Auditory Cortex
Background: In the auditory system, the detection and perception of a sound's spatial location must be computed and processed entirely within the brain, as space is not mapped onto its sensory epithelium. In the auditory cortex (AC), initial studies of spatial tuning generally supported a model in which spatial information is represented in the relative firing rates of two opposing, hemispheric channels. However, these studies were performed exclusively under anaesthesia, and more recent psychophysical and electrophysiological studies in the awake AC have provided some evidence for a third, frontal channel. The confusion over the number of spatial channels represented in the AC stems at least in part from the lack of a direct comparison of individual neurons between wakefulness states.
Methods: We therefore employed longitudinal in vivo two-photon calcium imaging in the AC of CBA/CaJ mice with the genetically-encoded calcium indicator GCamp6s. A total of eight imaging sessions were performed (interleaved between four anaesthetized and four awake sessions). Spatial tuning was probed with a five-loudspeaker free-field array spanning 120 ° of horizontal angular space (30 ° ipsilateral to –90 ° contralateral), and analyses were performed on neurons that could be re-found on all sessions.
Results: We found that spatial tuning between wakefulness states and across sessions was generally unstable, although the population under anaesthesia as a whole exhibited a robust contralateral bias, thus supporting the two-channel model of other studies using anaesthesia. However, in the awake state, a subpopulation of neurons became robustly tuned to the front of the animal. This subpopulation was remarkably stable, consisting of largely the same neurons across sessions. Interestingly, these neurons did not have stereotyped spatial tuning under anaesthesia. Many were actually non-responsive or not spatially tuned while anaesthetised.
Conclusions: Our findings thus provide direct evidence for a third, frontal spatial channel in the awake mouse AC. The precise manner in which anaesthesia specifically suppresses frontal tuning remains unclear, but recent reports of spatial tuning in the awake mouse inferior colliculus show no such robust frontal tuning, tempting us to speculate that it in fact arises de novo in the AC. Future studies promise further insights into the behavioural significance of frontal spatial tuning in mammals.

SU196. Noise-Induced Hearing Loss Strengthens L2/3 Excitatory Synapses on L2/3 Interneurons in Mouse Auditory Cortex

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Category: Primary Auditory Cortex
Background: Hearing loss associated with hyperexcitability-related disorders such as hyperacusis and tinnitus leads to plastic changes in the auditory cortex. It has been shown that hearing damage enhances the activity of the auditory cortex and generates a compensatory reorganization of its neuronal circuits. For its part, neuronal microcircuits of the primary auditory cortex are the first cortical processing pathway, being crucial for auditory signaling transduction. Likewise, synaptic transmission changes may modify sensory perception through microcircuits reorganization. Therefore, understanding the mechanisms underlying synaptic transmission is a key step in hearing loss study. This work evaluates the effect of noise-induced

hearing loss (NIHL) on the dynamics of excitatory synapses of layer 2/3 microcircuits formed by excitatory principal cells (PN) to PN, somatostatin (SOM), and parvalbumin (PV) cells
Methods: Hearing loss was generated by exposing mice bilaterally to an octave band (8-16 kHz) noise at 100 dB SPL for 2 hours. The effect of NIHL over excitatory synapsis was evaluated on Day1 and Day3 after noise exposition (NE). We used optogenetic approaches to tag, PN-PN, PN-PV, or PN-SOM synapses. In vitro dual patch-clamp recordings were performed between pairs of these synapses. The excitatory synapses were light stimulated by expressing CamKlla-dependent ChR2 on PN cells. The amplitude ratio of excitatory postsynaptic currents (EPSCs) responses among PN→PN, and PN→PV or PN→SOM synapses was compared between Sham and NE groups of mice. Additionally, to evaluate whether NE-induced synaptic changes are pre or postsynaptic, miniature EPSCs (mEPSCs) were assessed.
Results: While NE enhances EPSCs amplitude on Day1 and Day3 of PN→PV connections, PN→SOM synapses increase only on Day3, suggesting that NE strengthens both excitatory synapses. To know the extent of NE-induced synaptic strength, we tagged and simultaneously recorded both PV and SOM cells in the same mice and compare their EPSCs responses, revealing that PN→PV are stronger than PN→SOM connections.
Furthermore, mEPSCs assessment revealed that on Day1 NE decreases mEPSCs frequency in PV and SOM cells, likewise reduces mEPSCs amplitude in PN cells, suggesting that PN-PV synaptic strength mainly is due to alterations in the quantal content (n*Pr) at presynaptic level, whereas PN→SOM synapses did not change due to compensatory effects at both pre and postsynaptic levels. On Day3 NE enhances mEPSCs amplitude in PV and SOM cells, indicating that postsynaptic changes are responsible for the synaptic strength on PN→PV and PN→SOM connections. Likewise, NE on Day3 increases mEPSC frequency in PV cells, suggesting that changes of quantal content at presynaptic level support stronger PN→PV compared with PN-SOM connections.
Conclusions: Altogether these findings propose that Noise-Induced Hearing Loss causes plastic changes in synaptic transmission by intensifying excitatory connections from PN to PV and SOM interneurons in Layer 2/3 of primary auditory cortex.

SU197. Effect of Noise on Neural Encoding of Speech at Auditory Cortex in Individuals With ANSD

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Category: Primary Auditory Cortex
Background: Individuals with Auditory neuropathy spectrum disorder (ANSD) witness pervasive difficulty understanding speech in the presence of noise. Despite some of them having relatively good speech perception in quiet, they have ~15 dB of signal-to-noise ratio (SNR) loss (Jaisinghani and Manjula, 2020). As endorsed, the speech perception ability is dependent on the neural detection of time-varying spectral and temporal cues (Tremblay et al., 2004), and these can be indicated by the Cortical Auditory Evoked Potentials (CAEP) (Rance et al., 2002). It was hypothesized that robust effects of noise observed on speech perception of ANSD could also be seen in their neural encoding at the auditory cortex. To date, no studies have examined the effect of noise on cortical responses in ANSD. Here, the CAEP for speech in quiet and noise were compared to uncover the possible mechanism behind their poor perception in noise. In addition, we checked for any existing correlation between CAEP measures in noise and SNR-50. If present, it would help in predicting their behavioral scores by physiological measure.
Methods: Repeated measures design was framed to compare the CAEP of individuals with ANSD in quiet and noise. Sixteen adult native Kannada speakers with a confirmed diagnosis of ANSD were recruited as participants. All had up to a moderate degree of SNHL, with a minimum of 50% speech identification scores (SIS) in quiet, in the better, or test ear. Before CAEP, SNR-50 was measured. The SNR-50 procedure was identical to our previous study (Jaisinghani and Manjula, 2020). Later the CAEP were recorded from four scalp electrode locations Fz, Cz, C3, and C4, in quiet and in noise (+10 dB SNR) using multi-channel AEP equipment.
Results: CAEPs could be reliably recorded for all participants in quiet. Whereas in noise, CAEPs could be recorded for all except three participants. The morphology of CAEP in noise was noted to be poorer than that of CAEP obtained in quiet, indicating that neural synchrony worsens with noise in this population. Further, a significant delay in P1, N1, and P2 latencies of CAEP in noise was noted compared to CAEP in quiet (based on Wilcoxon signed ranks test). These findings also support that background noise worsens the already disrupted cortical neural synchrony due to the line-busy effect. No significant relationship (based on

Pearson Product moment correlation) was found between SNR-50 and any of the CAEP parameters, which can be attributed to a high degree of inter and intra-individual variability in their speech in noise-induced neural synchrony.
Conclusions: CAEP in noise (stimulus complexity) is a more sensitive tool to measure cortical neural synchrony deficit in individuals with ANSD. Also, the electrophysiological evidence of disrupted cortical neural synchrony in them with the advent of noise is noted.

SU198. A Subcortical Auditory Model With Efferent Gain Control Explains Perceptual Enhancement
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Category: Psychoacoustics
Background: A target embedded in a background sound "pops out" when it follows a precursor consisting of the background alone. This phenomenon is known as auditory enhancement. This paradigm not only enhances the detection of the target (signal enhancement) but also increases the effectiveness of the target as a forward masker (masker enhancement). The focus of this study was on masker enhancement, for which the underlying mechanism is not well understood. We tested the hypothesis that the dynamics of the efferent system explain masker enhancement.
Methods: We used a subcortical auditory model with medial olivocochlear (MOC) efferents that received both ascending inputs from wide-dynamic-range brainstem cells and descending inputs from the midbrain. We replicated the psychoacoustic study in Kreft and Oxenham (2019, JASA 146:3448-56). Each stimulus had a precursor (500-ms duration), followed by a masker (100 ms), and a tone probe (20 ms), each separated by 20-ms gaps. The masker was four equal-amplitude, logarithmically spaced sinusoids geometrically centered around the target frequency. The precursor was a copy of the masker with or without the target-frequency component, and the probe was a brief tone pip at the same frequency as the target. The probe-detection thresholds were measured for listeners with hearing loss, and then for normal hearing listeners at (A) the same sound level (SPL), (B) the same sensation level (SL), and (C) the same SPL and SL, by using threshold-equalizing noise (TEN) noise, as for listeners with hearing loss. The model's probe-detection threshold was estimated using a decision variable based on the maximum rate of the model inferior colliculus (IC) responses to the probe. Responses were summed across nine frequency channels spanning the frequency range of the stimulus components, such that there was always a channel at each component frequency, as well as channels centered between components. A two-interval, two-alternative, forced-choice task was simulated, using the method of constant stimuli.
Results: The simulation results from the model with the MOC efferents exhibited the same trends as reported in the psychoacoustical study. The efferent model with hearing loss had no enhancement. The efferent model for normal-hearing had significant enhancement in the configuration for which SPL was matched, but no enhancement for the conditions in which either SL or both SPL and SL were matched to the experiments with hearing loss. However, the model without efferents failed to capture any of the effects described above.
Conclusions: The simulations showed that the model with MOC efferents was able to simulate the masker-enhancement effect observed in both groups of listeners and all three sound level conditions, whereas the model without efferents failed to do so. The current findings support the hypothesis that efferent activity could explain the auditory-enhancement effect.

SU199. How the Brain Tracks Structure in Rapid and Slow Sound Sequences - A MEG Study in Humans
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Category: Psychoacoustics
Background: Human listeners are automatically tuned to patterns within rapidly evolving sound sequences. This sensitivity can be measured with MEG/EEG, and is hypothesized to rely on fast memory formation associated with tracking transition probabilities within the stimulus sequence. Our previous work focused on fast sequences (tone pips presented at a rate of 20Hz). Here we extend the investigation to slower sequences (4Hz) that enable us to also isolate brain responses to individual tones. Using MEG, in passively listening naïve subjects, we ask the following questions:

(1) Are listeners able to automatically track repeating patterns in slow tone sequences?
(2) Which brain networks are engaged?
Methods: We used sound sequences that consisted of 50 ms tone pips, drawn from a frequency pool of 20 log-spaced values ranging from 222 Hz to 2000 Hz. The orders in which these tone-pips were successively distributed defined random and regular condition; the regular sequences (REG) were structured as 6 repeats of 10 frequencies. The random sequences (RAND) consisted of a random succession of frequencies. Two timing profiles were created: in 'fast' sequences tones pips were presented in direct succession (20Hz rate); in 'slow' sequences tone pips were separated by a 200 ms silent gap (4 Hz rate).
Naïve subjects (N=22) listened passively (while performing a simple visual task) to sound sequences (all conditions presented in random order), while their brain responses were measured with MEG.
Results: MEG results demonstrate that, passively elicited MEG brain responses show significantly stronger sustained response magnitude in REG relative to RAND patterns. This is observed even in the slower sequences, despite the long durations of the pattern (2500ms), revealing the auditory brain's remarkable implicit sensitivity to complex patterns. Importantly, brain responses evoked by single tones exhibited the opposite pattern - stronger responses to tones in RAND compared to REG sequences. The overall response pattern is not consistent with increased gain on predictable sensory information (e.g. as hypothesized by predictive coding) but is in-line with increased inhibitory activity in REG sequences.
Conclusions: MEG data demonstrate that passive listeners are tuned to the emergence of structure in complex sound sequences even for long (2500 ms) pattern durations. The observation of simultaneous but opposing DC and evoked response effects reveal concurrent processes that shape the representation of unfolding auditory patterns.
This work was supported by a BBSRC grant to MC.

SU200. Auditory Intuitive Physics Revealed by Sensitivity to Physical Inconsistencies in Contact Sound Sequences

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Category: Psychoacoustics
Background: Many sounds that we hear in everyday life are generated by physical interactions between objects. Humans are known to infer physical variables from visual scenes, but the extent to which they do so from sound remains unclear. The issue has historically been difficult to study, in part because previous sound synthesis models could not replicate the acoustic richness of real-world sounds, limiting the ability to generate and manipulate the sounds of physical interactions. Using recently published methods for synthesizing realistic contact sounds, we probed for auditory intuitive physics by testing human sensitivity to physical inconsistencies in sound.
Methods: We used a physics-based generative model to synthesize realistic impact, scraping and rolling sounds from simulated physical scenes. In a first experiment, participants identified which of two sound clips was generated with physically inconsistent sets of input parameters. The inconsistency could occur in one of five physical variables (Mass, Stiffness, Bounce Height, Restitution and Resilience, and Shape). In a second experiment, participants identified which of three sounds came from a different object. Each of the three sounds was generated from a different latent physical scene that was randomly initialized, such that participants had to abstract the object properties to perform the task.
Results: We found that human listeners were sensitive to inconsistencies in all of the tested physical parameters. Participants were also able to discriminate objects from the sounds they made.
Conclusions: Our results suggest that human listeners infer physical variables from sound and store them over time in object representations. Moreover, the object representations are at least somewhat invariant to the latent scene and types of physical interactions. The results suggest that humans possess auditory "intuitive physics", and show how realistic sound synthesis models facilitate its study.

SU201. Modulation of Binaural Cues Reveals Functional Boundaries for Auditory Stream Segregation

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Category: Psychoacoustics
Background: The process of auditory stream segregation relies on various acoustic features, including spatial cues caused by binaural disparities. While much is known about listeners' ability to discriminate

small differences in interaural time (ITD), interaural level (ILD), and interaural correlation (IAC), less is known about how the central auditory system uses these cues to functionally determine the perception of integrated or segregated sound streams.
Methods: Using the ABA auditory stream segregation paradigm (Bregman, 1990), we presented listeners with continuous repetitions of ABA triplets (narrowband noise in low-high-low configuration with a 6-semitone frequency separation) with a slow modulation (50 sec period) of ITD, ILD, or IAC cues carried by the A-component of the ABA triplets. During each experimental trial, participants were instructed to continuously respond with a button box whether they perceived integrated (button-1) or segregated (button-2) auditory streams, and electroencephalography (EEG) signals were measured using a 64-channel cap.
Results: Participant response patterns analyzed as a function of binaural cue, predictably showed more segregation when the A-component carried a more lateral cue while the B-component was at the midline, than when both cues were centered at the midline (ILD and ITD = 0). Using logistic regression, binaural cue segregation boundaries were calculated for ILD (mean: 4.7 dB, ILD) and ITD (mean: 383 µs), and a significant correlation was observed between these cues ($r2=0.65$, $p<0.05$). EEG responses measuring the global field potential showed significantly larger responses to triplets that were perceived as segregated than integrated, and for triplets directly before a switch in perception was reported, in agreement with previous results (e.g., Higgins et al., 2020). For trials where IAC was modulated a general pattern emerged, where more switches were observed when A and B components were both centered (IAC = 1) than when the A-component was more diffusely correlated (IAC < 0.9).
Conclusions: By measuring binaural cue segregation boundaries, these results demonstrate an alternate approach for understanding the functional use of binaural cues for auditory stream segregation. The observed correlation between ITD and ILD segregation boundaries may indicate consistent within-listener use of binaural cues for stream segregation.

SU202. Stimulus History Effects on Temporal Pitch Discrimination in Acoustic and Electric Hearing
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Category: Psychoacoustics
Background: One goal of temporal pitch tasks in cochlear implant (CI) recipients is to estimate their upper limit of temporal pitch perception, which is typically in the range of 200-400 pulses per second (pps). To minimise the use of non-pitch cues, experimenters often adopt a “mixed-standards” procedure, whereby the standard rate changes from trial to trial. However, previous research with normal-hearing (NH) listeners has shown that trial-by-trial variation of the feature under investigation may introduce stimulus history effects, such that the judgement on a given trial is affected by the stimuli present on previous trials. Here, we demonstrate and measure stimulus history effects on temporal pitch discrimination by both CI recipients and NH listeners.
Methods: Cochlear-device CI recipients and NH listeners indicated which stimulus in a 2-interval forced-choice trial had the higher pitch. A mixed-standards procedure was used in which 2-interval-trials were presented in consecutive pairs, with the same standard and signal rates used for the 2 trials in each pair. In CI recipients, 400-ms pulse trains were presented via direct stimulation of a single apical electrode. Standard pulse rates were 100, 200, 300, 400 and 500 pps, and signal rates were 35% higher. In NH listeners, band-pass-filtered harmonic complexes were presented monaurally over headphones and in a low-pass-filtered TEN background to elicit pitch based on a purely temporal code. Standard F0s were 100, 200, 300, 400 and 500 Hz, and signal F0s were based on rate-change difference limens obtained in an adaptive staircase procedure, in which all harmonics were summed in sine phase. In the main NH experiment, 400- and 500-Hz stimuli were presented in random phase so as to reduce pitch strength and mimic the upper limit in electric hearing.
Results: In both CI recipients and NH listeners, temporal pitch discrimination significantly worsened with increasing rate. Furthermore, both groups showed significantly higher performance when the within-trial rate change was in the same direction as the change in the standard pulse rate relative to the previous pair of trials, compared to when the two types of change were in opposite directions. In CI recipients, this stimulus history effect was greatest at high rates. For both groups, the direction of between-pair rate changes influenced pitch judgements for both the first and second trials in each pair, but the effect was larger for the

first trial. Broadly similar stimulus history effects were also revealed by a re-analysis of published temporal-pitch-ranking data obtained with the widely-used Midpoint Comparison Procedure.
Conclusions: Stimulus history affects pitch judgements in temporal pitch discrimination tasks in both CI recipients and NH listeners. Moreover, stimulus history effects extended over at least two trials, with the effect of a given stimulus decreasing with increasing distance from that stimulus.

SU203. Adaptation to Noise in the Detection of Spectral, Temporal and Spectro-Temporal Modulations
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Category: Psychoacoustics
Background: Noise adaptation is defined as the improvement in auditory function as the signal of interest is delayed from the noise onset. While it is known that adaptation to noise occurs in word recognition and temporal modulation detection, it is yet unknown if it also occurs in spectral or spectro-temporal modulation detection. This work aimed at investigating if noise adaptation occurs in spectral, temporal, and spectro-temporal modulation detection and whether the magnitude of adaptation in those tasks correlates with adaptation to noise in word recognition.
Methods: 16 normal-hearing volunteers participated in the experiments. Stimuli were presented monoaurally to their left ear. In the modulation detection tasks, the signal was a 200-ms spectro-temporal modulated ripple noise. As low temporal and spectral modulation frequencies are essential for speech recognition, the spectral modulation rate was 2 cycles/oct, the temporal modulation rate was 10 Hz, and the spectro-temporal modulations consisted of the combination of these two modulations. In the speech recognition task, the signal consisted of disyllabic words unprocessed or vocoded to maintain only envelope cues. The two tasks (modulation detection and speech recognition) were performed in quiet and in white noise (at 60 dB SPL) for noise-signal onset delays of 50 ms (early condition) and 800 ms (late condition). In the modulation detection tasks, the signal level was 60 dB SPL (0 dB SNR) and the modulation depth (dB) was varied adaptively to calculate the threshold depth at 71% correct detection. In the speech recognition tasks, the speech level was adaptively varied to calculate the SNR at 50%-word recognition. Adaptation was calculated as the threshold difference between the early and late conditions.
Results: Mean adaptation was statistically significant in spectral [2.2 dB; $p<0.001$] and temporal [1.95 dB; $p<0.001$] modulation detection but not in spectro-temporal modulation detection [-0.07 dB; $p=1.0$]. Mean adaptation in word recognition was significant for vocoded words [2.26 dB, $p<0.001$] but not for natural words [0.69 dB, $p=0.15$]. Adaptation in natural and vocoded word recognition was not correlated with spectral modulation detection ($r=-0.17$, $p=0.55$; and $r=-0.19$, $p=0.48$, respectively), temporal modulation detection ($r=-0.01$, $p=0.96$; and $r=0.15$, $p=0.60$, respectively), or spectro-temporal modulation detection ($r=-0.4$, $p=0.13$; and $r=-0.35$, $p=0.20$, respectively).
Conclusions: Adaptation to noise occurs in spectral and temporal modulation detection but is less (or zero) when spectral and temporal modulations are simultaneously present. More data are needed to elucidate the relationship between adaptation in the detection of spectral, temporal and spectro-temporal modulations with adaptation in word recognition. [Supported by the University of Salamanca and Banco Santander to DLR and the Spanish Ministry of Science and Innovation (grant PID2019-108985GB-I00) to EALP].

SU204. Loudness Dominance and Perceptual Dissimilarity
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Category: Psychoacoustics
Background: Psychophysical experiments displaying loudness dominance suggest that auditory attention tends to be directed to the more intense elements of a sequence of sounds. Lutfi and Jesteadt [2006, J. Acoust. Soc. Am., 120(6), 3853-3860] demonstrated a disruption of loudness dominance when the high- and low-intensity sounds were perceptually dissimilar (e.g., tone vs. noise). Potentially, perceptual differences in the sequential sounds that support auditory segregation also reduce loudness dominance. This possibility was examined in the current study.
Methods: The stimuli were sequences of five sounds with levels that varied: high - low - high - low - high. There were two general experiments, both were single interval, yes-no, intensity discrimination tasks. In Experiment 1, the signal to be detected was an increment to both the high- and low-intensity elements. The

signal increment to the low-intensity elements was proportionately larger than the high-intensity elements, which for an ideal observer would yield greater weighting of the low-intensity elements. In Experiment 2, the signal increment was applied only to the low-intensity elements. In both experiments, sounds (high vs. low) were systematically varied in several perceptual dimensions. The thresholds (as ΔL in dB) for the low-intensity elements were compared across conditions.
Results: Thresholds were highest when high- and low-intensity elements had the same composition (e.g., harmonic complexes with the same fundamental frequency [f0]). Manipulations in the perceptual features of the sound elements, high-intensity vs. low-intensity, did not always change thresholds. Thresholds were lowest when (i) the elements were tones or harmonic complexes vs. broadband noise, and when (ii) the elements were harmonic complexes that differed in both f0 and harmonic number (i.e., non-overlapping frequency regions). Thresholds were higher when the elements had (i) differences in spectral shape (tilt vs. no tilt) and (ii) different f0 but the same harmonic numbers. Thus, we observed a reduction in loudness dominance due to differences in frequency regions.
Conclusions: The results indicated that difference in frequency regions leads to a release from loudness dominance, whereas timbral or pitch difference does not.

SU205. Modeling Feature-Based Auditory Attention With Deep Neural Networks

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Category: Psychoacoustics
Background: Attentional selection allows human listeners to successfully recognize speech in noisy environments (the "cocktail party problem"). Although attentional listening abilities have been characterized to some extent in humans, we lack quantitative models of auditory attention that are capable of explaining attention-mediated behavior. We also lack normative models of attention that reveal how attention should influence neural representations to enable selective listening. Inspired by known neurophysiological effects of attention, we introduce a model of auditory attention by adding a feature-based attentional gain module to a neural network, optimizing both the network and the gain module to perform a word recognition task in the presence of competing sounds.
Methods: We built a deep neural network optimized to perform an attentional word recognition task on audio signals, reporting words spoken by a cued "target" talker in a multi-source mixture. The model implemented feature-based attentional gain at each network stage via learnable logistic functions operating on the time-averaged feature activations of a cued talker. Features of the cued talker could thus generate high gains, while those absent from the cued talker could generate low gains, to an extent determined by parameters optimized to maximize correct recognition. Task performance was measured by word recognition accuracy as a function of target-distractor ratio (SNR) in both speech-on-speech and speech-in-noise settings.
Results: The model successfully performed the word recognition task in both distractor settings. In the presence of competing talkers the model correctly reported the words of the cued talker and ignored the distractor talker(s). Similar to humans, the model showed higher accuracy with single-talker distractors than with multi-talker distractors. The model's internal representations revealed that attentional selection occurred only at later model stages.
Conclusions: We provide a framework to quantitatively model feature-based auditory attention by optimizing a deep neural network to perform an attentional word recognition task. The model provides hypotheses for how attention might be expected to modulate neural responses at different stages of the auditory system, and can help understand the conditions in which attentional selection is intrinsically difficult.

SU206. Transient Neurogenin1 Upregulation and Optogenetic Modulation of Plp1- Positive Inner Ear Glia in a Mouse Model of Auditory Neuropathy

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Category: Regeneration

Background: Sensorineural hearing loss is irreversible and can be caused by loss of auditory neurons. Regeneration of neurons from inner ear glial cells (Schwann cells) may offer a future alternative to implantable hearing devices or may aid in improving the efficacy of cochlear implants.
Neurons and Schwann cells in the peripheral nervous system are closely related and originate from a common progenitor. Prior work with our transgenic mouse model demonstrated that in the postnatal murine inner ear, Proteolipid 1 (Plp1)- expressing inner ear glial cells expressed proneural markers after transient Lin28 overexpression in vitro and in vivo. This was accompanied by upregulation of proneural transcription factor Neurogenin1 (Neurog1), a key reprogramming factor in the central and peripheral nervous system. In this study, we aimed to transiently overexpress Neurog1 in lineage traced or optogenetically modified inner ear glial cells in vitro and in vivo to study the phenotype and functional response of inner ear glial cells.
Methods: Spiral ganglion glia were isolated from the cochleae of neonatal Plp1-CreER;Tdtomato and Plp1-CreER;Ai32ChR2-YFP/ChR2-YFP mice. We established an in vitro glial culture model for spiral ganglion glia and transduced fluorescence activated cell sorting (FACS) purified glial cells with Neurog1-TetO-Neongreen or Neurog1-TetO-mScarlet lentivirus, which allowed for Doxycyline dependent, transient Neurog1 expression in glial cells. Viral transduction and neural gene and protein expression were analyzed with FACS, histology, and quantitative PCR (qPCR).
Using our established in vivo model of auditory neuropathy, neurons in 6 week old Plp1-CreER;Tdtomato or Plp1-CreER;Ai32ChR2-YFP/ChR2-YFP mice were ablated. We stereotaxically injected Neurog1-TetO Lentivirus into the deafferented auditory nerve trunk and transiently overexpressed Neurog1 in transduced cells. Transduction efficacy, gene and protein expression were analyzed using qPCR and histology. Functional responses were assessed using electrically evoked auditory brainstem responses (ABRs) and optically evoked ABRs.
Results: Transient upregulation of Neurog1 after lentiviral transduction in vitro induced proneural gene expression in Plp1- positive inner ear glial cells. Similarly, successful transduction and transient in vivo upregulation of Neurog1 led to expression of (pro-) neural genes in Plp1- positive inner ear glia in the cochlear modiolus 8 weeks after auditory nerve damage. Blue light stimulation at the level of the round window after 8 weeks of incubation revealed successful optogenetic modulation of Plp1-ChR2 expressing cells.
Conclusions: Transient lentiviral overexpression of Neurog1 initiates expression of proneural markers in Plp1-positive cells in a mouse model of auditory neuropathy in vivo. Optogenetic modulation of photosensitized reprogrammed Plp1 cells indicated that glial cells may obtain primitive proneural features in vivo.

SU207. Automatic Extraction of Symptoms in Otology Clinic Letters Using Natural Language Processing

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Category: Clinical Otolaryngology and Pathology
Background: Hearing loss is the most common sensory disorder in humans and a huge amount of electronic healthcare data relating to this condition exists. Most of this data is in free text format, such as clinic letters or ward round notes, limiting its secondary use for quality improvement and research. Structured data, such as a list of diagnostic codes assigned to a patient record at the end of an hospital admission, is more amenable to analysis. Therefore, this study developed a set of natural language processing (NLP) tools to produce structured data on hearing loss symptomology from free text. NLP is a field related to artificial intelligence that focuses on developing methods for computers to understand and interpret human language.
Methods: Six key symptoms related to hearing loss were chosen: hearing loss, tinnitus, otalgia, otorrhoea, impairment of balance and vertigo. A dataset of 1000 clinic letters from the otology department at the Royal National Throat, Nose and Ear Hospital in London was used. The first of two NLP tools was designed to identify symptoms in free text by matching them to a dictionary of related terms derived from SNOMED-CT. For example, the dictionary for otorrhoea included the terms ‘discharge’ and ‘wet ear’. The letters were annotated by an otolaryngology resident and audiologist for the correct symptoms, and this gold standard document was used to augment the dictionary with new terms. The second tool was a set of three deep machine learning models designed to contextualise each extracted symptom by assigning three labels: firstly, whether the symptom was affirmed or negated, secondly whether the symptom was experienced by

the patient or someone else (eg. a relative) and finally the laterality of the symptom (left ear, right ear or bilateral).
Results: The performance of both tools was tested on a subset of the clinic letters that was not used during model development. A confusion matrix was produced, describing the number of true positive, true negative, false positive and false negative instances. The F1 score was used as the main performance metric, calculated as the harmonic mean of the precision and recall. The F1 score for symptom extraction (first tool) was 0.78 and for contextualisation of extracted symptoms (second tool) was 0.71.
Conclusions: An NLP pipeline capable of producing structured symptom data was successfully created and shown to perform well on real life data. Downstream applications for this pipeline include deep semantic searches, for example searching a patient record for previous symptoms affecting a particular ear, and cohort identification for clinical trials, whereby researchers can identify eligible patients with a particular hearing loss phenotype. For these ambitions to be realised, testing of the external validity using datasets from other hospitals is required.

SU208. Promoting Hair Cell Differentiation and Maturation by Transcriptomic and Epigenetic Regulation in Immortalized Multipotent Otic Progenitor (iMOP)-Derived Organoid.

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Category: Regeneration
Background: Hair cells (HCs) in the cochlea convert sounds into neural signals that are eventually relayed to the auditory cortex. Damage and loss of HCs by repeated exposure to loud sounds, aging, or congenital disease results in hearing loss. The molecular and epigenetic mechanisms responsible for regulating HC differentiation are still being determined. To investigate the molecular changes that occur during the transition from otic progenitors into nascent hair cells, we used immortalized otic progenitor cells (iMOPs) as an in vitro model system. With this system, different small molecule treatments can be administered to identify cellular and epigenetic mechanisms of HC differentiation.
Methods: Immortalized otic progenitor cells (iMOPs) were cultured either in the presence or absence of FGF to maintain cells in a proliferative state or to promote differentiation, respectively. iMOPs were collected ten days following FGF withdrawal for immunohistochemistry or RT-qPCR to determine changes in protein or mRNA levels of hair cell or supporting cell-specific genes during differentiation followed by small molecule treatment. To identify candidate genes involved in hair cell differentiation, RNA-seq was performed using proliferative iMOPs and differentiated iMOP organoids. Differentially expressed genes from RNA-seq were used for gene ontology (GO) analysis to provide clues to identify changing biological processes and cellular functions during iMOP organoid differentiation.
Results: In differentiation of iMOP organoids, we confirmed that iMOP cells can generate cells that express neuronal, SC, and HC markers within a single organoid. Immunohistochemistry and RT-qPCR showed increased protein and transcript levels, respectively, for HC and SC specific genes in differentiated iMOP organoid compared to proliferative cells. Furthermore, we observed the expression of HC genes and the presence of hair bundles in organoids. Gene ontology analysis revealed enrichment for biological processes such as inner ear development, auditory receptor cell development, and cochlea development. Transcripts from key genes, Gfi1, Pou4f3, and Atoh1, that are central to HC differentiation were upregulated in iMOP organoids. Furthermore, we showed that differentiated iMOP organoids respond to small molecules that inhibit Notch or Wnt signaling pathway (CHIR99021 or DAPT respectively) resulting in increased HC specific transcripts. Addition of histone deacetylase inhibitors after or in tandem with blocking the Notch or Wnt signaling, further increased HC specific gene expression.
Conclusions: Taken together, we generated hair cell-like cells during iMOP differentiation. iMOP cells are responsive to small molecules that target pathways implicated in hair cell regeneration. Using this iMOP organoid model system, we hope to delineate the epigenetic landscape changes that prevent or promote efficient HC production as well as different subtypes of hair cells.

SU209. Regeneration of Spiral Ganglion Glial Cells After Ablation in Adult and Aging Mouse DTA Model

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Category: Regeneration

Background: PLP/CreERT;ROSA26-eGFP-DTA (DTA) mouse is an established animal model of cell-specific ablation of the myelin Proteolipid Protein 1 (Plp1)-expressing glial cells in the nervous system upon induction with tamoxifen administration. DTA mice developed a demyelinating disease characterized by significant motor and physiological defects by 5 weeks post-induction (p.i.), followed by a full recovery of symptoms a few weeks later (Traka et al., 2010). DTA mouse model was used here to analyze regeneration of glial cells in the PNS, including Schwann cells and satellite cells within the spiral ganglion and auditory nerve of mature-adult and aged DTA mice to better understand the remyelination processes that occurs in patients recovering from acute peripheral demyelinating disease. Previous work has shown that robust Schwann cell regeneration and axonal remyelination occur few weeks after Schwann cell ablation in young DTA mice (Wan and Corfas, 2017). The impact of aging on glial cell regeneration and ultimately remyelination in the auditory system has not been previously investigated.
Methods: Mature adult (4-6 months) and aged (9-12 months) female and male DTA mice were treated with tamoxifen for three and four continuous days respectively to induce recombination and expression of the diphtheria toxin A subunit (DTA) and subsequent death in Plp1-expressing glial cells (Traka, 2019). DTA mice were sacrificed at different time points post-induction (1, 3 and 5 weeks p.i.) to analyze glial cell regeneration and remyelination within the auditory system. Untreated PLP/CreERT;ROSA26-eGFP-DTA littermate mice that were used as controls. Inner ears were fixed, then spiral ganglion and auditory fibers were dissected and immunostained either as whole mount or sectioned and then immunostained for markers expressed in different types of glial cells, as well as for myelin and node of Ranvier. Proliferation in glial cells was analyzed using the 5-ethynyl-2′-deoxyuridine (EdU) cell proliferation marker injected 24 hours before sacrificing mice.
Results: Preliminary data show that Schwann cells density within the auditory fibers in mature-adult DTA mice is similar to controls at both 3w and 5w p.i., indicating robust Schwann cell regeneration occurring in these animals. Satellite cell density (as indicated by Sox2+ cells) within the spiral ganglion showed a reduction compared to controls at 3w p.i. but returned to control levels at 5w p.i. indicating a relative delay in satellite cell regeneration. Analysis of glial cell regeneration and remyelination in auditory system of aged DTA mice is still underway.
Conclusions: Robust Schwann cell regeneration occurs after cell ablation in the auditory system, yet satellite cells take more time to regenerate.

SU210. Nestin-Expressing Cells Are Mitotically Active in the Mammalian Inner Ear

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Category: Regeneration

Background: Nestin is an intermediate filament protein whose expression is associated with pluripotency in a wide variety of stem cells throughout the body. A growing body of evidence suggests nestin may be involved in hair cell development. The objective of this study is to investigate whether nestin-expressing cells residing in the murine inner ear have a role in early postnatal hair cell regeneration.
Methods: Two nestin reporter mouse models were used in this experiment. In Nestin-GFP mice, all nestin-expressing cells fluoresce green and lose fluorescence when nestin expression ceases. In the lineage-tracing model, Nestin-CreRosa-tdTomato, any cell that expresses nestin at the time of tamoxifen injection will produce fluorescence which will be passed to all its progeny. Both mice were bred with Pou4f3-huDTR mice, which undergo selective hair cell ablation upon injection with diphtheria toxin.
In a preliminary experiment to assess which cells express nestin after ablation, Nestin-GFPxPou4f3-huDTR mice underwent hair cell ablation on postnatal day 1 (P1) and were euthanized on P8. Their cochleae were immunostained and whole mounted for cell counting.
Mitotic activity was investigated using the lineage-tracing mouse. Nestin-CreRosa-TdTomato mice underwent tamoxifen injections on P2. EdU was injected on days P5/6/7, and the mice were euthanized on P7 to identify whether nestin-expressing cells were mitotically active in the early postnatal mice. Their cochleae and vestibular organs were immunostained and whole-mounted for cell counting.
Nestin-expressing cell phenotypes were quantified after ablation in the Nestin-CreRosaxPou4f3 mice. They underwent tamoxifen injection on P2, ablation on P3, and were euthanized on P10. Their cochleae were immunostained and whole-mounted for cell counting.
Results: Nestin-GFPxPou4f3 mice demonstrated nestin expression in some repopulated hair cells.

In the Nestin-CreRosa-TdTomato mouse, three morphotypes of nestin-expressing cells were identified, bipolar, unipolar, and abnormal cells. All three morphologic subtypes were EdU-labeled. Mitotic activity was noted in 13.1+/-11.5% of the nestin-expressing cells in the organs of Corti, 3.7+/-4.5% of those in the utricles, 7.9+/-9.2% of those in the saccules, 15.3+/-15.3% of those in the cristae, 20% of those in the one spiral ganglion assessed, and 18% of those in the one Scarpa's ganglion assessed.
In the Nestin-CreRosaxPou4f3 mice, none of the repopulated hair cells in the organ of Corti arose from the nestin-expressing cells and the proportions of the three morphologic subtypes remained the same between unablated and ablated organs of Corti.
Conclusions: Nestin-expressing cells were identified in the cochlea, vestibular organs, and their respective ganglia. After cochlear hair cell ablation, nestin-expressing cells did not appear to react to this specific insult. However, nestin-expressing cells in all inner ear tissues exhibited some degree of mitotic activity, supporting the potential for some stem cell capabilities, though perhaps not for hair cell regeneration.

SU211. Mandarin Tone Perception for Tone Agnosics and Amusics

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Category: Speech Perception
Background: Congenital amusia, a neurogenetic disorder, mainly affected perception and production of music. Listeners with congenital amusia are called amusics who have normal hearing, intelligence, and musical exposure, but are difficult to perceive, appreciate, and remember music. Several studies on tone language users (e.g., Mandarin Chinese, Cantonese, and Thai) suggested that congenital amusia impaired listeners' lexical tone perception at phonemic and syllabic levels. In addition, a subgroup of congenital amusics in listeners with tone languages have deficits in both musical perception and tone perception and are named tone agnosics. As a previous study in our lab indicated that tone agnosics relied on semantic cues in words to recognize Mandarin tones, the hypothesis of this study was that when perceiving Mandarin tones, tone agnosics may benefit from processing both vowel and tone information at the same time. That is, when being asked to identify vowel plus tone versus identify only tones for the same Mandarin speech stimuli, tone agnosics were expected to perform better for tone identification in the vowel-plus-tone task than in tone only task.
Methods: Three groups of young listeners were recruited in this study: normal control, pure amusics (only congenital amusia with normal tone perception), and tone agnosics. There were 20 Mandarin stimuli: five Mandarin vowels x four lexical tones. Listeners had three identification tasks: vowel-plus-tone, tone only, and vowel only. The order of the three tasks was randomized for each listener.
Results: First, for tone identification either in vowel-plus-tone or tone only task, normal control and pure amusics showed significantly higher performance than tone agnosics with no difference between the two former groups. However, for vowel identification either in vowel-plus-tone or vowel only task, there was no significant listener group effect.
Second, for tone identification, tone agnosics and pure amusics showed significantly higher scores in the vowel-plus-tone task than in the tone only task, while normal control had similar scores between the two tasks. For vowel identification, no group had a significant difference between the vowel-plus-tone task and the vowel only task.
Conclusions: These results suggested that tone agnosics had significant deficits in Mandarin tone perception compared to pure amusics, indicating that their main struggle in Mandarin speech recognition may be primarily due to their difficulty in tone processing. Their tone deficits influences both speech and music perception. Tone agnosics' better tone identification performance in the vowel-plus-tone task than in the tone only task suggest that they may take vowel and tone information integrated when processing Mandarin syllables.

SU212. Audiometric Markers of Cochlear Synaptopathy and Speech in Noise Deficits in Humans

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Category: Speech Perception
Background: Cochlear synaptopathy has been shown to precede hair cell loss and threshold shift over age. Cochlear synaptopathy is assumed to alter auditory information processing, whether accompanied by threshold elevations or not, and is a predicted contributor to speech-in-noise difficulties. It is crucial to understand the impact of cochlear synaptopathy on speech coding to develop effective therapeutic interventions.
Methods: Here, we examine young, middle-aged, and elderly individuals with and without hearing impairment for characteristic features of cochlear synaptopathy using pure tone audiometry, DPOAE (IO, DP-Gram, Level-Maps), ABR, ASSR, speech comprehension (OLSA), psychoacoustic test of speech in noise and a Custom Questionnaire for hearing self-assessment in normal-hearing and hearing-impaired people of different ages.
Results: We will present first data of a larger clinical study in which we gain indications of speech comprehension problems through the comparison of different audiometric and psychoacoustic techniques with speech in quiet and speech in noise tests in subjects of different ages.
Conclusions: We will discuss the results in the context of the opportunity to use objective functional audiometric biomarkers for speech discrimination disorders in the future, particularly aiming to develop a model for speech coding in the brain (SCB-model) that can be used for intervention strategies in the clinic in the future.

SU213. Tone Language Experience but Not Lexicality or Phonotactics Influences the Frequency Following Response

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Category: Speech Perception
Background: Auditory processing at the level of the brainstem has been shown to be affected by speech and language experience. However, it is not yet known whether the encoding of speech and lexicality is differentiated, and whether phonotactics are encoded, at the level of the brainstem. In the current study, the frequency following response (FFR) to speech sounds was used to understand whether the brainstem can differentiate the perception of (a) Speech sounds with vs. without lexicality; and (b) Phonotactically legal vs. phonotactically illegal sounds.
Methods: In order to investigate these research questions, we compared 30 Mandarin and 30 non-Mandarin speakers on the FFRs collected in response to Mandarin speech sounds that varied in terms of their lexicality and phonotactic validity. In addition, all subjects were behaviorally tested on an auditory lexical decision task, a word-likeness task, and a tone identification task.
Results: Our behavioral analysis revealed that the Mandarin group had significantly higher tone identification accuracy for all tokens compared to the non-Mandarin group, regardless of if the token was a real word, nonword (lacked lexicality), or contained phonotactic violations. Also, only Mandarin speakers comparatively rated real words higher and responded to real words with higher lexical decision accuracy compared to non-words and phonotactic violations. For our electrophysiological data, using a support vector machine learning classification of the whole FFR waveforms, we found that the Mandarin group outperformed the non-Mandarin group on identification of the respective Mandarin stimuli category, regardless of the lexicality status of the stimuli. However, using the traditional FFR analyses including pitch strength, pitch error, or stimulus-to-response correlation, we failed to find any significant interactions with word type.
Conclusions: In sum, although our FFR results replicate the previous general findings on the effect of language experience on modulation of the FFR, our findings do not support the effect of language experience on fine-grained distinction between lexically and phonotactically legal words from their respective nonword counterparts. However, our behavioral data exhibits that Mandarin speakers are indeed treating real words, nonwords, and phonotactic violations differently.

SU214. Directional Effect of Target Position on Spatial Selective Auditory Attention

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Category: Speech Perception

Background: Spatial selective auditory attention plays a key role in listening in a mixture of competing speech sounds. Previous neuroimaging literatures found alpha power lateralization when listeners directed their auditory attention to either left or right focus, and greater cortical representation of the attended speech envelope versus the ignored speech envelope (also called 'neural speech tracking'). However, little is known about the neural activities with attentional focus directed on speech sounds behind listeners. The purposes of this study are to examine the directional effect of four different target positions (left, right, front, and especially back) on spatial selective auditory attention by assessing 1) behavioral performance in spatial selective hearing, 2) scalp-topography of alpha power and 3) neural speech tracking. It is expected that listener performance and neural correlates will be poorer when the target is behind the listener causing the relatively adverse signal to noise ratio as well as front-back confusion.

Methods: For fifteen young adults (18 – 35 yrs), scalp-topography of alpha power in response to attended target speech versus unattended distractor will be measured in a behavioral auditory attention task. Neural speech tracking will also be calculated by cross-correlating the temporal envelopes of the sentence stimuli and EEG data. In the task, participants are asked to indicate target words (e.g., color and number) of the coordinate response measure sentences, routed from the target speaker position cued by a pure tone, in the presence of a competing sentence in the opposite direction. This task has two listening conditions: 1) fixed target position and 2) switching target position condition in which the target position randomly changes in the left-right or front-back configuration. Neural correlates of auditory attention to the four different target positions will be compared within and between listening conditions. Correlations between behavioral performance and EEG analyses will be assessed.

Results: It is hypothesized that alpha power will be biased to the attended target direction on the scalp-topography. That is, when directing auditory attention to the back azimuth of the auditory scene the peak cortical band power may be more posterior (e.g., parietal region) relative to its position with left or right focus. Our working hypothesis is that alpha power and neural speech tracking will be the most robust for attended target streams in the front position, followed by lateral and the weakest in back. Also, it is expected that neural speech tracking will be poorer in the switching condition than in the fixed condition.

Conclusions: Given the expected outcomes, the current study will address neural signatures of spatial selective auditory attention to not only the lateral, but also front-back auditory scenes for further understanding of how auditory attention works in spatial selective hearing.

SU215. A Novel and Accurate Method for Predicting Speech Intelligibility in Various Types of Noise

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Category: Speech Perception

Background: The increasing interface between automatic speech recognition and the human voice suggests that such human and machine communication systems will benefit from an objective metric that predicts speech intelligibility under various types of noise and temporal distortion. However, most of the previous research on speech intelligibility measurements has focused on predicting the effects of individual noise or distortion processes and missed difficult conditions such as reverberation, center clipping and phase jitter. This study introduces an objective metric, the Spectrogram Orthogonal Polynomial Measure (SOPM), for predicting speech intelligibility for individuals with normal hearing.

Methods: Speech recognition scores were collected from normal-hearing listeners (N=10) using 720 IEEE sentences embedded in Gaussian noise and presented at 65 dB SPL. Speech intelligibility scores were measured in block-randomized sessions for SNRs from 10 to 10 dB in 5 dB increments. The SOPM metric is generated by using Krawtchouk moments to extract features from the spectrogram. The performance of the SOPM metric is examined for different noise types (stationary and fluctuating noise), distortions (peak clipping, center clipping and phase jitter), optimal time-frequency separation and reverberation conditions in both quiet and noisy situations. The metric predictions are validated with measured speech recognition scores as well as published data from a repository of speech intelligibility scores for HINT and TIMIT sentences. A two-way analysis of variance (ANOVA) was used to determine the effect of the metric and the

effect of parameters. A Tukey-adapted paired post-hoc comparison test was used to determine the differences between measured and SOPM predicted scores.
Results: Results to data show close agreement between measured and predicted speech intelligibility scores. This metric was sensitive to and accurate in the presence of various forms of distortions.
Conclusions: The present study proposes an objective metric, SOPM, to predict speech intelligibility for a listener with NH. The metric is based on the correlation between moment coefficients of the original and degraded spectrogram. The proposed metric is computationally cost-effective and performs modestly well in terms of predicting speech-intelligibility under a wide variety of realistic conditions.

SU216. Test Equivalency of the Spanish and English AzBio Sentences Among Bilingual Normal Hearers
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Category: Speech Perception
Background: Spanish is the second most common language spoken in the United States. Yet, access to validated culturally appropriate Spanish-language materials for use in the assessment of hearing-impaired individuals continues to be limited. The AzBio is a widely accepted measure of speech perception in noise used to determine cochlear implant candidacy. Recently a Spanish version of the AzBio was introduced, although little is known about test-language effects and the potential for influence on test performance. This study examined performance on a spectral discrimination task in normal hearing adult bilingual speakers and compared outcomes to speech perception in noise measured using the English and Spanish versions of the AzBio to determine if different language measures resulted in the same classifications of hearing performance.
Methods: Normal hearing bilingual adults were enrolled for study. Subjects were queried on Spanish and English fluency. All subjects underwent audiometric testing 250 – 20,000 Hz. Adaptive Hearing in Noise Test (HINT) and AzBio testing was assessed in both Spanish and English. The AzBio sentence lists were presented at a fixed SNR from -8 dB SNR to +8 dB SNR in 2 dB increments. The psychometric function was plotted for both languages and compared to performance on the adaptive HINT.
Results: Results indicate that normal hearing bilingual adults differed in their performance on the Spanish versus the English version of the AzBio speech perception in noise measure. Analysis of the psychometric function of the Spanish AzBio compared to the English AzBio demonstrated the Spanish AzBio yielded a steeper slope than the English AzBio. Language dominance was not a predictive factor in performance for either measure. HINT scores were predictive of the 50% correct score on AzBio in both languages. The spectral discrimination task suggested excellent performance on AzBio at higher SNRs in both languages.
Conclusions: The signal-to-noise ratio required to achieve 50% word understanding is significantly lower as measured by the Spanish AzBio compared to the English AzBio in normal hearing bilingual listeners. Results suggest that test-language has the potential to influence test performance. Further research is required to clarify the differences between the two measures and ensure the test measures are accurately reflecting functional performance.

SU217. Speech-In-Noise Performance Differences Measured by Linguistically Varying Assessments: Correlational Analysis of the WIN, DIN, and AEM Tests
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Category: Speech Perception
Background: Speech-in-noise tests are not commonly administered in clinic partially due to clinician perceived time constraints, uncertainty in which test material is most appropriate for a patient, and lack of materials clearly quantify degrees of speech-in-noise impairment. Speech-in-noise tests available today use a variety of target signals, such as digits, sentences, or monosyllabic words. Linguistic cues of speech, such as semantics, syntactics, word familiarity and word frequency, influence speech-in-noise performance; and, thus, may add to the clinician confusion regarding performance interpretation. Our study aimed to evaluate the performance between three speech-in-noise tests with varying linguistic cues: the Digits in Noise test (DIN), the Words in Noise test (WIN), and the American English Matrix Test (AEMT). To our knowledge,

the AEMT, has yet to be compared to other speech-in-noise tests. We hypothesized that performance would vary in threshold, but be correlated. Understanding correlational relationships on these tests will allow for standardization of degrees of speech-in-noise impairment and determine if these tests can be used interchangeably in clinic.
Methods: Native English speakers with normal hearing (n= 27) and sensorineural hearing loss (n= 32) were tested. The outcome of interest was performance on the DIN (most linguistic cues), the AEMT (some linguistic cues), and the WIN (least amount of linguistic cues). The DIN and WIN were presented in the presence of multi-talker babble and used a descending paradigm to derive the 50% correct threshold using the Spearman-Karber Equation. The AEMT was presented in an open set in the presence of steady-state speech-spectrum noise and used an automated adaptive procedure to converge to the 50% correct threshold.
Results: Speech-in-noise performance, reported as the signal to noise ratio (SNR) at which 50% correct word recognition is achieved (SNR-50), for the normal hearing listeners was the following: DIN: -12.6 dB SNR (SD = 1.94), AEMT: -7.8 dB SNR (SD = 4.22), and WIN: 4.8 dB SNR (SD = 1.65). Performance for the listeners with hearing loss was: DIN: -4.9 dB SNR (SD = 2.68), AEMT: -1.5 dB SNR (SD = 4.20), and WIN: 16.6 dB SNR (SD = 5.12). For both groups, performance increased with increasing linguistic cues. Strong positive and statistically significant correlations ($p<0.001$; Pearson product moment correlation) were observed with r values of: DIN/WIN: r= 0.849, AEMT/WIN: r= 0.848, and AEMT/DIN: r= 0.729.
Conclusions: All three speech-in-noise test materials were statistically correlated. All three tests provided a reliable representation of speech-in-noise performance, and were considered feasible to implement in the clinic (administration was less than 7 minutes each). With additional analyses to determine predicative validity, our findings indicate that these three tests can be used interchangeably in the clinic. Having several comparable speech-in-noise tests allows for a patient-centered approach to test selection.

SU218. Speech in Noise Perception and Neural Auditory Processing of Speech Among School-Age Children

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Category: Speech Perception
Background: The present study characterizes perceptual and neural auditory processing of speech in noise (SIN) assessed by the frequency following response (FFR) among early school-age children with normal hearing. The current study aims to investigate the correlation between neurophysiological and perceptual SIN perception and the contribution of working memory (WM) function.
Methods: Thirty-five children with normal hearing, between the ages 6-8 years participated in the study. SIN perception was evaluated using both electrophysiological and perceptual measures. For the electrophysiological assessment, brainstem responses elicited by an acoustic click and the frequency following response (FFR) by a speech syllable /da/ were collected in both quiet and under background noise. FFR latency and amplitude components were detected and analyzed including the fundamental frequency (F0). For the perceptual assessment, all participants underwent a sentences perception task at three signal to noise ratio (SNR) levels: +3dB, 0dB and -3dB. In addition, all participants completed a WM task using the digit span subtests of the Wechsler Intelligence Scale for Children.
Results: A significant correlation between the neural processing of F0 (assessed by the FFR) and SIN perception was observed. Participants who performed poorer on the sentences in noise task, also showed a larger decrease in F0 amplitude when switching from the quiet to the noise condition. In addition, a significant correlation between WM function and SIN perception was observed; children who performed better on the digit-span forward subtest demonstrated better performance on SIN tasks.
Conclusions: A cognitive-perceptual-neural relationship was observed at early school ages. Better speech in noise perception is related to neural processing of F0 and working memory function. These two factors may account for the individual differences in SIN perception among normal hearing children.

SU219. The Stability of the Speech-To-Song Illusion: Effects of Individual Differences

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Category: Speech Perception
Background: Music and language are easily distinguishable for the average listener despite sharing many structural and acoustic similarities. In the Speech-to-Song (STS) illusion, multiple repetitions of a natural

spoken utterance can give rise to a perceptual switch wherein the stimulus begins to sound like song to the listener. Although prior work suggests that both musicians and non-musicians experience the STS illusion (Vanden Bosch der Nederlanden et al. 2015a), some evidence shows that relative to non-musicians, musicians may require more repetitions to experience the STS illusion (Besson, Chobert and Marie, 2011; Dalla Bella, Peretz, and Aronoff, 2003; Falk et al., 2014), perhaps due to more robust musical representations or more refined selectivity of an auditory signal as speech- or song-like.
Methods: In our study, we measured the STS illusion by presenting listeners with stimuli known to elicit the STS illusion and asking them to rate the degree to which each repetition sounded song-like. We also administered the Goldsmiths Musical Sophistication Index (Gold-MSI), a speech prosody test (PEPS-C), and a tonality test (from Corrigall and Trainor, 2015).
Results: Although data collection is ongoing, initial results suggest that musicality (reflected in the Active Engagement subscale of the Gold MSI) negatively predicts the rate of STS transformation, as found in prior work. Individuals who gave higher ratings overall also tended to get higher scores on the musicality and music training subscales of the Gold-MSI as well as higher scores on the speech prosody task. These findings provide initial support for the possibility that individual differences in musical skill, music aptitude, and sensitivity to speech prosody may predict the experience of the STS illusion.
Conclusions: Understanding how the auditory system processes musical and linguistic stimuli may have implications for the development of effective hearing devices or for diagnostic assessments for individuals with communicative deficits.

SU220. Does Linguistic Complexity and Statistical Learning Interact to Maximize Learning in the Brain?
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Category: Speech Perception
Background: One of the longstanding debates in the area of speech and language acquisition is whether it is the linguistic complexity (Maggu et al., 2019) or statistical learning (Romberg and Saffran, 2010) that drives speech and language acquisition. Recently, there has been a growing interest in studying the interactive effects of linguistic complexity and statistical learning on speech and language development (Adriaans and Kager, 2010). However, whether these interactive effects maximize learning, is currently unclear.
Methods: In the current study, we examine whether a combination of linguistic complexity and statistical learning maximizes learning in a passive listening paradigm (i.e., listening while asleep), as assessed via frequency following responses (FFR). More specifically, we examine the generalization learning due to passively listening to 500 repeated presentations of pre-voiced dental-retroflex contrasts (i.e., linguistically complex stimuli; e.g., d̪a-ɖa) on the perception of voiceless dental-retroflex contrasts (i.e., linguistically simple stimuli; e.g., t̪a-ʈa) and vice versa while they are being recorded on FFRs.
The current study is aimed at recruiting 40 adults participants (18-35 y) with normative hearing abilities who are classified into four groups, namely, Complex_Stat (n=10) from whom FFRs are being recorded for complex stimuli pair first followed by the simple stimuli pair with the stimuli presented in a statistical learning fashion; Simple_Stat (n=10) from whom FFRs are being recorded for simple stimuli pair first followed by the complex stimuli pair with the stimuli presented in a statistical learning fashion; Complex_Rand (n=10): from whom FFRs are being recorded for complex stimuli pair first followed by the simple stimuli pair with the stimuli presented in a random fashion; and Simple_Rand (n=10): from whom FFRs are being recorded for simple stimuli pair first followed by the complex stimuli pair with stimuli presented in a random fashion.
Results: Preliminary results so far indicate that the Complex_Stat group (i.e., presented with complex stimuli first) demonstrate enhanced FFR amplitudes for the simple stimuli pairs, indicating generalization learning to the simple stimuli by exposure to complex stimuli first, within a statistical learning paradigm. In comparison, Simple_Stat group (i.e., presented with simple stimuli first) do not exhibit generalization learning to the complex stimuli i.e., complex stimuli pairs in this group did not show enhanced FFR amplitudes. However, both these groups with stimuli presentation in statistical learning fashion are exhibiting enhanced FFR amplitudes as compared to the groups where stimuli were presented randomly (Complex_Rand, Simple_Rand). Further, there is no difference between the random presentation groups on their FFR amplitudes.

Conclusions: Overall, the preliminary findings reveal that the interaction of complexity and statistical learning maximizes generalization learning in the brain. The findings of the current study have implications towards development of therapy approaches for speech sound disorders.

SU221. An Animal Model of Tinnitus Induced by Chronic Stress: A Behavioral Study

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Category: Tinnitus
Background: Introduction: Recent pathophysiology on tinnitus in human and animal studies proposed the possible association between tinnitus and stress. This study was designed to establish a novel animal model of tinnitus induced by chronic stress and to investigate the possible mechanism as well as psychological conditions related with tinnitus after chronic stress exposure.
Methods: Rats were exposed to restraint stress for 2 hours a day for 10 days. The gap response of prepulse inhibition acoustic reflex (GPIAS) was measured on the last day of stress to detect tinnitus and the rats were divided into tinnitus and non-tinnitus groups. Hearing tests of distortion product otoacoustic emissions (DPOAE) and auditory brainstem response (ABR) were performed. The different behavioral tests of elevated plus maze (EPM) and forced swimming test (FST) were conducted to observe anxiety and depression of the rats to investigate the relationship between tinnitus and their psychological conditions.
Results: After the chronic restraint stress, tinnitus was observed in 64.3% of the rats. Hearing test results of the tinnitus group showed no difference compared to the control and non-tinnitus groups, indicating that their tinnitus was induced only by stress. Tinnitus group showed significantly decreased time spent in the open arm compared to non-tinnitus and control groups in EPM study ($P<0.05$) and significantly increased immobility time in FST study ($P<0.05$), which demonstrate their increased anxious and depressed conditions after chronic stress.
Conclusions: A rat model of tinnitus induced by chronic stress was firstly established and an association between tinnitus and psychological symptoms of anxiety and depression were observed in this study. Further research to investigate the pathomechanism in the brain regarding tinnitus induced by chronic stress and its causal relationship with other psychological symptoms will be needed.

SU222. The Changes of Intrinsic Properties in IC Neurons Underlie Hyperactivity After Unilateral Acoustic Trauma

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Category: Tinnitus
Background: Neuronal hyperactivity has been associated with many brain diseases. In auditory system, hyperactivity has been seen as a potential mechanism underlie the hyperacusis and/or tinnitus. Many studies have shown that sound overexposure leads to hyperactivity in central auditory system which is reflected in an elevation of neuronal spontaneous firing, bursting and increased synchrony. Inferior colliculus (IC) is a major integrative center of the central auditory system. It receives and integrates ascending as well as descending information from many auditory as well as from non-auditory brain structures. Our previous study has shown increased spontaneous firing and bursting events in IC neurons after sound exposure, but how the hyperactivity is developed in the IC is still unclear. One of the possibilities for that is the intrinsic properties of IC neurons are altered after exposure. In this study, we examined the intrinsic properties in IC neurons following a sound exposure.
Methods: A total of 26 CBA/CaJ mice were used in this study. All animals were between 5-12 months old. The sound exposure was performed in adult animals (at least 2 months old). One octave narrowband noise centered at 12.5 kHz (8–17 kHz) was presented at the level of 116 dB SPL unilaterally for 1 h under ketamine/xylazine anesthesia. Intracellular recordings were conducted with quartz micropipettes filled with 1 M potassium acetate having impedance around 250 MΩ in unanesthetized mice. Electrodes were inserted into the IC via a small opening (≈100 μm) in the skull. Spontaneous and sound evoked activity to pure tones 100 ms duration presented at different sound frequencies at the level of 55 dB SPL were recorded. We examined and compared spontaneous firing rates (SFRs), resting membrane potentials (RMPs), and half width of action potentials in IC neurons before and after exposure.

Results: We found that after sound exposure the SFRs were increased and RMPs were depolarized in IC neurons with BFs at/above center frequency of sound exposure. The half width of action potentials was also significantly decreased after exposure. All these intrinsic properties changes were evident on both contralateral and ipsilateral sides of IC but the effect on the ipsilateral IC was more robust.
Conclusions: Our research suggests that the changes of intrinsic properties in IC neurons, at least partially, underlie the hyperactivity after unilateral sound exposure.
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SU223. Tinnitus Emerges Independently of Elevated Inferior Colliculus Spontaneous Activity and Thalamic Dysrhythmia After Noise Trauma

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Category: Tinnitus
Background: Current pathophysiological models of tinnitus presume that increased spontaneous activity of auditory neurons underpin auditory phantom perception. While elevated spontaneous rates have been reported in different nuclei along the auditory pathway, their relationship to tinnitus induction, developmental time course, and spiking characteristics across regions remains unexplored. Contradictory findings emerge across animal species, time scales, and different methods of tinnitus induction and assessment.
Methods: Thus, to achieve a coherent understanding of the neural underpinning of tinnitus, we set out to systematically investigate tinnitus correlates in a single animal model with extended temporal and spatial windows. Thus, we examined the development of tinnitus from 0–10 hours after noise trauma. Tinnitus was inferred using a deep neural network classifier previously trained on behaviorally confirmed dorsal cochlear nucleus (DCN) single-unit recordings. We recorded from multiple auditory regions simultaneously—cochlear nucleus, inferior colliculus (IC), and medial geniculate nucleus (MGN)—and examined their interdependence.
Results: Our results show: 1) while tinnitus-like activity emerged in DCN immediately after noise trauma, contralateral IC activity remained unchanged after noise trauma or tinnitus; 2) contralateral MGN activity increased but spiking was not directly drivien by DCN spiking; 3) MGN oscillatory activity was abolished by noise trauma itself, independent of tinnitus development.
Conclusions: These findings question the involvement of IC in tinnitus generation and the relationship between auditory thalamic dysrhythmia and tinnitus.

SU224. Sazetidine-A, a Desensitizing Nicotinic Agent, Ameliorates Subcellular Evidence of Tinnitus

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Category: Tinnitus
Background: Tinnitus, commonly known as ringing of ears afflicts approximately 15% of the total world population, of which up to 36% of suffers experience a significant negative impact on their quality of life. Like chronic pain, tinnitus pathology is believed to be initiated by partial peripheral deafferentation of sensory neurons resulting in maladaptive plastic changes, altering central auditory/somatosensory, limbic and attentional systems. Similarities in the pathological markers for tinnitus and chronic pain include increased activity in certain neuronal populations within the auditory and somatosensory cortices respectively.
Cichon et al. (2017) recording from somatosensory cortex in a neuropathic chronic pain model, found significant increases in excitatory activity of layer 5 principal neurons (PNs) and vasoactive peptide positive (VIP) neurons. Consistent with these findings, the present studies in the primary auditory cortex (A1) of animals with behavioral evidence of tinnitus, found significant increases in excitatory input and decreased inhibitory input onto layer 5 PNs. These same A1 circuits showed significantly increased excitability of VIP neurons in tinnitus animals. Desensitizing nicotinic acetylcholine agents have shown efficacy in preclinical treatments of chronic pain and may be effective in the management of tinnitus.
Methods: 3-4 months old Long Evans (LE) rats were unilaterally noise exposed to 116 dB narrowband noise centered at 16 kHz for an hour. Tinnitus was assessed using an established conditioned suppression operant model (Bauer et al., 1999), and the animals were classified as control, exposed tinnitus and exposed

non-tinnitus. The ability of Saz-A, a nicotinic acetylcholine receptor (nAChR) desensitizing agonist, to normalize hyperexcitability in A1 layer 5 PNs was examined using in vitro whole-cell patch clamp recordings.
Results: Whole-cell patch clamp recordings from A1 layer 5 PNs collected spontaneous postsynaptic excitatory/inhibitory currents (sEPSC/sIPSC). Bath application of Saz-A did not alter the previously observed tinnitus-related increases in sEPSC frequency onto layer 5 PNs. However, PNs were hyperpolarized following Saz-A application, suggesting a link to increased inhibitory currents. A1 Layer 5 PNs showed significant tinnitus-related decreases in sIPSC frequency. This disruption in the excitatory/inhibitory homeostasis in animals with behavioral evidence of tinnitus was tested with bath application of 500 nM Saz-A. Saz-A significantly increased sIPSC frequency in tinnitus animals, with no changes in sIPSC frequency observed in control animals. Consistent with the ability of Saz-A to normalize rat behavioral evidence of tinnitus (see ARO poster, Ling et al.) the present studies find that Saz-A can normalized subcellular tinnitus-related changes in A1 of animals with behavioral evidence of tinnitus.
Conclusions: Together these studies support sazetidine-A (Saz-A), an nAChR desensitizing agonist's ability to ameliorate tinnitus in rats with behavioral evidence of tinnitus (Patent#US17/428,164). Future preclinical/clinical trials are needed to determine the efficacy of Saz-A in the management of tinnitus.

SU225. Preclinical Studies on Sazetidine-A, a Potent Nicotinic Desensitizing Agent, for Tinnitus Treatment: Behavioral and Pharmacokinetic Studies

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Category: Tinnitus
Background: Tinnitus impacts between 10-20% of the population. Individuals most troubled by their tinnitus have their attention bound to and distracted by, their tinnitus percept. While numerous treatments to ameliorate tinnitus have been tried, no therapeutic approach has been clinically accepted. Sazetidine-A (Saz-A), a nicotinic cholinergic receptor (nAChR) desensitizing agonist, has been shown effective in preclinical neuropathic pain studies and has potential to alleviate tinnitus in a condition-suppression noise-exposure model of tinnitus (Patent#US17/428,164). Saz-A targets α4β2 nAChRs, showing similar desensitizing properties in rat and human studies. Here we present results from 2 preclinical tinnitus studies, using subcutaneous (SubCu) or oral administration of Saz-A. Subsequently, concentrations of Saz-A in plasma and neocortex using different drug concentrations, routes of administration (Oral, Subcu and IP) and times-post administration were examined.
Methods: Chronic tinnitus was induced in Long Evans (LE) rats using a unilateral exposure to 116 dB (SPL) 1/3rd octave band-limited noise for one hour. Tinnitus was assessed using an operant conditioned-suppression paradigm. Tinnitus scores were assigned to control and sound-exposed rats before and after Saz-A administration. Separate sets of LE rats were used to assess Saz-A blood and neocortex concentrations in pharmacokinetic studies. Blood levels were assessed using liquid chromatography mass spectrometry (LC-MS), similar to Caldarone et al. (2011) with neocortex concentrations assessed using LC-MS and competition radio-ligand binding paradigm similar to Hussmann and Kellar (2012).
Results: To approximate future human Saz-A clinical trials and to avoid stress caused by SubCu injections prior to rat's behavioral testing, an oral (gel wafer) administration method for Saz-A delivery was developed. Saz-A was administered 1 hr prior to tinnitus testing. A prior study found SubCu injection 1.0 mg/kg of Saz-A effectively reduced the separation between control and tinnitus suppression curves suggesting reduction in behavioral evidence of tinnitus. The current oral Saz-A study used 16 (8 control unexposed and 8 sound-exposed) LE rats which showed suppression plots consistent with behavioral evidence of tinnitus. Similar to the prior study, oral administration of 1.0 mg/kg of Saz-A reduced behavioral evidence of tinnitus. Pharmacokinetic study of Saz-A levels in blood plasma and neocortex using various administration routes and times found: Saz-A blood plasma levels were the highest with IP injection, SubCu injection levels were 21% of IP, with oral administration 10% of the IP blood levels. Significantly, oral and SubCu Saz-A neocortical concentrations were similar. Consistent with prior studies, the present time-course data showed that Saz-A remained in neocortex longer than in plasma. Neocortical Saz-A levels remained high with the peak about 1 hour following oral administration.

Conclusions: These studies advance Saz-A as a potential drug to treat tinnitus and provides new pharmacokinetic data for subsequent Saz-A preclinical studies.

SU226. Sound Evoked Changes After Long Duration Sound as a Test for Tinnitus

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Category: Tinnitus
Background: An objective, non-invasive, electrophysiological test is needed for efficient tinnitus research. In wild type, CBA/CaJ mice, a long-duration sound (LDS) can alter both spontaneous firing rate and responses to sound in the inferior colliculus (IC). Specifically, the majority of sound-driven responses are suppressed while a subset are facilitated after the LDS. We believe that because tinnitus animals show increased spontaneous activity in the auditory system, the LDS-generated changes will be less apparent than in non-tinnitus animals.
Methods: Here, we recorded auditory brainstem responses (ABRs) before and after the LDS and show that there are tinnitus-specific differences. Awake CBA/CaJ mice received a unilateral sound exposure that resulted in mice with and without behavioral evidence of tinnitus. ABR responses to tone pips at three or more frequencies were collected from tinnitus, non-tinnitus, and unexposed control mice. We quantified the effect of LDS-changes and calculated a tinnitus score based on peak-trough amplitudes for each ABR wave.
Results: The tone-pip ABRs evoked by sounds in the exposed ear for tinnitus and non-tinnitus mice showed that non-tinnitus mice had significantly lower scores than tinnitus mice. That is, non-tinnitus mice had more suppression after LDS than tinnitus mice. At higher frequencies at later waves, the effect was more significant. However, there was no significant difference between tinnitus and the control. A correlation analysis of pre-LDS and post-LDS waveforms showed a significantly bigger difference in non-tinnitus mice than in tinnitus mice. A differential time frequency analysis analyzing the spectrum of the ABR waveforms over time showed tinnitus specific ‘hotspots’ at tinnitus frequencies, but not at non-tinnitus frequencies.
Conclusions: Responses to the LDS show tinnitus specific changes that may be a basis for an electrophysiological test for tinnitus.

SU227. Computational Models of Active Human Motion Control

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Category: Vestibular: Basic Research and Clinical
Background: The dynamics of vestibular processing vary dramatically among individuals, as well with age and disease state. For example, the time constant of decay for perceived angular velocity during a constant velocity stimulus (“velocity storage time constant”) is reduced in the elderly and in peripheral vestibular damage. Despite these differences, it is not completely known how the dynamics of vestibular processing contribute to active control of motion, e.g., self-motion during gait, and control of vehicles such as automobiles, airplanes, and spacecraft. Vestibular function is often implicated in errors in active control of motion, including falls and spatial disorientation.
We developed computational models of human control of their own motion from within a joystick-controlled machine. In these closed-loop models, machine motion results in afferent signals to peripheral and central vestibular processing. The brain uses these to determine appropriate motor command efferents, which lead to joystick inputs that result in machine motion. We aimed to A) develop a closed-loop human-machine model that includes state-of-the-art spatial orientation and motor-control components, and B) validate and refine this model using human experiments.
Methods: Modeling (Matlab/Simulink) and experimental validation were performed only for an Earth-vertical yaw rotation task to isolate vestibular organ stimulation to semicircular canal cues. Healthy subjects (18-40 years) were seated in a standard clinical motorized rotary chair (Neurokinetics) in complete darkness. Subjects were instructed to use a joystick to minimize chair velocity while experiencing a pseudo-random sum-of-sines “disturbance” ranging between 0.004-1.7 Hz. Experimental data was used to enhance and tune the model.

Results: The closed-loop model predicted wide variations in performance (nullification of disturbances) by frequency. In particular, the models predicted that human performance was mediocre for rotation control in the dark. The most effective nullification was predicted to occur in a small band of medium-range frequencies (0.01-0.1 Hz), due to combined high-pass filter vestibular dynamics and low-pass filter motor control dynamics.
Human experimental data closely followed the overall frequency response predicted by the model. One major innovation that improved model predictions was the requirement that sensory information reach a minimum threshold before determining a motor command, as sensory feedback is likely corrupted by neural noise. This greatly increased the agreement between experimental results and model predictions. Model parameters of central nervous system (CNS) delay and motor control responsiveness were also tuned to enhance model prediction accuracy.
Conclusions: The model accurately predicts variations in human task performance by frequency, and specifically mediocre control at certain frequencies. These are novel models of human-vehicle systems that increase understanding of how humans perform motion control tasks. With this, performance decrements in self-motion and vehicle control, such as imbalance and/or spatial disorientation, can be more accurately predicted and potentially mitigated.

SU228. Low-Cost Pupil Tracking With Machine Learning

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Category: Vestibular: Basic Research and Clinical
Background: Eye-tracking technology has improved dramatically in the past decade to improve virtual reality, on-screen gaze estimation, and assistive technologies. However, little research has been conducted to measure the vestibulo-ocular reflex (VOR), which is responsible for maintaining clear vision while head movement occurs. By utilizing the advances in eye tracking technology, measuring the VOR has become much more practical and can aid in diagnosing various eye, ear, and neurological conditions. Current research for measuring VOR is done with scleral coils or video-oculography (VOG) with markers. Current methods are highly accurate but are painful for the test subjects and expensive. To avoid painful and potentially damaging methods we proposed a pain-free low-cost procedure for measuring VOR through VOG without markers. While the absence of markers limits the accuracy, a machine-learning object detection model was utilized to minimize the gap in accuracy.
Methods: The low-cost machine learning VOG device without markers is run through a Raspberry Pi4 attached to a 3D-printed binocular-like headset. The headset has two cameras and an inertial measurement unit (IMU) embedded. The IMU is responsible for tracking the head's rotation. The cameras were used to train the machine learning object detection model with 1800 annotated images for 5000 steps. This model outputs a bounding box around the pupil which is used to track pupil rotation. By finding the ratio of eye rotation to head rotation, an individual's VOR can be calculated.
Results: The VOG device without markers is capable of tracking 2D eye rotation, which allows for an approximation of an individual's VOR. The accuracy of the bounding box was found to be approximately 90%. However, the model has slight difficulties tracking the pupil near the edge of the eye. This leads to a drop in accuracy and is partially responsible for the device being slightly less accurate than current methods of pupil tracking.
Conclusions: A low-cost machine learning VOG device without markers was created to aid in vestibular-ocular research to aid in diagnosing the severity of stroke, concussion, and vertigo. While this method is less accurate than current methods, it is pain-free and provides reasonably accurate VOR estimations for a much lower price. Accuracy can be improved in several ways such as training the model with more images near the edge of the eye, higher speed and resolution cameras, or by also tracking the torsional rotation of the pupil.

SU229. Hyperpolarization-Activated Currents in Zonally-Identified Vestibular Calyx Terminals

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Category: Vestibular: Basic Research and Clinical

Background: Calyx-shaped terminals make afferent synapses with type I hair cells in vestibular epithelia and express diverse ionic conductances that are responsible for action potential generation and regularity of discharge. Quantal and non-quantal modes of synaptic transmission have been described at the type I hair cell/calyx synapse and may be influenced by hyperpolarization-activated, cyclic nucleotide-gated (HCN) channels (Contini et al. 2022). There are four known isoforms (HCN1-4), which exhibit differences in activation kinetics, voltage-dependence and cyclic nucleotide sensitivity. HCN1 channels underlie hyperpolarization-activated current (Ih) in vestibular hair cells, but subunits underlying Ih in mature calyx terminals and cell bodies of vestibular ganglion neurons are unclear. Here we examined calyx terminal HCN channel expression in central and peripheral regions of the crista.
Methods: Whole-cell patch clamp methods were used to record Ih in calyces in slices prepared from gerbil crista (ages 1-8 weeks) as previously described (Meredith and Rennie 2015). The HCN channel blocker ZD7288 (100 uM) was applied extracellularly and dibutyryl-cAMP (db-CAMP, 0.5 mM) was added to the patch electrode solution in some experiments. Immunohistochemistry was performed on crista wholemounts or slices using antibodies against Myosin7a, Tubulin beta 3 and HCN2 subunits.
Results: A slowly activating, non-inactivating inward current (Ih) was present at hyperpolarized potentials in most calyces tested in peripheral (34/42 (81 %) and central crista zones (24/28 (86 %). Peak Ih obtained with 1s voltage steps from -80 to -140 mV in peripheral zone calyces (-164 pA; median) was not significantly different from peak currents in central zone calyces (-137 pA, P = 0.279, Mann-Whitney Rank Sum test). Activation kinetics of calyx Ih did not vary between crista zones, but were slower than kinetics in type I and type II hair cells. ZD7288 blocked Ih in all calyces tested and resulted in a hyperpolarization of the resting membrane potential. Peak Ih amplitude increased with intracellular db-cAMP and activation kinetics became faster. In current clamp, calyces from both zones showed three categories of action potential firing. In response to a depolarizing current pulse ~ 70% of cells fired a single action potential, whereas ~17% showed an evoked action potential followed by membrane potential oscillations. A third group of calyces showed spontaneous firing (13%). In phasic cells removal of Ih resulted in increased action potential latency to peak. Immunostaining with an antibody against HCN2 subunits showed localization within calyx terminals.
Conclusions: We found that Ih is prevalent in mature calyx terminals across crista regions with no clear regional differences. Ih was enhanced by db-cAMP and immunostaining showed the expression of HCN2 subunits in calyx terminals. Functional roles of HCN-mediated currents in synaptic transmission at the type I hair cell calyx synapse will be further explored.
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SU230. Comparison of Enhancement of the Vestibular and Cochlea Perilymph Between Gadobutrol and Gadoterate Meglumine at Magnetic Resonance Imaging in Meniere's Disease

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Category: Vestibular: Basic Research and Clinical
Background: To compare the degree of enhancement of the perilymph between gadolinium-based contrast agents Gadobutrol, (Gadovist, Bayer Ltd., Germany) and Gadoterate meglumine (Dotaram Guerbet Ltd., France)) in patients with Meniere's disease (MD) at 3-T magnetic resonance imaging (MRI).
Methods: 20 subjects were clinically diagnosed with MD according to the criteria of the 2015 Classification Committee of the Barany Society (CCBS). Four hours after intravenous (IV) contrast agent (Gadovist and Dotaram) injection, MRI scans were performed with a 3-T MR unit (Siemens, Germany) using a receive 32-channel phased-array coil. We independently measured the signal intensity ratio (SIR) by using region of interest analysis (1cm diameter) and performed a visual assessment in order to evaluate the perilymph of the vestibule and cochlea.
Results: Gadobutrol and gadoterate meglumine show enhanced contrast differences in MD patients. Differences in endolymphatic structures visualization were found between gadobutrol and gadoterate meglumine in the MD patients. gadoterate meglumine allowed a better assessment of semicircular canals in vestibule. Contrast effect of gadoterate meglumine seemed to be slicly slightly better than that of gadobutrol in the cochlea, but there was no significant difference.
Conclusions: Gadoterate meglumine was more visible than gadobutrol and had stronger contrast intensity, it would be better to use this contrast agent for MD patient diagnosis.

SU231. Supporting Cell Calcium Transients in Mammalian Vestibular Organotypic Cultures

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Category: Vestibular: Basic Research and Clinical

Background: The mammalian vestibular system consists of five organs working in concert to create our sense of balance, head position, and spatial orientation." In several evolutionarily conserved species including the mouse, the two vertical semicircular canals, the anterior (AC) and posterior (PC) canals, contain an anatomical feature known as the eminentia cruciata. This centrally located region contains a cluster of progenitor-like supporting cells. These supporting cells exist in close proximity to sensory hair cells, but with unique morphologies, and are referred to as, clinocytes and clino2 cells. Here, we used a transgenic mouse with the genetic calcium indicator GCaMP5G to track calcium transients in these supporting cells and the surrounding sensory epithelia in organotypic cultures. Supporting cell calcium transients were imaged using a two-photon microscope under different growth conditions and show adaptive growth properties and signaling mechanisms.

Methods: The sensory epithelia of the anterior, posterior, and horizontal canals were harvested from Gad2-Cre::GCaMP5G-tdTomato young adult transgenic mice of either sex [1]. These tissues were dissected in artificial perilymph at 4°C [2]. Each crista was cultured separately in 35mm dishes at 37°C, 5% CO2 in NeuroCult Complete™ (STEMCELL Technologies) supplemented with epithelial growth factor (EGF), fibroblast growth factor (bFGF), 0.2% heparin solution, either with or without the addition of leukemia inhibitory factor (LIF) and streptomycin 25µM. Brightfield images and tdTomato and GCAMP5 fluorescence images were captured every 24 hours using a Leica DMIL LED microscope with CoolLED pE-300 fluorescence (J. Shepherd). Media was replaced every 48 hours and grown for up to 14 days in vitro. Calcium transients in the sensory epithelium were imaged by two-photon microscopy (Bruker) on days 12 and 14 post-culture.

Results: Live cell imaging with GCaMP5G shows supporting cells and surrounding sensory epithelia viability up to 14 days in vitro under different growth conditions. Clino2 cells and clinocytes maintain their robust intracellular calcium transients. Marked morphological changes were also observed throughout 14 days in all three semicircular canal crista cultures.

Conclusions: The vestibular supporting cells, clinocytes, located in the vertical semicircular canals of young adult Gad2-Cre::GCaMP5G-tdTomato mice actively demonstrate intercellular and intracellular calcium transients up to 14 days in culture. Clinocytes are dynamic and undergo morphological changes within their niche and throughout the sensory epithelium in culture. They are resistant to a low concentration of antibiotics and are able to grow in vitro. Future studies will aim to elucidate these morphological and putative functional changes at different ages and growth conditions.

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SU232. Effects of Age on the AMcVEMP Temporal Modulation Transfer Function

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Category: Vestibular: Basic Research and Clinical

Background: Cervical vestibular evoked myogenic potentials (cVEMPs) reflect saccular stimulation that result in an inhibitory muscle reflex recorded over the sternocleidomastoid muscle, and these responses are used to study basic vestibular function and also in clinical applications. For decades, cVEMPs have used transient stimuli such as clicks and tonebursts to evoke onset responses. Recently, amplitude-modulated tones have been used to elicit cVEMPs (AMcVEMPs), which reflect phase synchrony and nonlinearities from the human otolith reflexes that cannot be tapped using other existing techniques. AMcVEMP temporal modulation transfer functions (TMTFs) of different analysis techniques have been established for young, healthy adults but there is no information regarding the effects of age-related degradation. The purposes of this study were to 1) characterize the effects of age for AMcVEMP TMTF, and 2) determine evidence of vestibular non-linearity in young, middle-aged and older adults.

Methods: Our preliminary data collection, to date, has included 12 young (20-39 years), 11 middle age (40-59 years) and 9 older adults (>60 years) with no history of vestibular lesions or middle-ear pathologies.

Stimuli were amplitude-modulated tones with a carrier frequency of 500 Hz and modulation frequencies (MF) ranging from 11 to 397 Hz. Stimuli were presented using a B81 transducer at 65 dBHL. AMcVEMPs were recorded from the sternocleidomastoid muscle using surface electrodes. Response analysis used FFT-based-approach; analyses included amplitude, signal-to-noise ratio and phase coherence. For non-linearity, responses rates were calculated at the harmonics of the MFs.
Results: Significant age-related degradation in the amplitude, SNR and phase coherence were seen. AMcVEMPs were elicited across a wide range of MFs (11-263 Hz) for young adults; in middle-aged and older adults, amplitude, SNR and phase coherence were robust across a narrower range of MFs, resulting into narrower TMTF shapes. The shape of the AMcVEMP TMTF varied for different measures across the groups. With increasing age, there also occurred an upward shift in the MF that elicited the most robust AMcVEMP. The most robust AMcVEMP were seen at 79 Hz for young and middle-age and at 113 Hz for older adults. Robust responses were also present at the harmonics of MFs in most young, some middle-aged and fewer older adults indicating loss of vestibular non-linearity as an effect of aging.
Conclusions: Aging causes a significant decline for AMcVEMP response; however, the effect of aging is not uniform across MFs and across measures. AMcVEMP TMTF gets narrower with age, with an upward shift in the best MFs. AMcVEMP can also be used to determine evidence of vestibular non-linearity. Our data suggest a loss of vestibular non-linearity as a function of aging. Results from this study enhance our understanding of age-related changes in vestibular system. Expansion of AMcVEMP to clinical population may lead to a deeper understanding of the pathological disorders.

SU233. Changes in Perceived Timing of Galvanic Vestibular Stimulation Relative to Visual, Auditory, and Vibrotactile Stimulations

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Category: Vestibular: Basic Research and Clinical
Background: Various sensory information involved in balance maintenance is often integrated together with different timing characteristics when they are processed from peripheral to central stages. However, related sensory cues must coincide both in spatial and temporal aspects for this sensory integration process to be maximally effective. The aim of this study is to measure the perceived timing of galvanic vestibular stimulation (GVS) relative to other sensory modalities such as visual, auditory, and proprioceptive (vibrotactile) stimulations.
Methods: Sixteen healthy adult subjects (7 males, 9 females, aged 22-47 years) participated in the temporal measurements of sensory perceptions. We first measured simple reaction times (RTs) to galvanic vestibular, visual, auditory, and vibrotactile stimuli with a stimulus duration of 1.5 sec. From these RT results, we made predictions about the relative timing of these stimuli necessary for them to appear simultaneous. We then presented GVS-vibration, GVS-light, and GVS-sound stimulus pairs and used the psychophysical method of simultaneity judgments to compare the measured and predicted temporal relationships for the perception of simultaneity.
Results: Simple reaction times for GVS (1238 ± 344 ms) were significantly longer than for vibration (339 ± 39 ms), light (262 ± 72 ms), or sound (208 ± 32 ms). Simultaneity judgments indicated that GVS had to occur 170 ms before light, 260 ms before vibration, and 400 ms before sound to be perceived as simultaneous with them, respectively. This temporal lead of the GVS in multisensory interaction was significantly less than the relative timings predicted by RT differences between GVS and other sensory stimulation.
Conclusions: The findings in this study demonstrate that the GVS needs to precede other balance-related sensory inputs to some extent in order for their stimulus pairs to be perceived as simultaneous, and this GVS time delay is reduced compared to when it was perceived alone. This implies that temporal characteristics of the GVS might be compatible with an incomplete tendency to compensate for differences in processing times during the multisensory integration process.

SU234. Vestibular Hair Cell Survival in Usher Syndrome Type 1 Patients

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Category: Vestibular: Basic Research and Clinical
Background: Usher syndrome is the leading cause of combined hereditary deafness and blindness. It is classified into three subtypes based on severity, with type 1 the most severe form. Usher type 1 patients typically have profound hearing loss at birth; experience progressive loss of sight by retinitis pigmentosa beginning around age 10, worsening over several decades; and display developmental delay in balance-related behaviors, with balance deficit increasing in severity over time. Surprisingly, vestibular deficits and their associated pathologies are poorly understood. However, given recent progress in inner ear gene therapy for Usher syndrome, it is critical to better characterize the otopathology in Usher type 1 patients in order to identify opportunities for therapeutic intervention. Here, we utilize the human temporal bone collection at Massachusetts Eye and Ear to investigate the survival of hair cells in the vestibular organs of Usher syndrome type 1 patients.
Methods: There are three Usher type 1 cases in the Massachusetts Eye and Ear temporal bone collection (aged 64-84). These archival specimens were diagnosed as type 1 based on clinical evaluation of symptom severity and onset. In one specimen, linkage analysis points to a locus that includes Ush1F and Ush1D. Hair cell survival, and stereocilia morphology, in the vestibular organs of these three cases were assessed by differential interference contrast microscopy, and in one instance, transmission electron microscopy. Data from Usher cases were compared to age-matched normal specimens.
Results: In all cases, we saw many surviving vestibular hair cells carrying intact stereocilia bundles. Hair cells were observed in all vestibular organs, with the exception of the saccule in which no sensory epithelium was detected. In one specimen, hair cells were examined by transmission electron microscopy, enabling a high-resolution assessment of stereocilia bundle morphology. Mature, coherent, bundles were observed on both type 1 and type 2 hair cells. Thus, vestibular hair cells in Usher syndrome type 1 patients survive for many decades.
Conclusions: These data, obtained in 64–84 year-old patients, suggest there is an extended postnatal therapeutic window for gene therapy to rescue vestibular function. The survival of hair cells into later life, even in the most severe cases of Usher syndrome, is strong evidence for the presence of vestibular hair cells in early development, in this number or greater. This presents the exciting opportunity to rescue the sense of balance in patients with Usher syndrome, providing the potential to significantly improve quality of life in these individuals.

SU235. Vestibular Rehabilitation Can Be Performed More Accurately With the Help of an Automated Inertial Measurement Unit Sensor-Based Guiding System

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Category: Vestibular: Basic Research and Clinical
Background: Vestibular rehabilitation is an effective treatment method for unilateral and bilateral vestibulopathy. The conventional generic vestibular rehabilitation is usually performed at home by patients, after being educated with an illustrated handout. Some doctors and patients find this method unreliable because the printed media-based instructions are not intuitive nor motivating. The biggest problem of the handout-based vestibular rehabilitation is that there is no feedback even when the patients are not properly performing the rehabilitation exercise. The objective of this study was to validate the feasibility of an inertial measurement unit (IMU) sensor-based vestibular rehabilitation guiding system (IMU-rehab). The subjects' performance was compared with that of conventional vestibular rehabilitation based on an illustrated handout (CON-rehab).
Methods: Twenty-four normal subjects were prospectively recruited after informed consent. The subjects were educated on how to perform the generic vestibular rehabilitation: adaptation exercise, balance and gait exercise. An illustrated handout was provided so that the subject can always refer to the handout when needed. The subjects were first asked to perform the rehabilitation exercise on their own with the help of the illustrated handout (CON- rehab). Then the subjects were asked to perform the same rehabilitation exercise, now with the IMU sensor-based vestibular rehabilitation guiding system. The IMU system recorded the subject's head movement position and speed. The system not only gave audio instruction on how to perform each rehabilitation step, but it also provide audio feedback if the head movement was too slow or fast. The

accuracy of the vestibular exercise was graded by a single expert based on how well each rehabilitation exercise was performed (A, B, C, D, and F).
Results: The performance of vestibular rehabilitation was significantly better in the IMU-rehab condition compared to the CON-rehab condition. As for the adaptation exercise, the performance of CON-rehab was grade A and D in 50.3% and 12.8% of the subjects, respectively. When IMU-rehab was performed in the same subjects, it improved to 70.2% and 0%, respectively. As for the balance and gait exercise, the performance of CON-rehab was grade A and D in 54.1% and 4.6% of the subjects, respectively. When IMU-rehab was performed in the same subjects, it improved to 75.0% and 0%, respectively.
Conclusions: The IMU sensor-based vestibular rehabilitation guiding system was able to lead the subjects to perform a more precise vestibular rehabilitation exercise. The speed of head movement was adequately controlled with the system thanks to the individualized feedback. Also, all the step of vestibular rehabilitation was not omitted thanks to the audio instructions. The IMU sensor-based vestibular rehabilitation may be superior to the conventional vestibular rehabilitation based on an illustrated handout.

SU236. Peripheral Vestibular Injury and Dysfunction is Associated With Reduced Walking Speed in Noise-Exposed Rats

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Category: Vestibular: Basic Research and Clinical
Background: The vestibular system integrates signals related to vision, head position, gravity, motion, and body position to provide stability during motion through the environment. Disruption in any of these systems can reduce agility and lead to changes in one's ability to safely navigate their environment. Causes of vestibular decline are diverse; however, excessive noise exposure can lead to otolith organ dysfunction (Stewart et al., 2020). We hypothesize in the current study that noise exposure can be a source of vestibular damage that leads to decreased vestibular-mediated agility, while tracking vestibular short-latency evoked potential (VsEP) responses in an age matched cohort. We aim to determine the impact of noise exposure on vestibular-mediated motor performance by tracking noise-induced changes in VsEP in parallel with motor performance in a balance beam crossing task.
Methods: Rats are trained to cross a 1.2-meter balance beam. All 10 balance beam trials, regardless of outcome, are recorded and analyzed for each rat. Each balance beam crossing is objectively timed, using two sensors that mark start and stop times. When rats demonstrate stable crossing times and their crossing scores demonstrate consistent performance and proficiency, they are exposed to noise (120 dB SPL, 1.5kHz 3-octave band noise) or sham conditions. Following noise exposure, rats continue to perform the balance beam crossing task 2 times per week, and if deficits are not present, rats may be re-exposed to noise. Sham rats were exposed to the same conditions as noise rats with the exception of noise. Rats are being tracked continuously into late adulthood; however some rats' ears are being collected prior to late adulthood to track progressive changes in vestibular sensory epithelia related to aging and intense noise exposure.
Results: Rats' crossing behavior rapidly stabilizes and remains proficient beyond the first year of life in the absence of noise exposure. Deficits in motor performance became apparent after noise exposure, evidenced by increased crossing times. In an age-matched cohort of rats, VsEP deficits followed a similar timeline of impairment and recovery following noise exposure. As expected, vestibular sensory epithelia become more severely damaged with repeated noise exposures; however a single noise exposure is sufficient to cause permanent damage to vestibular sensory epithelia.
Conclusions: These findings show the impact noise has on the vestibular system, not only at cellular and physiological levels, but at a functional level that may have implications for people that have experienced noisy environments.

SU237. The Development of a Clinical Test of Spatial Orientation

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Category: Vestibular: Basic Research and Clinical
Background: Vestibular perception is often assessed using sophisticated machine driven equipment, presenting a challenge for clinic friendly testing. Supra-threshold spatial orientation tests appear to be more clinic friendly and suggest some ability to discriminate vestibular performance deficits. However, the

current clinic friendly tests use are susceptible to feedback bias that could mask deficits in rotational spatial perception. Here we report a manually driven rotary chair assessment of spatial orientation perception and correlate those results with other multisensory clinical perceptive tests.
Methods: Healthy adults (n=26, 14 females), mean age 25.0 (±9.9), were seated in a salon chair wearing blindfolds and noise-attenuating headphones. Subjects experienced 36 pseudorandomized rotations about the vertical axis (18 clockwise) varying between 60-300 degrees at 30-degree intervals [60 120 150 210 240 300] corresponding to the hour positions on a clock face. Subjects were instructed to imagine themselves facing 12 o'clock prior to each rotation. Subjects verbally reported the direction they turned, the clock hour mark they pointed to as the hour hand, and their confidence [0 (not confident) to 10 (completely confident)]. No feedback was provided. Spatial error was the difference between actual and reported position. Subjects then completed a joint position error test, subjective visual vertical, and dynamic subjective visual vertical in virtual reality, and vestibular-ocular reflex (VOR) function was quantified with video head impulses. Mixed model ANOVAs examined differences in spatial errors and confidence ratings and Principal Component Analysis (PCA) examined the relationships between different perceptual tests.
Results: Average spatial errors were [19.3, 27.5, 28.5, 46.2, 56.0, 46.5] degrees for each rotation size. Average confidence was [7.2, 6.4, 6.6, 5.9, 5.9, 5.7] for each rotation size. Absolute error was significantly greater for large rotation sizes (>180°) compared to small rotation sizes (<180°) ($t=10.73$, $p<0.001$). Confidence was significantly lower for large rotation sizes ($t=-10.46$, $p<0.001$). Neither spatial errors, nor confidence differed based on repetition number. Exploratory PCA identified 2 orthogonal components explaining 70% of the total variance: 1) Verticality + Head Orientation; 2) Spatial Orientation.
Conclusions: This study reports a manual rotary chair test that captures spatial orientation perception in a manner not previously portrayed. Results should be parsed into two error scores representing rotations smaller and larger than 180 degrees. Preliminary results suggest a shorter version of the test would yield similar results. Exploratory PCA suggests that spatial orientation may be an independent percept from head on body position and verticality. A clinical version of this test may enhance the diagnostic and treatment approaches for individuals with dizziness.

SU238. Spatial Localization of Sound and Light With Bilateral Cochlear Implants in Children With Concurrent Auditory and Vestibular Impairments

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Category: Vestibular: Basic Research and Clinical
Background: This study aims to determine whether spatial localization and visuospatial abilities in children with bilateral cochlear implants (CIs) are affected by concurrent vestibular impairments. Vestibular impairments often occur in children using CIs (20-70%), likely due to the close anatomical proximity of the vestibular and auditory systems and the potential for CI insertion to affect the vestibular end organs. Saccular input provides an indication of postural position in space and labyrinthian input stabilizes the visual field during head movement. We hypothesized: 1) that the loss of two essential sensory systems in children (auditory and vestibular) increase their risks of impaired sound localization relative to hearing and age-matched peers; and 2) visuo-spatial abilities are particularly poor in children with CIs who also have impaired vestibular-ocular reflexes.
Methods: Participants were 12 children (M:9, F:3) who received bilateral CIs simultaneously (mean(2.96) age at CI = 5.99 years) or sequentially (mean(4.02) age at first CI = 3.47 years and mean(5.32) age at second CI = 7.42 years) and who underwent vestibular and balance testing. Spatial localization was measured in response to sound (level roved white noise), or visual stimuli (blue light) presented at stationary locations or while moving by 20° or 40° within a horizontal 120° arc. Head and eye movements were not restricted; head movements were monitored using gyroscope and accelerometer and eye movements were measured by pupillometry. Outcome measures were analysed by mixed linear models with fixed factors of vestibular group, age, and sex and a random intercept for each child.
Results: Vestibular impairment was identified in 5/12 (42%) of the children with bilateral CIs (VI group) and normal vestibular function was measured in the other 7 children (no-VI group). The VI group had confirmed diagnoses of Usher Syndrome (n=3), or congenital cytomegalovirus (n=2). Localization of stationary sound was significantly poorer in the VI than no-VI group ($F(2)=2.42$, $p<0.02$). Of the 5 children

in the VI group, 5 (100%) had abnormal vestibular-ocular reflexes. In the VI group, 2/5 (40%) children demonstrated abnormal eye tracking to light compared to 1/7 (14%) in the no-VI group. Quantification of head and eye displacement compared to auditory and light targets are being analyzed for comparison between the VI and no-VI groups.
Conclusions: These preliminary findings in a small group of children with bilateral CIs suggest that concurrent vestibular impairments affect both auditory and visual localization.

SU239. A Study on the Change of Balance Function and Histopathological Findings of Vestibular Organ in Type II DM Mice

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Category: Vestibular: Basic Research and Clinical
Background: Type II diabetes mellitus (DM) is associated with multiple vascular complications. Several studies revealed that DM increases the risk of vestibular dysfunction. First, we aimed to evaluate the changes in vestibular function and vestibular organ histopathology in type II DM mice. Second, the effect of high-fat diet-induced hyperglycemia on vestibular function and histopathology was also evaluated.
Methods: C57BL/6J mice and C57BLKS/J-db/db mice were utilized as normal and type II DM models. The rotarod test and inclined balance beam test were utilized. The time to fall (TTF) was recorded in the rotarod test with three modes (constant-low speed, constant-high speed, and accelerating speed). The time to transverse (TTT) was recorded in the inclined balance beam test with four conditions [wide beam + light on (1), wide beam + light off (2), narrow beam + light on (3), and narrow beam + light off (4)]. In addition, we calculated somatosensory, visual, and vestibular scores using the division formula (e.g., Vestibular point = condition 4 / condition 1). The tests were conducted 12, 22, 31, and 40 weeks after birth, and we checked both body weight and blood glucose level over the same time interval.
After 40 weeks, we performed histopathological evaluation of the vestibular organ of each mouse. We divided all mice into three groups: the normal mice with normal diet group (Group N), normal mice with high-fat diet group (Group H), and type II DM mice with normal diet group (Group D). The differences in balance test and histopathology results were analyzed.
Results: Group D showed significantly higher body weight than the other groups at 12 weeks ($p<0.001$), and Group D showed significantly lower body weight than the other groups at 31 and 40 weeks ($p<0.001$). Group D showed the highest blood glucose level in all experimental periods ($p<0.001$).
In the rotarod test, Group D showed significantly poorer results than the other groups at all time points. Group D also showed significantly poorer results on the inclined balance beam test than the other groups at all time points. Finally, Group D showed significantly poorer vestibular score results than Group N ($p<0.001$) and H ($p<0.001$) at 31 and 40 weeks.
In Group D, the mean number of vestibular hair cells in the macula of saccule was significantly smaller than that of other groups ($p=0.042$). Group D also showed a significantly decreased mean nuclear area than other groups ($p=0.030$). But there was no difference in the mean area of the nucleus ($p=0.690$) or area ratio ($p=0.507$) between Groups N and H.
Conclusions: The results of this study suggest that hyperglycemia induced by type II DM imparts vestibular dysfunction and histopathological vestibule alterations in model mice. However, only hyperglycemia in normal mice did not affect vestibular dysfunction.

SU240. Vestibular Histology Changes in a Viral Infection Model for Sensorineural Hearing Loss

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Category: Vestibular: Basic Research and Clinical
Background: Lassa Virus (LASV) is the causative agent of Lassa fever (LF), which causes hearing loss (HL) and vestibular dysfunction. The exact mechanism leading to these symptoms remains unclear. Our previous study with LF model mice showed that CD4 T cells play a key role in LASV-induced HL and inner ear damage. While LASV is required to be handled in the BSL4 setting, ML29, a vaccine candidate for

LASV, allows for handling at a lower biosafety level. Here we investigated ML29 infected mice as a potential surrogate for studying LASV-induced inner ear dysfunction in a regular lab setting.
Methods: Stat1-KO mice were intraperitoneally inoculated with PBS or ML29, with anti-mouse CD4 and/or CD8 mAb injected for T-cell depletions. The auditory and vestibular behavior was assessed weekly for three months, and the temporal bones were harvested at the end of three months post-infection. We conducted histological studies of the temporal bones. Paraffin-embedded inner ear thin sections were stained/labeled with H and E, CD3 antibody, and LASV nucleoprotein antibody. Vestibular structures were assessed for structural damage, hemorrhage, lymphocyte infiltration, and the presence of CD3-positive lymphocytes and LASV antigen.
Results: Hearing loss and abnormal vestibular behavior were seen in mice treated with ML29. The majority of mice treated with ML29 developed imbalances. Severe hearing loss was observed in mice depleted with CD8, while other groups showed mild to moderate hearing loss. Lymphocyte infiltration was seen in ML29 infected mice in most vestibular structures except for saccule and superior/inferior vestibular ganglions. Severe vestibular structural damage and the presence of LASV antigen were observed in the CD8-depleted group. No LASV antigen was seen in saccules of the CD4 or CD4/8 depleted groups. Infiltration of CD3+ lymphocytes was seen in crista ampullaris and utricle of CD8-depleted mice.
Conclusions: ML29 infection in Stat1 KO mice induced abnormal behavior suggestive of vestibular dysfunction, which correlated with histological changes in the vestibular organs.
Similar to the LASV-infected mice, CD4 immune responses contributed to ML29 infection-induced vestibular damages.
ML29 is a suitable surrogate model for studying LASV infection-induced hearing loss and vestibular dysfunction, which can be handled in the biosafety level 2 laboratory.

SU241. The Value of Saccade Metrics and VOR Gain in Detecting a Vestibular Stroke

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Category: Vestibular: Basic Research and Clinical
Background: A normal video Head Impulse Test (vHIT) is the gold standard in the emergency department to rule-in patients with an acute vestibular syndrome (AVS) and a stroke. We aimed to compare the diagnostic accuracy of vHIT metrics regarding the vestibulo-ocular reflex (VOR) gain and the corrective saccades in detecting vestibular strokes.
Methods: Prospective cross-sectional study (convenience sample) of patients presenting with AVS in the emergency room of a tertiary referral center between February 2015 and May 2020. We screened 1677 patients and enrolled 76 patients fulfilling the inclusion criteria of AVS. All patients underwent vHIT including an automated data analysis and a manual analysis. A delayed MRI served as a gold standard for vestibular stroke confirmation.
Results: Out of 76 patients, 51 were diagnosed with vestibular neuritis and 25 patients with vestibular strokes. The overall sensitivity and specificity for detecting stroke with an automated VOR gain (best cut-off of 0,665) was 83,3% and 88,4% respectively, compared to 87,5% and 75% for saccade analysis by an expert and 83% and 59% for the automated saccade analysis. Gain misclassified 13,15% of the patients, manual saccade analysis 21% and automatical saccade analysis 32,8%.
Conclusions: We found an excellent accuracy of vHIT for the diagnosis of vestibular strokes provided that the VOR gain and not the saccades are evaluated. Nonetheless, saccades provide an additional and important information in vHIT evaluation. The automated saccade detection algorithm are not yet perfect compared to the analysis by an expert, however, this might be a valuable tool for future vHIT evaluations by non-experts.

SU242. Developing a Measure of Self-Motion Perception to Assess Vestibular Spatial Function

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Category: Vestibular: Basic Research and Clinical

Background: Vertigo is a debilitating symptom experienced by approximately 40% of individuals within their lifetime, with varying central and peripheral etiologies. Those with vestibular disease often have decreased vestibulo-ocular reflex (VOR) gain compared to healthy controls; hence, current therapeutic approaches to vestibular rehabilitation often emphasize adaptation of the VOR. However, patients with vestibular disease often have persistent deficits in non-specific dizziness or self-motion perception even after completing vestibular rehabilitation. There is a paucity of research surrounding vestibular spatial perception as it relates to vertigo, including its durability over repeated measurements. The current study seeks to determine the test-retest reliability and minimal detectable changes in vestibular spatial perception within subjects over time.
Methods: Eleven healthy subjects without a diagnosis of vertigo or other vestibular disease were recruited for the present study from July through September 2022. Participants completed baseline training in video head impulse testing and a novel rotary chair paradigm; these tasks were repeated during a second visit. Vestibular spatial orientation perception was assessed through yaw rotary motion in a motorized chair; subjects were rotated a maximum of 16 degrees in either direction from center. Following each rotation, subjects indicated perceived spatial orientation by rotating a chair-fixed dial to indicate the front of the room. Perceived spatial orientation was regressed against actual spatial orientation with the slope of the line of best fit representing spatial orientation accuracy. Intraclass correlation analysis was conducted to determine consistency of self-motion perception within individuals across the two study visits.
Results: Eleven subjects with a mean age of 22.8 (standard deviation 4.2, range 18-31) were enrolled, including 7 women (63.6%) and 4 subjects (36.3%) identifying as Black, Asian, or multiracial. Study visits were separated by an average of 10 days (standard deviation 4.2, range 7-19). The intraclass correlation coefficient for self-motion perception across the two study visits was 0.643, demonstrating moderate reliability. Moreover, there was no statistically significant change in slope of the line of best fit between the two study visits (paired-t statistic = -0.43; $p = 0.67$). The minimal detectable changes in vestibular spatial perception ranged from 0.399 – 0.601 among single-visit and pooled data.
Conclusions: Spatial orientation demonstrates moderate test-retest reliability among healthy individuals. Measurement of spatial orientation among patients suffering from vertigo might provide additional clinically relevant information not necessarily captured by current diagnostic techniques focused on VOR; this could potentially inform vestibular rehabilitation approaches, particularly among individuals with intact vestibular reflexes who have benefitted marginally from therapies grounded in VOR adaptation. Further study is needed to determine the durability of test-retest reliability among both healthy and symptomatic individuals, as well as whether self-motion perception can be readily adapted as an innovative avenue for vestibular rehabilitation.

MONDAY, FEBRUARY 13, 2023

POSTER SESSION 3

MO1. Age-Related Cortical Markers of Spectral and Temporal Envelope Change Detection

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Category: Aging
Background: Our ability to process auditory spectral and temporal cues is essential for daily speech communication especially in background competition. Although advancing aging may impact the perception of relevant spectral and temporal envelope cues, the underlying cortical activity linked to potential perceptual changes has not been well characterized. Some neuroimaging studies have shown hemispheric asymmetries for processing spectral versus temporal cues, and it is hypothesized that advancing age may alter these asymmetries due to compensatory mechanisms needed to maintain perceptual performance. Here, we evaluate this hypothesis by measuring detection thresholds for spectral modulation (SM) and temporal modulation (TM) as well as multi-channel EEG simultaneous with behavior to evaluate perceptual sensitivity and cortical responses to changes in SM and TM cues in younger and older listeners with normal hearing.
Methods: Participants included 16 young (19 to 25 years old; 11 females), and 16 older normal- hearing listeners (60 to 80 years old; 14 females). SM detection thresholds were measured at 0.5 and 2.0

cycles/octave, and TM detection thresholds were measured at 4 and 32 Hz in both age groups. Cortical evoked responses (N1, P2, sustained potential – SP) to changes in SM and TM cues were measured at suprathreshold levels simultaneous with behavior via 64-channel EEG using an acoustic change complex paradigm (ACC) in both passive and active attention conditions. Cortical activity was analyzed at both sensor- and source-localized levels.
Results: Behavioral threshold measures were consistent with previous studies in which SM and TM detection thresholds varied with modulation frequency, but no statistically significant differences were observed between age groups. Sensor-level analyses of evoked responses for SM and TM encoding showed a significant main effect of age, where younger adults had significantly larger N1, P2 and SP amplitudes than older adults. Further, active attention produced larger P2 and SP amplitude relative to passive conditions for younger but not older listeners. Hemispheric asymmetry based on source-localized responses was evaluated with the lateralization index and showed that cortical activity for both SM and TM cues was greatest in the right hemisphere for both groups, but asymmetry was reduced in older listeners for some conditions.
Conclusions: The results demonstrated that SM and TM detection thresholds were not significantly different between age groups, but cortical evoked response amplitudes were reduced in older versus younger listeners and those responses did not change with attention in the older group. Likewise, hemispheric asymmetry was observed toward the right hemisphere for both SM and TM cues in both age groups but was somewhat reduced in the older group. The observed age-related differences in cortical but not behavioral measures is consistent with the hypothesis that older adults may engage compensatory neural mechanisms to support processing and perception of SM and TM cues.

MO2. Age-Related Changes in the Neural Processing of Envelope and Fine-Structure Cues, Assessed in the Mongolian Gerbil

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Category: Aging
Background: Aging is associated with a decreased ability to process complex sounds in challenging listening conditions, even in individuals with normal hearing thresholds. Optimal speech in noise intelligibility requires accurate neural representations of envelope and temporal fine structure (TFS) cues present in speech. Aging is also associated with a progressive loss of cochlear synapses which is undetected by threshold audiograms and may contribute to speech in noise difficulties. However, the effects of cochlear synaptopathy on the neural coding of envelope and TFS cues are unclear. Here, we use Mongolian gerbils (Meriones unguiculatus) to examine age-related changes in the neural coding of stimulus envelope and TFS cues, and their relationship to cochlear synaptopathy. Mongolian gerbils were chosen for their low-frequency hearing, which makes them a viable model for testing TFS processing, and cross-species comparisons with humans.
Methods: Scalp-recorded phase-locked neural responses to the stimulus envelope of amplitude modulated tones (envelope following responses, EFRs) and to the frequency modulation of a low-frequency tone (frequency modulation following responses, FMFRs) were used to assess envelope and TFS cues respectively, in an age-graded series of Mongolian gerbils. EFR stimuli varied in amplitude modulation rates to emphasize peripheral vs. central generators. FMFR stimuli varied in FM depths and rates to probe the limits of fine structure processing. Additionally, auditory brainstem responses (ABRs) assessed changes in hearing thresholds with age. Cochlear synapses and sensory hair cells were quantified using cochlear whole-mounts immunostained for markers of pre-synaptic ribbons (CtBP2), post-synaptic glutamate receptors (GluA2) and hair cell bodies (MyosinVIIa).
Results: Hearing thresholds measured by ABRs showed minimal changes with age. ABR wave 1 amplitudes decreased progressively with age, while wave 5 amplitudes were unaffected or increased. Preliminary results suggest that EFRs to fast AM frequencies (~500Hz) shows age-related decreases while EFRs to slower AM frequencies (~40Hz) are unchanged or enhanced. Gerbils also exhibited robust FMFRs to low frequency (500Hz) tones. FMFRs revealed that TFS processing may be affected earlier than envelope cues, with age. Immunostaining of cochlear elements revealed a progressive loss of synapses with age. Ongoing analysis is looking at the relationship between cochlear synapse loss, and reduced envelope and TFS processing with age.

Conclusions: These results suggest that age-related synaptopathy alters complex sound encoding in the peripheral auditory pathway. These deficits are exacerbated by reduced envelope and TFS cues. Responses evoked from more central generators suggest the presence of some compensatory mechanisms with age.

MO3. Aging Leads to Impairment of Spatial Hearing Abilities in the Mongolian Gerbil

Matthew Sergison*[1], John Peacock[1], Monica Benson[1], Nathaniel Greene[1], Achim Klug[1], Daniel Tollin[1]
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Category: Aging

Background: Aging in humans is known to effect spatial hearing and speech in noise recognition, often even when hearing thresholds are normal. The Mongolian Gerbil (Meriones unguiculatus) is an animal with a hearing range similar to that of humans, providing a translational model to research how aging can lead to dysfunction of auditory pathways. However, whether aging gerbils exhibit spatial hearing deficits similar to what is seen in humans is not well known. Spatial deficits could feasibly be caused by dysfunction of the auditory brainstem, the first site of binaural processing in the auditory pathway. Here, we combined electrophysiological recordings and animal behavior to examine if aging gerbils show a decrease in spatial hearing abilities compared to young gerbils.

Methods: We performed auditory brainstem responses (ABRs) on a cohort of young (2-10 month) and aged (33-24 month) gerbils. ABRs provide a way to non-invasively measure synchronized activity from the auditory brainstem nuclei, and allow us to calculate the binaural interaction component (BIC), a biomarker for binaural processing in the brainstem.

Results: We find a reduction of ABR wave amplitudes in aged animals compared to young animals, indicating a reduction of synchronous activity in the aged auditory brainstem. We also find a reduction of BIC amplitude and less BIC modulation by interaural time differences (ITDs) in aging animals, indicating deficiencies of binaural processing. However, aged gerbils showed normal ABR audiograms, indicating that these deficits are not the result of inner or outer hair cell loss. We then ran young and gerbils through a number of spatial hearing tasks that utilize the prepulse inhibition of the acoustic startle response (PPI). PPI requires no training and also offers a high-throughput measure such that a large number of animals can be tested in a relatively short timeframe. In our first set of experiments, we measured spatial acuity by presenting broadband noise that swapped speaker locations, acting as a prepulse, prior to presenting a startle stimulus. PPI of the startle response increased monotonically with wider angles of speaker swaps in young gerbils, but not in all aging gerbils. Additionally, to mimic detecting speech in noise, spatial release from masking ability was assessed. A broadband chirp was presented from the midline in the presence of broadband masking noise at different angles. PPI was largest when the maskers were at sources farther from the target and decreased as the masker intensity increased and were moved spatially closer to the target. We again saw this effect was strongest in young gerbils, with impaired detection abilities in the aging gerbils.

Conclusions: Collectively, this data demonstrates that aging leads to dysfunction in auditory brainstem physiology, and impairs spatial hearing abilities in gerbils across a multitude of conditions. [Supported by R01-DC017924]

MO4. Ergothioneine Treatment Helps Delay Presbycusis in CBA/CaJ Mice

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Category: Aging

Background: L-ergothioneine (EGT) a naturally occurring amino acid, is found in high concentrations in certain foods such as mushrooms, and was utilized as a treatment for age-related hearing loss (ARHL). Because of EGT's abilities as an antioxidant/anti-inflammatory agent, it was hypothesized that its treatment would target some root causes of ARHL such as oxidative stress, chronic inflammation, and others.

Methods: L-Ergothioneine (EGT) was used to supplement 25-26 months old male and female CBA/CaJ mice over a 6-month treatment period. Animals were divided into three groups: Control, Low- Dose (35 mg/kg/week – maintenance dose after first week of daily IP injection) and High-Dose groups (70 mg/kg/week – maintenance dose after first week of daily IP injection).

Physiology: Auditory Brainstem Response (ABR) and distortion product otoacoustic emissions (DPOAEs) were used to evaluate hearing function at various time points in the 6-month long study.
Liquid Chromatography-Mass Spectroscopy: Like hearing measurements, EGT uptake was measured longitudinally using whole blood samples collected at various time points in the study using LC-MS technique.
Molecular Biology: At the end of the study, cochlear tissue samples were treated to measure expression levels for different biomarkers related to anti-aging mechanisms like oxidative stress, mitochondrial health, and inflammation, using RT-PCR techniques.
Furthermore, to determine correlations between EGT levels and hearing loss in humans, the National Health and Nutrition Examination (NHANES) data base was analyzed. The survey has mushrooms (a measure of EGT intake, as mushrooms have the highest concentration of EGT) in their dietary base, a 24-hour dietary recall, and various hearing measurements including hearing thresholds.
Results: For the mouse study, control groups for males and females showed typical increases in ABR and DPOAE thresholds over the 6 months tested. Similar aging effects were observed for DPOAE amplitudes. For both low-dose and high-dose treatment groups, a distinct sex difference was observed for the response to the treatments for ABRs and DPOAEs. Male mice showed statistically significant therapeutic effects, i.e., slowing down the progression of ARHL. Interestingly, treated female mice did not show these physiological improvements like their male counterparts. LC-MS results revealed higher concentrations of EGT in whole blood samples in EGT treatment groups vs controls. RT-PCR analysis of cochlear tissue showed the therapeutic effect of EGT, as it downregulated inflammation (TNF- α) and apoptosis (Cas-3) markers and upregulated antioxidant (SOD2) and mitochondrial health (PGC1α) biomarkers. Moreover, the NHANES human data analysis showed that results (hearing thresholds) were roughly the same for both groups (mushroom consumption and no mushroom). Further analysis is being conducted to observe correlation between hearing thresholds at different frequencies and mushroom consumption for NHANES human data.
Conclusions: The study demonstrates that EGT can provide protection in treating certain key aspects of ARHL in aging mice; clinical implications need further investigation.

MO5. Cochlear and Auditory Nerve Contributors to Neural Presbyacusis
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Category: Aging
Background: Neural presbyacusis, defined broadly as deficits in auditory nerve (AN) function and structure observed in older adults, is pervasive and negatively impacts speech recognition in noise. However, the extent to which AN deficits are dependent on a loss or dysfunction of the AN only or are also associated with well-established deficits in cochlear function is largely unknown. Accumulating evidence suggests that deficits in AN function are not associated with elevation of pure-tone thresholds. Building upon our previous work showing deficits in AN response amplitude (CAP-N1) and synchrony (PLV) in older adults, we examined the extent to which different measures of the AN response are associated with age-related changes in cochlear function measured via distortion product otoacoustic emissions (DPOAE) and/or specific to structural changes in the AN. Structural changes were assessed using diffusion tensor imaging (DTI) to reveal potential deficits in myelin of the AN.
Methods: Participants included groups of older (55+) and younger (18-30 y/o) adults. AN compound action potentials (CAP-N1) were elicited by a 110 dB pSPL click. We examined the effects of age on the CAP using a suprathreshold multi-metric approach that included novel measures of intertrial phase-locking (PLV) and peak amplitude. We collected DPOAE input/output functions and estimated DPOAE peak strength from a "strength function", calculated as 20x the log of the converted DPOAE signal in dB relative to the overall pressure level of the stimulus (L1 + L2). In a subset of participants, we collected DTI of the AN.
Results: Older adults exhibited significantly reduced CAP-N1 response amplitudes, decreased neural synchrony (PLV), elevated pure-tone thresholds, and decreased DPOAE peak strength as compared to younger adults. In younger but not older adults, neural synchrony and CAP response amplitudes are tightly coupled. Elevated pure-tone thresholds were associated with weaker DPOAE peak strength but were not associated with PLV or CAP amplitudes. In older adults, weaker DPOAE peak strength predicted smaller CAP amplitudes. DPOAE peak strength was not related to PLV. Preliminary results for the DTI estimates are promising and show higher values of fractional anisotropy (FA) associated with stronger neural synchrony across younger and older subjects.

Conclusions: Our results suggest that neural presbyacusis, as assessed by CAP response amplitudes and neural synchrony, and DPOAE peak strength, may reflect multiple underlying pathologies in the cochlea and AN. Associations between DPOAEs and CAP suggest that age-related deficits in cochlear function may impact AN response amplitudes, but not neural synchrony, and that DPOAE peak strength may be more sensitive in identifying associations between cochlear and AN function than pure-tone thresholds. In addition, deficits in neural fiber integrity (FA) contribute to poorer neural synchrony but do not affect cochlear function.

MO6. Open Board

MO7. Modulation of Acoustic Startle Response by a Background Noise Changes Over the Lifespan of the CBA/CAJ Mouse

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Category: Aging

Background: It is well known that the strength of the acoustic startle reflex (ASR) is sensitive to the level of ambient noise. This noise-modulated startle (NMS) has been investigated in multiple mammalian species, generally following an inverted-U curve. In rats, early work found an optimal level of startle enhancement at 60-75 dB SPL, or about 50 dB lower than the startle eliciting stimulus. The effect is also observed in humans but with a lower optimal background level of about 40 dB SPL. NMS appears to be independent of a functioning amygdala or medial frontal cortex. While the ontogeny of the effect has been investigated in rats, its long-term persistence as an animal ages has not been previously reported. The focus of this work is to assess whether aging modulates the impact of a background floor in a mouse model of age-related hearing loss.

Methods: We measured the ASR in CBA/CaJ mice from 4 to 27 months old in 3 conditions, in quiet or with a 45 or 65 dB SPL white noise background. A white noise startle elicitor stimulus (SES) was delivered with an intensity varying between 65 to 115 dB SPL, with 20 trials per intensity level. A tactile startle elicitor (air puff) was also delivered in quiet or with the same background noises. Motor responses were classified by a custom automatic, machine learning classifier program as either startles or non-startles, and pooled into 3-month age groupings. ASR peak amplitude and latency were analyzed.

Results: In younger animal (ages 6-14 months) tested in a 65 dB background noise, startle amplitude was significantly increased at SES levels above 85 dB, compared to the quiet condition. At SES levels below 95 dB there was no statistically significant effect. At 15 months of age and older, the effect of the background was to decrease amplitude at SES levels between 75 dB and 100 dB SPL. Latency of the peak startle response in 6-14 month old animals was increased by presence of the background noise at low SES intensity and decreased at high SES, compared to no background. At ages greater than 14 months, latencies to low intensity SES were significantly greater in the background condition than in quiet. However, at high SES levels there was no difference between conditions. Importantly, there was no effect of background noise on tactile startle response.

Conclusions: The results indicate that NMS effectiveness decreases with increasing age in CBA/CaJ mice. We also report the novel finding that the effectiveness of NMS depends on the level of the SES, leading to a complex interaction of background noise level, SES level and age. Because no enhancement of tactile startle occurred, the NMS does not appear to be explained by a general arousal effect.

MO8. Age-Related Reductions in Electrocochleographic Phase-Locked Responses

Miguel Temboury Gutiérrez*[1], Jonatan Märcher-Rørsted[1], Gerard Encina-Llamas[1], Jens Hjortkjær[1], Michael Bille[2], Jesper Borchorst Yde[2], Torsten Dau[1]
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Category: Aging

Background: The frequency following response (FFR) is a potential that reflects synchronous neural activity phase-locked to the fine structure of acoustic stimuli. Brainstem FFR amplitudes have been shown to reduce with age, even when hearing thresholds are clinically normal. This reduction has been attributed to desynchronized neural activity at brainstem ('central') processing stages, but recent modeling work suggested that early neural degeneration at the level of the auditory nerve (AN) could explain this central FFR reduction. If age-related loss of AN fibers drives age-related reductions in the brainstem FFR, the FFR

may have potential for diagnosing AN damage. However, to verify this hypothesis, responses to the same stimuli recorded simultaneously from the AN (i.e., auditory-nerve neurophonics, ANN) and the brainstem (i.e., the FFR) need to be compared in younger and older participants.
Methods: We recorded potentials in 15 young and 14 older participants with clinically normal or near-normal audiometric thresholds (<25 dB HL from 0.125 to 6 kHz). Potentials from the cochlea were measured using a tympanic membrane (TM) electrode referenced to an electrode placed on the ipsilateral mastoid. Traditional brainstem responses were recorded simultaneously with a mastoid-to-vertex montage. Auditory brainstem responses (ABR) and compound action potentials (CAP) were measured in response to 115.5 dB ppeSPL, 100-μs clicks. FFRs and ANNs were derived as the phase-dependent response, (C-R)/2, to 100 dB ppeSPL, 10-ms pure tone bursts at 516, 1032 and 3096 Hz. FFRs and ANNs were also measured using 85 dB SPL, 250-ms tone bursts at 516 and 1086 Hz.
Results: FFRs to 250-ms tones were significantly reduced with age in both recording montages, suggesting that isolated peripheral responses were reduced in a manner similar to brainstem scalp-recorded FFRs. CAP amplitudes were also reduced in the older participants, while no significant age-effects were observed in ABR wave-V amplitudes. The ANN response (TM-to-mastoid montage) to the 10-ms tone bursts was significantly reduced at 1032 and 3096 Hz. Due to the limit of AN phase-locking, responses to 3096-Hz tones may mainly reflect hair cell activity (i.e., the cochlear microphonic, CM). Since peripheral responses to 3096-Hz tones were more than 10 dB smaller than the responses to 516 and 1032-Hz tones, this may indicate that the ANN (measured at 516 and 1032 Hz) is minimally contaminated by the CM.
Conclusions: We observed reduced ANN and CAP amplitudes in older normal-hearing participants using a TM electrode recordings. FFRs recorded using a classical brainstem montage were similarly reduced. We suggest that the reduction in scalp brainstem FFRs is driven, at least in part, by age-related degeneration of the level of the AN, deeming the FFR as a potential biomarker of AN damage.

MO9. Changes in the Oscillatory Activity of Executive Attentional Networks Following Neurofeedback Training of Auditory Selective Attention

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Category: Auditory Cortex and Thalamus: Human Studies
Background: To improve the attentional modulation of cortical auditory evoked responses, a neurofeedback training paradigm was developed.
Methods: Two concurrent speech streams – a female voice repeating “Up” five times and a male voice repeating “Down” four times – were played from left and right loudspeakers, respectively. Neurofeedback was given to the participants (i.e., if the "up" stream was attended, a visual object on the computer screen would move upward, and vice versa.) Over the course of four weeks, subjects repeated "neurofeedback training" in four sessions.
When attending right and ignoring left during the last experiment compared to the first, subjects showed strengthened alpha oscillation in the right parietal cortex during the after-cue-before-sound period, showing that spatial inhibitory processing to suppress sound inputs from the left was improved.
Results: After the four weeks of training, the temporal cortex showed increased attentional modulation of beta oscillation, indicating enhanced neural activity to forecast the target. Additionally, there was an improvement in the strength of attentional modulation on cortical evoked responses to sounds.
Conclusions: These findings demonstrate how neurofeedback training effectively enhances the top-down processing in executive cortical network for auditory selective attention.

MO10. Processing of Auditory Semantic Novelty in Human cortex: An Intracranial Electrophysiology Study

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Category: Auditory Cortex and Thalamus: Human Studies
Background: Semantic novelty paradigms are useful tools to probe the cortical circuits involved in language processing. Aberrant detection of semantic novelty is a feature of neuropsychiatric disorders, including autism and schizophrenia, and disorders of consciousness. This investigation took advantage of the superior spatio-temporal resolution of intracranial electroencephalography to examine semantic novelty processing in a large cohort of subjects with comprehensive electrode coverage.

Methods: Subjects were adult neurosurgical patients (N = 39; 19 women) undergoing chronic invasive monitoring for medically intractable epilepsy. Cortical activity was recorded using depth and subdural electrodes (>5700 contacts), with extensive coverage of the superior temporal plane, superior temporal sulcus, lateral temporal, frontal, parietal and limbic cortices. Stimuli were monosyllabic words from three semantic categories, presented in two auditory target detection tasks. Each task included common words "cat", "dog", "five", "ten", "red", and "white" (20 exemplars each by different talkers; 6 female), and ten novel words (all by men), five of which were in the target category. Cortical activity was examined as averaged evoked potentials (AEPs) and event-related band power in broadband gamma (30-150 Hz) and alpha (8-14 Hz) bands. Effects of semantic novelty were defined as differences between responses to common and novel words. Significance of novelty effects was established using cluster-based permutation tests.
Results: Semantic novelty increased the difficulty of the task, as indexed by lower target hit rates and longer reaction times in response to novel targets. Physiologically, effects of semantic novelty were observed as augmented gamma power and greater alpha power suppression. These effects were broadly distributed across the cortex, particularly in the superior temporal plane, both banks of the superior temporal sulcus, insula, temporal, parietal, and prefrontal areas. Latencies of semantic novelty effects were shortest in auditory cortex and became progressively longer at higher levels of the auditory cortical hierarchy. Additionally, prominent AEP effects were generally observed in the same broadly distributed regions. Left hemispheric bias for AEP and alpha semantic novelty effects was observed in the auditory cortex, temporo-parietal auditory related areas, and prefrontal cortex.
Conclusions: This study provides a framework for understanding activation patterns of brain areas involved in processing of auditory semantic novelty. Novelty effects are broadly distributed and extend from early sensory areas into prefrontal cortex. Future studies will aim to disambiguate semantic novelty from more general auditory mechanisms. This work has the potential to clarify neural mechanisms associated with aberrant speech and language processing in clinical neuropsychiatric populations.

MO11. Central Gain is Significantly but Equivalently Elevated in Hidden Hearing Loss, Tinnitus, and Hyperacusis, Suggesting an Upstream Pathophysiology Unrelated to Any Particular Perceptual Phenotype

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Category: Auditory Cortex and Thalamus: Human Studies
Background: Adults with normal hearing often report irrepressible and distressing phantom percepts (tinnitus), distress, discomfort, or pain from moderately intense sound (hyperacusis), or difficulty suppressing distracting sound sources to encode attended sound features (hidden hearing loss, HHL). The outward phenotypes of tinnitus, hyperacusis, and HHL are distinct, yet each has been linked to a maladaptive central plasticity process – excess central gain. If central gain is equivalently elevated in each condition, then it cannot account for their distinct and independent symptoms and would be better likened to an "upstream" initiating event. Alternatively, if central gain were specifically elevated in one condition or in persons who reported a greater lifestyle burden related to their condition, it would be more akin to a downstream pathophysiology underlying a particular set of outward symptoms. Here, we address these possibilities by measuring central gain alongside psychoacoustic assays of tinnitus, loudness perception, and multi-talker speech perception in normal hearing adults with chronic tinnitus, hyperacusis, or HHL.
Methods: We recruited 55 participants with clinically normal hearing thresholds through 8kHz from four cohorts: young neurotypical, older neurotypical (ages 19-37 and 41-66, respectively), young tinnitus, and young hyperacusis. Central gain was measured with 64-channel scalp EEG in response to an amplitude modulated tone slowly swept up and down in intensity. This allowed us to efficiently derive the gain (i.e., slope) of the neural input-output function across an individualized continuum of intensities spanning minimum audibility to uncomfortable loudness levels. Psychophysical characterization of their tinnitus, hyperacusis, and HHL were quantified as the minimal masking threshold, cross-modal loudness matching, and multi-talker digit recognition accuracy, respectively. Lifestyle burden was estimated via the tinnitus handicap inventory, the modified hyperacusis questionnaire, and the speech, spatial, and qualities of hearing scale questionnaires, respectively.

Results: Sound intensity neural growth slopes were nearly twice as steep in older neurotypical, young tinnitus, and young hyperacusis participants compared to young neurotypicals. Excess central gain was equivalently elevated in each group. Individual growth functions were not correlated with questionnaire-based estimates of individual tinnitus, hyperacusis, or HHL lifestyle burden. Psychophysical assays of tinnitus masking, loudness growth, and multi-talker speech intelligibility showed group-specific deficits, but individual values were not correlated with either central gain or lifestyle burden.
Conclusions: Questionnaire and psychophysical characterizations speak to the distinct and independent perceptual disruptions imposed by tinnitus, hyperacusis, and age-related HHL. Neural growth slopes were significantly but equivalently elevated in all three conditions compared to young neurotypical participants, demonstrating that excess central gain alone cannot account for the perceptual qualities or lifestyle burden of any particular disorder. Instead, excess central gain is likely a byproduct of central disinhibition that may or may not be mechanistically linked to downstream processes that produce the distinct manifestations of each disorder.

MO12. Age-Related Changes in the Processing of Broadband Noise Stimuli along the Auditory Hierarchy: Evidence From fMRI

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Category: Auditory Cortex and Thalamus: Human Studies
Background: Older listeners without clinically defined hearing loss often report listening difficulties in everyday life. Age-related neural degeneration in the cochlea may not be detected by clinical audiometry but may still lead to altered temporal processing in the ascending auditory pathway. The notion of a central gain proposes a compensatory mechanism that enhances evoked responses in the central auditory system. Yet, it is unclear whether gain mechanisms selectively affect the processing of different temporal modulations at different stages along the auditory pathway. Here, we use functional magnetic resonance imaging (fMRI) to investigate whole-brain blood oxygen-level dependent (BOLD) responses to amplitude modulated sounds in young and older listeners with normal age-corrected audiograms.
Methods: We report data from 65 subjects that took part in an fMRI experiment in which they were presented with broadband noise stimuli. The noise stimuli were either unmodulated or contained imposed sinusoidal amplitude modulations at a rate of 4 Hz or 80 Hz. BOLD fMRI contrasts between the responses to the noise stimuli and silent “baseline” trials were used to map correlates of sound responsiveness in the auditory brainstem and cortex. Contrasts between BOLD fMRI responses to the modulated vs. unmodulated noises were used as measures of sensitivity to the imposed modulations.
Results: Analyses indicate that the magnitude of the BOLD contrasts between sound stimulation and baseline trials decreases with age in regions of interest in the auditory brainstem. The analyses further suggest that the overall magnitude of the BOLD contrast between the responses to unmodulated noise and baseline trials increases with age in some auditory cortical regions of interest. BOLD correlates of differences in sensitivity to different amplitude modulation rates tended to diminish with age in some auditory cortical regions.
Conclusions: The present study explored linear associations between age and BOLD fMRI correlates of baseline “sound responsiveness” and sensitivity to temporal amplitude modulations. Our analyses suggested a reduced responsiveness in the auditory brainstem regions and an enhanced responsiveness to unmodulated sound stimuli at later a stages with advancing age. However, auditory cortex may display reduced differential sensitivity to different temporal modulations in older listeners.

MO13. Predicting Speech Perception in Noise With the Acoustic Change Complex

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Category: Auditory Cortex and Thalamus: Human Studies
Background: Over the past two decades the acoustic change complex (ACC), which is a cortical potential evoked by a change in an ongoing sound, has been widely studied in experimental settings. Showing

correlations with psychophysical outcomes, it appeared as a promising tool for objective measures of suprathreshold hearing performance without, however, breaking through in the clinical practice. Using electrode configurations and equipment as used in clinical practice for auditory brainstem response (ABR) recordings (Vonck et al., JARO 20: 489-498, 2019), we recorded ACCs to frequency changes in normal-hearing and hearing-impaired subjects with the objective to examine to which extent ACC measures can predict speech perception in noise performance.
Methods: ACCs were recorded in 13 adult subjects with sensorineural hearing loss (SNHL) and 24 age-matched normal-hearing (NH) subjects with ages across all subjects ranging from 20 to 66 years. The stimuli consisted of a 3 s base tone, an upward frequency sweep of 3 ms towards a 300 ms target tone 12% above the base; they were presented to the better ear. Base frequencies were 0.5, 1, 2 and 4 kHz. Responses were recorded using Ag/AgCl electrodes placed on Cz (active), contralateral mastoid (reference) and forehead (ground). Filtering was applied between 0.01 and 100 Hz, and waveforms were averaged over 100 recordings. Speech reception thresholds (SRTs) of the better ear were measured using Dutch standardized sentences presented in a background of stationary speech-shaped noise. Hearing loss (HL) was quantified by the pure tone thresholds across 0.5, 1, 2 and 4 kHz.
Results: In almost each subject we could record clear and large ACC waveforms with amplitudes around 10 µV for NH subjects and around 7 µV for SNHL subjects (Vonck et al., Hear Res 420: 108508, 2022). The SRT scores correlated very well to ACC latencies and to a lesser extent to ACC amplitudes. Using multiple regression analysis we found that when averaging the ACC and HL outcomes over 1, 2 and 4 kHz SRT could be explained for 87% by ACC latency (35%) and HL (52%). Considering only the ACC measures over those three frequencies, SRT could be explained for 74% by latency (60%) and amplitude (14%).
Conclusions: The ACC to fast and large frequency changes (a few semi-tones) can be used to predict speech perception in noise. The predictive value using the latency is better than the ACC threshold, which moreover requires more time (Vonck et al., Hear Res 401:108154, 2021). When confirmed in validation studies with larger numbers of subjects, it can aid clinicians in their evaluation of auditory performance and higher order processing, in particular when behavioral testing is unreliable.

MO14. Neuromagnetic Representation of Musical Roundness in Auditory Cortex

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Category: Auditory Cortex and Thalamus: Human Studies
Background: The perception of roundness in music is based on (1) consonance and dissonance, (2) the musical context, and (3) the listener's personal background. Many neurophysiological studies have focused on one or another aspect but only a few combined them all. In this study we performed an experiment that took all three into account using a well-balanced four-chord progression paradigm which tried to come as close as possible to real music. Musical aptitude and psychoacoustic ratings of subjects were matched with auditory evoked responses of the auditory cortex measured with magnetoencephalography (MEG).
Methods: Based on the musical aptitude test "Advanced Measures of Music Audiation" (AMMA), we divided 30 subjects into 15 more and 15 less gifted individuals. All subjects participated in an MEG experiment in which they heard various four-chord progressions played by three different instruments. Finally, subjects rated all four-chord progressions on a Likert scale from 1 to 7 according to their personally perceived roundness. All sequences were created according to the rules of traditional western music theory. They contained dominant chords with different amounts of dissonance on the third position (CHORD3) and found their resolution on the fourth position. The statistical analyses focused on the auditory N1m component in relation to the evaluation of roundness ratings for single chords, perceived roundness over whole cadences and the impact of musical aptitude (MUS).
Results: Psychoacoustic results showed a clear gradation of the roundness ratings for CHORD3 and different cadences but surprisingly, there was no significant effect of CHORD3 for N1m amplitudes on the third position. However, on the fourth position the N1m amplitude was significantly larger if rounder chords were played on the position before it. On an individual level, rounder cadences showed significantly larger N1m amplitudes on the fourth position compared to less round rated cadences. There was no global effect of musicality but a tendency of enhanced N1m amplitudes. High AMMA listeners showed more differentiation in the N1m for different roundness scores, although only the interaction effect of CHORD3 and MUS on the fourth position reached significance.

Conclusions: The results of this study emphasize that future auditory research should be based on more natural designs. Compared to previous studies on consonance and dissonance, this concept seems not to be reflected in the N1m amplitude as an absolute value but rather depends on the musical context and resolution. N1m amplitudes become larger when a chord or cadence is perceived as round. More gifted listeners show better differentiation of differences in roundness perception, which is reflected in the psychoacoustic and partly in the neurophysiological data, but a global effect of musicality on the N1m could not be reproduced.

MO15. Recurrent Network of Neurons Can Produce Selectivity to Temporally Separated Sounds as a Whole

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Category: Auditory Cortex and Thalamus: Structure and Function

Background: Sound-based communication depends on the percept of a sequence of sounds at multiple time scales. Sequences of phonemes, syllables or words are such examples. The underlying neural mechanisms responsible for stringing together such sequences as a whole are not known. The above is true for human speech and the songbird, and likely for other species. We hypothesize that two temporally separated sound tokens in a sequence (A-B) can be associated together if neurons, through plasticity, develop selectivity to A and B presented sequentially. The initial response of neurons to overall A-B is weak which, with training, changes to strong responses and not to the individual sounds A and B.

Methods: We use a two layer network, one putative thalamic layer and another putative cortical Layer-IV. The network is arranged into columns of sets of neurons selective to sounds with a stimulus parameter varying column to column. Each cortical column has excitatory neurons (EXs) and inhibitory neurons (INs, parvalbumin+, PV and somatostatin+, SOM) with SOM and EX having extra-columnar projections unlike PV. SOM and PV connections were as typically reported. We consider a column to be selective to A and another to B, with selectivity decaying away from the respective columns. All neurons were modelled as generalized integrate and fire neurons with inhibitory rebound capability. Recurrence and inhibitory rebound in the layer are the key properties to allow associations of two temporally separated sounds.

Results: We simulated multiple cases of separations between A and B and in each with SOM turned off. The latter mimics the optogenetics experiments in our earlier study. The network is presented A-B multiple times and we find that gradually selectivity to A-B emerges in neurons in the columns at the flanks of tuning to A and B. However with SOM turned off this selectivity emerges faster. The initial response to A and B individually, were lower in those neurons. The same effect was stronger with greater separation between the column tuned to A and that to B. A larger inhibitory rebound in EXs is observed in the case SOM is turned off due to PV being disinhibited, as SOM has strong projections to PV compared to PV on itself. Since the inhibitory rebound can match with presentation of the second token (B) after the first (A) token, synapses get strengthened for those activated in an appropriate time window with selectivity to A-B emerging.

Conclusions: We thus propose a possible network mechanism of associating a sequence of sound tokens with the simplest case of two tokens, which may be extended to longer sequences and remains to be tested.

MO16. Silencing Auditory Cortex Impairs Learning and Expression of a Socially Rewarded Auditory Behavior

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Category: Auditory Cortex and Thalamus: Structure and Function

Background: While socially-significant sounds that evoke natural behavioral responses in animals are usually assumed to be fixed and species-specific, they often require experience and learning to become meaningful for a listener. One example of social sound learning comes from the mouse maternal model of communication, in which mothers or virgin co-carers use distal cues to locate and retrieve their pups back to the nest. Traditionally, ultrasonic vocalizations emitted by pups serve as a localizing signal, but mothers can also learn to associate synthetic sounds with pup retrieval. How and where this association is formed between novel auditory stimuli and social communicative significance is still poorly understood. While much research has implicated auditory cortex (ACx) in associative sound learning that guides behavioral actions in a variety of paradigms, not all auditory tasks have been found to require ACx - either to learn or express the behavior. Here, we tested how silencing ACx alters an animal's ability both to learn a new sound

that reliably predicts where pups will be found, and to express the auditory behavior after it has been learned.
Methods: We first asked whether ACx is necessary for expression of the learned behavior using chemogenetic inhibition. ACx of naïve, female virgin mice (N=6) were bilaterally injected with an adeno-associated virus carrying a silencing DREADD (i.e., designer receptor exclusively activated by designer drugs). After three weeks of expression, those animals were trained in a T-maze to enter one of the two arms cued by an amplitude-modulated band-pass noise and rewarded with pups, which were then retrieved back to the nest in the main stem.
Results: Within 8 days, most animals learned to use the sound to locate pups. After the task was learned, we temporarily inactivated the ACx by injecting clozapine-n-oxide (CNO) and found the performance significantly decreased ($p < 0.05$).
Next we tested the effect of ACx inactivation during learning. CNO or saline was injected 30 min prior to each daily training session. Our preliminary data show that after eight sessions, the CNO animal group (N=12) showed a significant impairment in performance ($p < 0.05$) compared to the saline group (N=6).
Conclusions: Together, our results suggest that ACx activity is necessary for both learning a socially rewarded acoustic cue and expressing recognition for the sound in the behavior soon after learning. Continuing studies are examining how this ACx activity is being dynamically used for learning and guiding approach to a pup during behavior, ultimately helping to inform us about sensory cortical function during communication behaviors.
The work is supported by NIDCD R01 DC008343.

MO17. Sound-Evoked Facial Movements: A Sensitive Assay of Mouse Hearing That Provides an Attractive Alternative to Acoustic Startle Audiometry, Auditory Brainstem Response Testing, or Operant Behavioral Testing

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Category: Auditory Cortex and Thalamus: Structure and Function

Background: While the auditory brainstem response (ABR) can provide an indirect, physiological proxy for hearing, direct measures of hearing can only be obtained through the study of behavior. In genetically tractable rodents, operant behavioral hearing tests are the gold standard, but can require several weeks to obtain a single data point. Involuntary behaviors such as isoluminous pupil fluctuations, acoustic startle reflex, or pre-pulse inhibition of startle can be measured quickly but are only sensitive to sound levels well above hearing threshold or habituate quickly. Here, we introduce a new involuntary behavioral measure – quantitative facial videography – that is far more sensitive than other reflexive behaviors and provides a rapid, non-habituating readout of acquired and inherited auditory hypersensitivity.
Methods: Adult mice were head-fixed atop a piezoelectric force plate during high-speed (150 Hz) videography of the face and pupil. Facial movements and pupil dynamics were quantified in 63 mice via motion energy analyses and DeepLabCut, a markerless body position estimator based on deep neural networks. Auditory sensitivity was tested through systematic variation of stimulus center frequency, intensity, and spectral bandwidth. Combined videography and force plate measurements were made before or after acoustic trauma. Additional experiments investigated conditioned changes in facial movements during auditory fear learning, in mice with an autism risk gene mutation (Ptchd-1 KO), and tested the involvement of auditory cortex in facial sound responses via interleaved trials of bilateral cortical silencing in parvalbumin (PV)-Cre mice crossed to a channelrhodopsin reporter line.
Results: Thresholds for sound-evoked facial movements were approximately 40 dB lower than the startle reflex and equivalent to ABR thresholds measured in the same mice. Facial movement amplitudes grew monotonically with sound level and spectral bandwidth and did not habituate. Following noise-induced acoustic trauma, facial movements were attenuated for high-frequency stimuli within the range of sensorineural hearing loss but heightened for spared, low-frequency stimuli. Ptchd1-KO mice exhibited exacerbated facial reactivity and pupil dilation, consistent with an autism hyperacusis phenotype. Facial movements elicited by sounds paired with tail shock were rapidly and persistently attenuated, providing a sensitive index for auditory fear learning. Optogenetic silencing of the auditory cortex had no discernable impact on sound-evoked facial movements.

Conclusions: Quantitative analysis of rapid and involuntary changes in the mouse face and pupil provide an index of behavioral sound registration that is distinct from the acoustic startle reflex. Facial movements are a sensitive assay of noise-induced hearing loss but also capture acquired and inherited auditory hypersensitivity, which is generally not captured by the ABR. Due to the simplicity of this approach, requiring only a computer and a video camera, this method avoids several limitations of ABR and acoustic startle reflex testing and could be broadly useful for rapid assessments of hearing status.

MO18. Cortical Parvalbumin GABA Neurons Regulate the Perceptual Volume Knob: Bi-Directional Changes in Loudness Perception via Reduced or Enhanced PV-Mediated Inhibition in the Auditory Cortex

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Category: Auditory Cortex and Thalamus: Structure and Function

Background: Noise-induced sensorineural cochlear damage triggers maladaptive central plasticity and perceptual hypersensitivity (McGill et al., eLife 2022). Hyperactivity, hyperresponsiveness, and hypersynchrony in cortical pyramidal neurons largely arises from reduced feedforward inhibition from parvalbumin+ (PV) cortical GABAergic neurons (Resnik and Polley, eLife, 2017; Resnik and Polley, Neuron, 2021), and so PV-mediated inhibition has been implicated in loudness hyperacusis, but this has not been directly tested. Here, we describe a two-alternative forced-choice (2AFC) classification task in head-fixed mice to probe changes in loudness perception after noise-induced cochlear sensorineural damage or direct activation of cortical PV neurons.

Methods: We trained head-fixed mice (N=26) to classify 11.3 kHz tones as soft versus loud across a 40-80 dB SPL range. We monitored behavioral classification before and for two weeks following noise exposure that caused either a 'pure' cochlear synaptopathy (N=6), a mixed sensorineural pathology (N=5), or in sham-exposed controls (N=6). To assess cortical PV neuron involvement in task performance, we interleaved trials with bilateral optogenetic PV stimulation with no-stimulation trials (N=6/3, channelrhodopsin/GFP control).

Results: At baseline or in sham-exposed or GFP-injected control mice, behavioral classification of soft versus loud varied smoothly and consistently across the sound intensity range, with a loudness categorization boundary near 60 dB SPL. After noise-induced cochlear synaptopathic or mixed sensorineural damage, mice developed a rapid but stable loudness hyperacusis to the spared mid-frequency tone, as reflected in a -9 dB shift in their loudness transition threshold. Bilateral silencing of auditory cortex in unexposed mice with normal hearing did not affect tone detection probability or behavioral reaction time. However, PV activation induced a strong bias for mice to report that moderate and high-intensity sounds were perceived as soft (+17 dB shift on average).

Conclusions: Here, we developed a 2AFC task that allows robust monitoring of animal loudness perception across weeks. Through our approaches, these data suggest that cortical PV neurons function as a perceptual volume knob; sounds are perceived as louder following acoustic exposures that reduce PV-mediated cortical inhibition but softer than normal when PV neurons are artificially activated. These data enrich a growing literature describing PV pathology as a critical point of dysfunction in a broad class of sensory hypersensitivity disorders.

MO19. Cell-Type- And Layer-Specific Synaptic Connectivity in the Auditory Cortico-Thalamo-Cortical Circuit

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Category: Auditory Cortex and Thalamus: Structure and Function

Background: The cortico-thalamo-cortical (CTC) circuit involves in the higher order of sensory processing. With the development of versatile Cre transgenic mouse lines and neuronal tracing, the CTC circuit has gained much attention in the recent years. Studies show cell-specific and area-specific synaptic connectivity properties in the CTC circuit, in motor, prefrontal, and somatosensory cortexes. However, how the hearing information is processed between the auditory cortex (AC) and the auditory thalamus – the medial geniculate body (MGB) remains understudied. Anatomical studies demonstrate reciprocal innervations between the deep layer of AC (layers 5 and 6) and MGB, in a cell-specific, and layer-specific pattern.

Nevertheless, little is known about the synaptic connectivity mechanism and the functional properties of the auditory CTC circuit.
Methods: In our study, we performed patch-clamp whole-cell-recording of acute brain slices that contain AC and/or MGB, using Ntsr-1 mice and neuronal tracers (e.g. AAV viruses, retrograde beads), to investigate the synaptic connectivity of the auditory corticothalamic and thalamocortical pathways. Excitatory post-synaptic currents (EPSCs) were evoked and collected from AC and MGB principal neurons using optogenetic stimulation of specific pathways.
Results: In the corticothalamic pathway, we observed significantly stronger excitatory synaptic responses in ventral (core-type) than dorsal MGB (matrix-type), using optogenetic stimulation of AC layer 6 axons. This is consistent with the anatomical data, where the majority of layer 6 axons were shown to project to the ventral subdivision of MGB. Our results are also similar to the findings in the somatosensory CTC circuit, where CT axon stimulation evoked stronger synaptic currents in VPL (core-type) than PO (matrix-type) (Guo et al, 2020). However, in the motor CTC circuit, optogenetically stimulating the CT axons showed weak thalamic neuronal responses in VL (core-type) in spite of strong anatomical innervation from the cortical area (Yamawakai and Shepherd 2015).
In the thalamocortical pathway, paired recording revealed the MGB neural projection evoked comparable excitatory synaptic responses in the AC layer 5 PT and layer 6 CT neurons. Comparable peak amplitude was also observed in the layer 5 and 6 principal neurons of the auditory and visual cortexes (Ji et al, 2016). Instead, the thalamic projection is found to be in favor of layer 5 PT neurons when the motor or prefrontal cortex axons are activated (Yamawakai and Shepherd 2015; Guo et al, 2018; Collins et al, 2018).
Conclusions: Our findings revealed a cell-specific and layer-specific synaptic mechanism in the auditory CTC circuit. The synaptic connectivity pattern in the auditory CTC shows similarities with other sensory cortexes but is distinct from the motor cortex. This study provides an essential functional mechanism of sensory processing in the higher order of hearing and assists in understanding the mechanism of hearing disorders. This work is supported by DC019618 (T.T.)

MO20. Auditory Phenotype in a Mouse Model of Neurofibromatosis Type 2 is Associated With Proliferation of Glial Cells and Dysregulation of the Merlin/NF2-Lin28-Let-7 Axis

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Category: Auditory Nerve
Background: Merlin, also known as neurofibromatosis type 2 (NF2), is a tumor suppressor gene, and its loss leads to development of Schwann cell tumors, predominantly of the vestibular portion of the 8th cranial nerves. Loss of Merlin/NF2 has been associated with dysregulation of the Lin28/let-7 axis leading to tumor formation. Lin28 increases proliferation and dedifferentiation via inhibition of pri-let-7 maturation in cells lacking Merlin.
Schwannomas of the vestibular nerve are typically associated with varying degrees of sensorineural hearing loss (SNHL), but the etiology of this impairment remains to be elucidated, although leading current theories implicate the secretion of pro-inflammatory and potentially neurotoxic factors.
In this study, we examined the auditory and vestibular nerves in a Periostin-Cre; Nf2flox/flox mouse model for NF2 to further investigate the underlying cochlear NF2 phenotype and the possibility of a dysregulated Lin28/let-7 interaction.
Methods: Periostin-Cre; Nf2flox/flox mice or littermate controls with FVB/NJ background were aged up to 11 months. We performed serial measurements of auditory brainstem responses (ABR) as a physiologic measurement of hearing sensitivity, and animals were sacrificed when the knockout animal displayed significant SNHL. Tissue was then processed for immunohistochemistry and cochlear whole mount analysis. Frozen sections of diseased and control animals were analyzed for glial and neuronal markers. Proliferation was assessed after 5-Ethynyl-2′-deoxyuridine (EdU)injections. Schwann cells and neurons were imaged with confocal microscopy and quantitative analysis was performed per 100 μm area in Rosenthal's canal, Scarpa's ganglion, and the auditory nerve trunk on serial sections. Cochlear whole mounts were stained for presynaptic CtBP2 and postsynaptic GluR2, imaged with confocal microscopy, and synapses were counted using AMIRA software. Glial cells of P0Cre; Nf2flox/flox; Tdtomato or P0Cre; Tdtomato animals at early

and late time points were isolated using fluorescence-activated cell sorting (FACS), and miRNA and mRNA were isolated, followed by quantitative PCR for Lin28 and let-7.
Results: At 10-11 months of age, ABR demonstrated significant hearing loss in all Periostin-Cre; Nf2flox/flox mice compared to control mice. A range of tumor sizes were observed within the labyrinth. As measured by EdU injections, all knockout animals displayed increased proliferation of glial cells within the cochlea, which was associated with increased loss of ribbon synapses, followed by neuronal loss compared to littermate controls. Lin28 was significantly upregulated in older knockout animals, with concurrent downregulation of let-7.
Conclusions: In this study, we demonstrate that the Periostin-Cre; Nf2flox/flox mouse model displays a cochlear phenotype that may be associated with dysregulation in the Lin28/let-7 axis after loss of Merlin. This could lead to loss of contact inhibition and increased proliferation of glial cells in the cochlea, initiating a loss of auditory synapses and neurons via mechanisms under active investigation. These findings may in part explain the sensorineural hearing loss in patients with vestibular schwannomas.

MO21. Sound Encoding of Vowels and Syllables in the Human Auditory Nerve
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Category: Auditory Nerve
Background: Speech perception relies on the detection of a variety of subtle acoustic spectro-temporal contrasts, but little is known about the speech processing in human cochlea. Here, we combine psychophysical experiments and computational modeling of the human cochlea to correlate perceptual performance and human auditory nerve responses to natural sounds expressing specific spectro-temporal contrasts.
Methods: Four pairs of stimuli were prepared. Two pairs differ in a spectral region below 1.5 kHz and are thought to primarily involve temporal fine structure (TFS) coding. The two other pairs differ in a spectral region beyond 1.5 kHz and are thus thought to primarily involve envelope coding. Furthermore, for each of the spectral regions, we used one pair of steady-state vowels (above 1.5 kHz: /i/–/y/; below 1.5 kHz: /o/–/u/), and one pair of syllables that differed in their consonant (above 1.5 kHz, /di/–/bi/; below 1.5 kHz, /du/–/bu/), thus requiring discrimination of a transient acoustic trait. Speech perception was assessed, in quiet, in typically hearing participants using forced-choice psychoacoustic method. The computational model considers the main biophysical properties of the human cochlea, including the place frequency map, the cochlear filters, the number of auditory nerve (AN) fibers per inner hair cell (IHC) all along the tonotopic axis, and the proportion of low-, medium- and high-spontaneous rate fibers per IHC. In response to a sound, this model simulates the auditory nerve neurophonic potential generated by the firing of AN fibers, as a proxy of the sound encoding process.
Results: The waveform of auditory nerve neurophonic to the vowels /o/ versus /u/ differs significantly ($r < 0.5$, coefficient of correlation) compared to those observed in response to the vowels /i/ versus /y/ ($r > 0.9$). This result is consistent with psychophysics data that demonstrate a better ability of participants to discriminate /o/ from /u/, than /i/ from /y/ ($\chi^2(1)=18.4$, $p < 0.001$). Similar results were observed with syllables, with a larger discrimination between /du/ against /bu/ compared to /di/ against /bi/, considering both auditory nerve neurophonic and behavioral data. Although these results may only concern the specific stimuli used in this experiment and cannot be generalized to all speech traits, it is striking that the simulated neurophonic response, which primarily captures the TFS, can predict the results obtained behaviorally.

Conclusions: These results suggest that neurophonic potentials recorded from the auditory nerve constitute a promising measure to examine speech processing in the human cochlea. Further investigations, including AN recording from patients who will undergo cerebellopontine angle surgeries are required to validate this hypothesis.
Work supported by ERA-NET Neuron grant CoSySpeech (G0H6420N).

MO22. A Mutation in ATP11A Causes Loss of Spiral Ganglion Neurons in Autosomal Dominant Auditory Neuropathy Type 2

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Category: Auditory Nerve
Background: Auditory neuropathy is a hearing disorder caused by a primary defect of sound encoding in spiral ganglion neurons (SGNs). Sound discrimination is more strongly impaired than sound perception thresholds, as the latter are mostly determined by active cochlear amplification.
Human non-syndromic autosomal dominant auditory neuropathy type 2 (AUNA2) has previously been mapped to chromosomal bands 12q24 or 13q34 (Lang-Roth et al. 2017). Affected individuals show an age-dependent increase in sound thresholds and poor or absent auditory brainstem responses despite relatively well-preserved otoacoustic emissions. However, speech perception is not as severely impaired as in other forms of auditory neuropathy.
Methods: We used a combination of whole genome sequencing, expression analysis, an in vitro flippase assay and phenotyping of a novel mouse model to decipher the disease mechanism of AUNA2.
Results: Affected individuals had a 5500bp deletion in ATP11A located in 13q34, expected to cause truncation of both possible isoforms. ATP11A is a P-4 ATPase which flips phosphatidylserine and phosphatidylethanolamine from the exoplasmic to the cytoplasmic leaflet of plasma membranes. The deletion preserves ATP11A expression but reduces flippase activity in vitro.
In mice, high levels of ATP11A expression are observed in all afferent spiral ganglion neurons. Conditional Atp11a knockout mice show an age-progressive loss of auditory brainstem responses despite preserved DPOAE, recapitulating the age-progressive human auditory neuropathy phenotype. In mutants, we observed a significant loss of hair cell ribbon synapses, suggesting loss of SGNs. Preliminary data indicate reduced action potential rates in SGN single unit recordings in vivo.
Conclusions: By multidisciplinary analysis, we show that AUNA2 is caused by a mutation in ATP11A which reduces flippase activity and likely causes progressive loss of SGNs. Possibly, the degenerative process preferentially affects the fraction of SGNs that have high spontaneous and evoked firing rates.

MO23. Ovary-Derived 17β-Estradiol Leads to Higher ABR Wave1 Amplitude and AMPA Receptor Subunit Transcription in the Female Mouse Cochlea

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Category: Auditory Nerve
Background: Females of multiple mammalian species have higher auditory brainstem response (ABR) wave 1 amplitude than males (humans: Kjaer, 1979; 1980; Wharton and Church, 1990; rats: Balogova et al., 2018; mice: Milon et al., 2018; Kobrina et al., 2020). Recently there has been increased interest in 17β-estradiol (E2) as a possible mediator of this sex difference since females have higher levels of circulating ovary-derived E2. However, there has been little progress elucidating the molecular mechanisms underlying higher ABR wave 1 amplitude in females. Our data show that female C57BL/6J mice have more GRIA3 and GRIA4 AMPA receptor (AMPAR) subunit mRNA in the cochlear spiral ganglion neurons (SGNs), especially GRIA3. More GRIA3 at the receptor channel would lead to faster kinetics and increased calcium permeability, which may contribute to increased SGN synchrony and larger ABR wave 1 amplitude in females. However, what contributes to the underlying sex differences in AMPAR subunit transcription in the mammalian cochlea is unknown. We hypothesize that the effects of ovary-derived E2 (gonadal sex) or X-linked genes (chromosomal sex) increases GRIA3 and GRIA4 mRNA in females and that this relates to differences in ABR wave 1 amplitude.

Methods: To test the role of gonadal sex vs. chromosomal sex on differences in cochlear physiology and AMPAR subunit transcription, we measured ABRs and quantified AMPAR subunit mRNA in SGNs using in situ hybridization in four-core genotypes (FCG) mice aged postnatal day 60. In viable male FCG mice, the testis-determining Sry gene was deleted from the Y-chromosome and reinserted onto the third chromosome. Breeding males of this genotype to wild-type females leads to the following phenotypes: 1) chromosomal female, gonadal female (XXF); 2) chromosomal female, gonadal male (XXM); 3) chromosomal male, gonadal female (XYF); and 4) chromosomal male, gonadal male (XYM). We can test the main effects of gonadal and chromosomal sex and their interaction on a trait by testing all FCG genotypes together.
Results: We found that XXF and XYF FCG mice have larger ABR click wave 1 amplitude than XXM and XYM FCG mice. Interestingly, preliminary analysis of AMPAR subunit mRNA expression does not entirely correlate with ABR data, as XXF have the highest levels of GRIA3 and GRIA4 mRNA, XYM are intermediate, and XYF and XXM mice have the least.
Conclusions: The ABR data support the hypothesis that high circulating E2 mediates increased ABR wave 1 amplitude in females. In contrast, in-situ hybridization data suggest that higher circulating E2 in females may contribute to more GRIA3 and GRIA4 mRNA, but only with XX chromosome complement, as XYF mice have lower levels of these subunits. Our data support an effect of gonadal sex on cochlear physiology, and the potential interaction of genetic and gonadal sex on AMPAR subunit transcription.

MO24. Electrically Evoked Auditory Brainstem Response Using Surface Electrodes at Different Cochlear Sites: A Comparison With Intracochlear Penetrating Electrodes

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Category: Auditory Nerve
Background: Transtympanic promontory stimulation test was thought to be an important surgical indicator for cochlear implantation, especially in patients at risk of poor auditory neuron functioning. However, the postoperative performances do not always correlate with the preoperative results from external cochlear stimulation test. The variable stimulatory point was suggested as a possible explanation. However, there have been no attempts to assess the predictability of the external cochlear stimulation test according to sites. In this study, we evaluate the effect of external cochlear stimulatory position on the electrically evoked auditory brainstem response (EABR).
Methods: EABR and eEABR to biphasic pulses were recorded from eight rats using the intracochlear and external surface electrodes, respectively. First, place-specific stimulation of the rat cochlea was achieved by positioning a ladle shape surface electrode outside the cochlea on each of the three turns, then penetrating a 4-channel multielectrode array into the scalar tympani of basal turn and the scalar vestibuli of middle turn. After a forward masking stimulus paradigm to minimize stimulus artifact contamination, latency and amplitude functions were obtained from the most prominent wave (P4) in all five different stimulation configurations. We also measured N3-P4 amplitude-growth functions (AGFs) on each electrode pair and calculated the slope of the AGF as the linear regression of amplitude with intensity, from 25 to 75% of its maximum.
Results: Preliminary results show that the slope of the eEABR AGF for a fixed reference electrode on the round window increased with increasing the distance from the round window to the surface electrode, but there is no statistically significant difference between apical and middle turn slopes. However, P4 latencies of eEABR revealed similar latencies regardless of the distance from the base. For intracochlear stimulation, the EABR AGF slope tended to increase with the distance between electrodes along the electrode array inserted at the basal turn, but not at the middle turn. Although the large variability in P4 latencies across individual ears, there is a trend toward longer latencies at the middle and shorter latencies to the basal with wide bipolar stimulation, while this trend was not observed with narrow bipolar stimulation. The slopes of the eEABR for basal stimulation were comparable with those of the EABR for narrow bipolar stimulation in the basal turn, while the eEABR slopes for middle and apical stimulation were in similar ranges of the EABR slopes for wide bipolar stimulation in the basal turn.
Conclusions: Both eEABR and EABR data varied depending on the stimulatory positions in a similar manner. The stimulating electrode positions would be associated the width of the current field and corresponding number of neurons at the modiolus, but further data are needed to better examine this factor.

MO25. Pseudo-Monophasic Pulses to Characterize Auditory Brainstem Implant-Evoked Responses in Mouse Inferior Colliculus

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Category: Auditory Prostheses

Background: The auditory brainstem implant (ABI) is currently the only device that restores hearing in human patients that suffer from Neurofibromatosis type-2, a genetic disease that causes growth of vestibular schwannomas on the eighth cranial nerve. The ABI uses a surface array of electrodes placed on the cochlear nucleus (CN), the first brainstem relay nucleus of the auditory pathway. ABI subject performance is low, with few subjects being able to perceive speech in open-set configuration. One hypothesis is that spread of stimulation within its two main subdivisions, DCN and VCN, prevents the speech signal from being intelligible. Pseudo-monophasic pulses have been proposed in cochlear implants as a potential solution to reduce current spread. They may also shed light on the polarity effect i.e. the phenomenon where different compartments of neurons (dendrites, somata, axons) can be more or less sensitive to the polarity of the leading phase (highest amplitude) of the stimulation pulse. We used pseudo-monophasic pulses with either cathodic- or anodic-leading phase to pursue the characterization of the evoked responses mediated by DCN vs. VCN stimulation in a mouse model of ABI.

Methods: We performed left-sided posterior fossa craniotomy and multichannel ABI array placement on DCN or VCN in thirty mice. Multi-unit responses were measured in the inferior colliculus (IC) with a 16-channel recording probe (Neuronexus). Two comparisons were made between the following trains of pulses: 1) pseudo-monophasic vs. biphasic pulse trains to study the impact of the pulse shape and 2) cathodic-first vs. anodic-first pulse trains to study the impact of polarity. All pulse trains were delivered in a bipolar fashion. We measured thresholds, dynamic range and spatial activation. VCN-stimulated responses were assumed to originate in that subdivision only. DCN-stimulated early responses were assumed to originate from DCN for low-level stimuli but late responses evoked by high-level stimuli were assumed to originate from the VCN (McInturff et al, 2022).

Results: 1) Late (but not early) responses to DCN stimulation by pseudo-monophasic pulses showed lower spread of activation across channels compared to biphasic pulses (n=5 mice). The effect was not large enough to significantly affect mean response threshold when channels were averaged. 2) Polarity had a strong effect on response: Early responses to DCN stimulation had lower thresholds for cathodic-first pseudo-monophasic pulses than for anodic-first pulses. These lower thresholds produced larger dynamic ranges. Late responses had no polarity preference. In contrast, VCN responses did not depend on polarity.

Conclusions: The results suggest that pseudo-monophasic pulses more selectively stimulate the CN. The strong polarity effects may shed light on which neural compartment is being stimulated by the ABI.

MO26. Variability in Cochlear Implantation Outcomes in a Large German Cohort With a Genetic Etiology of Hearing Loss

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Category: Auditory Prostheses

Background: The variability in cochlear implantation outcomes is largely unexplained and known clinical factors do not sufficiently predict cochlear implant performance. More recently, genetic factors have been proposed to impact cochlear implantation outcomes. The current hypothesis is that patients affected by genetic factors that primarily compromise the function of sensory cochlear tissue components have favorable outcomes as they are presumed of limited relevance to cochlear implant function. In contrast,

genetic mutations that functionally deteriorate the relevant neuronal components of the cochlea and the auditory pathway that are targeted by the electric stimulation of the cochlear implant may cause poor performance. We sought to verify this interpretation of cochlear implant outcomes within the background of a broad spectrum of confirmed genetic diagnoses in a large cohort of cochlear implant recipients.
Methods: This study included a large German cohort of cochlear implant recipients (n=123 implanted ears; n=76 probands) with a definitive genetic etiology of hearing loss according to American College of Medical Genetics /Association for Molecular Pathology guidelines and documented postoperative audiological outcomes. Postoperative cochlear implant outcome measures were based on at least one year of postoperative audiological follow-up for patients with postlingual hearing loss onset (> 6 years) and five years for children with congenital or pre-/perilingual hearing loss onset (≤ 6 years). Genetic analysis was performed based on single-gene screening, a custom-designed hearing loss gene panel, and whole genome sequencing.
Results: The genetic diagnosis of the 76 probands in the genetic cohort involved 35 genes and 61 different clinically relevant variants. With regard to implanted ears (n=123) the six most frequently affected genes affecting nearly one-half of implanted ears were GJB2 (21%; n=26), TMPRSS3 (7%; n=9), MYO15A (7%; n=8), SLC26A4 (5%; n=6) and LOXHD1 and USH2A (each 4%; n=5). Cochlear implant recipients with pathogenic variants that influence the sensory non-neural structures performed at or above the median level of speech performance of all ears at 70% (monosyllable word recognition score in quiet at 65 dB SPL). Our results shown that mutations in genes expressed in the spiral ganglion emerged as a significant factor more negatively affecting cochlear implantation outcomes as compared to all clinical parameters.
Conclusions: The analysis of the relationship of the molecular genetic diagnoses of a hereditary etiology of hearing loss and cochlear implantation outcomes in a large German cohort of cochlear implant recipients revealed significant variabilities. Poor performance was observed with genetic mutations that affected the neural components of the cochlea, supporting the “spiral ganglion hypothesis”.

MO27. 3D Basilar Membrane Templates to Assess the Position of the Electrode Array After Cochlear Implantation

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Background: Cochlear implant is an electronic device aiming the rehabilitation of hearing. During the surgery, the electrode array has to be inserted into the cochlea in an atraumatic fashion into the scala tympani. A postoperative CT scan is usually performed to assess the intracochlear positioning of the array. However, the metallic artifact of the array blurs the limits of the cochlea, and the position of the electrode array cannot be accurately determined. Here, we aimed to assess the accuracy of five ENT surgeons to determine the position of the electrode array using various 3D basilar membrane templates adapted to the proportions of the cochlea. The results of the analysis were blindly matched with the position of the electrode array determined by histology.
Methods: Ten 3D basilar membrane templates were generated from micro-CT imaging based on the dimensions of 100 cochleae. Cochlear implantations have been previously performed in temporal bone with Digisonic EVO or Advanced Bionics MidScala electrode arrays. For each type of arrays, a surgeon who didn’t participate on the analysis, selected 7 cochleae with a translocation of the array and 8 cochleae without translocation based on the histology results. Five ENT surgeons blindly determined the position of the electrode array on the imaging of these 30 implanted cochleae. The procedure consisted of four steps: 1. The selection of the appropriate basilar membrane template according to the cochlear dimensions; 2. The segmentation of the electrode array; 3. The fusion of the basilar membrane template and the 3D reconstruction of the electrode array using four landmarks; 4. The determination of the position of each electrode according to the basilar membrane. The results of the analysis were compared to those obtained by the histological analysis of the implanted cochleae.

Results: The time to analyze each cochlea was about 12 minutes. According to histology, surgeons were in almost perfect agreement to determine an electrode translocated to the scala vestibuli with the MidScala electrode array (Fleiss kappa = 0.82), and in a moderate agreement when using the EVO electrode array (Fleiss kappa = 0.42).
Conclusions: Our results indicate that a basilar membrane template can be used as a rapid and a reproductible method to assess the position of the electrode array after cochlear implantation using clinical imaging.

MO28. Coding Strategy for Opto-Electrical Hybrid Cochlear Implant

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Category: Auditory Prostheses
Background: Frequency-modulated phase coding is a novel strategy to encode acoustic information in future opto-electrical hybrid cochlear implants. The software controls pulse duration, amplitude, timing, and pulse rate. Compared to the already-approved methods on the market, the FMPC aims to increase performance for speech, speech-in-noise, and music perception.
Methods: Frequency selection: From the resulting gammatone-like spectrogram, a subset of frequency bands is selected. The number of frequency bands depends on the placement of the cochlear implant electrode and the width of the continuous frequency band. It is determined dynamically by the location of an electrode contact along the cochlea.
The decision to generate a pulse: The product of two terms, PowerProb*PhaseProb is compared with a random number (rn) of the interval $0 \leq rn \leq 1$, which is generated with a random number generator. A pulse is generated if this random number is smaller than the number calculated from the normalized rate and the phase. Wide frequency bands are stimulated electrically, and small frequency bands are optical. An electrical pre-pulse lowers the threshold of the optical stimulation.
PowerProb: Intensity mapping depends on five critical parameters: the spontaneous rate (a0), maximum rate (a1), the threshold for stimulation (a2), level for nonlinear behavior (a3), and a value describing the slope after the level for nonlinear behavior (a4). While parameter a0 shifts the curve towards larger values, a1 limits the rate to the number selected. The threshold has significant effects on the mapping. Low threshold values result in a rapid increase in the rate and quick saturation, whereas large threshold values slow the rise but limit the maximum achievable rate. Smaller effects are seen from the parameters a3 and a4. The selected values for a0=0 and a1=1 limit the rate R to the interval $0 \leq R \leq 1$. The input for the equation, the sound level p, is calculated from the acoustic power in each bin along a row of the gammatone-like spectrogram. In the present study, the length of one bin corresponded to 272 µs.
PhaseProb - temporal fine structure mapping: In single auditory nerve fiber recordings, the probability for another action potential is maximum at integer numbers of cycles following the last action potential after an action potential is generated. Our code has been developed similarly. PowerProb is then modified by multiplying it by PhaseProb, a number of the interval $(0 \leq PhaseProb \leq 1)$*wphase. The multiplier wphase is a factor that can increase or decrease the phase effect. Initially, wphase is selected to be 1.
Results: Pulse pattern map the intensity profiles seen in the spectrogram. Optical pulses are only produced for selected frequency bands. Pulse distribution is Poisson-like, with average pulse rates of about 150 Hz.
Conclusions: Performance in humans will be tested once the first opto-electrical hybrid cochlear implant is available.

MO29. Hearing Safety of Transcranial Ultrasound Parameters for a Novel Hearing Aid Technology

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Category: Auditory Prostheses
Background: Ultrasound (US) research has grown rapidly in the past decade, showing exciting potential therapeutic applications when used for noninvasively modulating brain regions and creating transient openings in the blood-brain-barrier for drug delivery. While investigating the abilities of US as a neuromodulation technique, our lab discovered that US applied to the head readily activates the auditory system, predominantly through vibrations of cerebrospinal fluid that then directly vibrate fluids within the cochlea via the cochlear aqueduct (Guo et al., Neuron, 2018). Due to the potential applications of US

induced auditory activation, our group is interested in characterizing safe parameter settings of US for the hearing system, particularly when used for neuromodulation, blood-brain-barrier opening, and hearing applications such as a novel hearing aid device.
Methods: To characterize safe parameter settings, we collected auditory brainstem responses (ABRs) and electrocochleography (ECochG) in response to air-conducted broadband noise and pure tones (2, 4, 8, 12, 20, and 30 kHz) at varying levels (10-80 dB SPL) before and after US stimulation in anesthetized guinea pigs. Both acute and chronic preparations were performed to fully characterize the potential for a parameter to cause damage. Stimulation was performed for 4 hours per session and consisted of 7 total sessions for chronic recordings. Control data was also collected for characterizing the stability of the recording protocol and for a standard noise-induced hearing loss (NIHL) comparison. We assessed ABR and ECochG thresholds, amplitudes, and latencies over time to identify changes that are associated with hearing damage or the experimental setup.
Results: Some tested US parameters showed neurophysiological changes associated with hearing loss especially with parameters using unmodulated and unramped ultrasound. Adding ramps to the unmodulated ultrasound parameters greatly reduced the extent of observed changes. Parameter settings used to effectively encode complex information (i.e., guinea pig vocalizations) to the auditory system do not show those neurophysiological changes associated with hearing loss at lower pressures and resembled the stability control data. Changes that occurred include threshold shifts that were most prevalent in the high frequencies and a reduction in ABR wave amplitudes. In more severe cases, some US parameter settings also caused threshold shifts in the middle frequencies. The patterns of hearing loss resembled those of standard NIHL.
Conclusions: Safe level ranges for US hearing were determined for chronic stimulation with increased pressure allowance when using amplitude modulated and ramped complex stimulations (guinea pig vocalizations) compared to unmodulated and unramped US. Future studies will include characterization of the safe and effective US parameters in large animal models that better mimic the head size of humans. Choice of center frequency and location of transducer on the head may have an impact on safe level ranges.

MO30. Evaluation of Auditory Performance Using Cochleo-Vestibular Implants (CVI)

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Category: Auditory Prostheses
Background: The Audio-Motion Processor (AMP) is the new wearable processor of the cochleo-vestibular implant (CVI), which allows parallel electrical stimulation of the auditory and vestibular systems. This is a new therapeutic option for patients suffering from sensorineural deafness and bilateral vestibulopathy (BV). The overall aim of this study was to evaluate the eventual negative impact of the electrical vestibular stimulation on auditory performance.
Methods: This study was conducted in four patients implanted with a CVI (MED-EL, Innsbruck, Austria). Stimulation could be controlled either by the AMP or with a standard cochlear implant processor (SP). We evaluated auditory performance in free field on the basis of pure tone audiograms (PTA) and speech audiograms (speech comprehension % at 65 dB SPL). Tests were performed in several conditions of hearing rehabilitation: (1) without any hearing rehabilitation device, (2) unilateral auditory rehabilitation with the patient's SP, (2) unilateral auditory rehabilitation with the patient's contralateral hearing aid only (cHA), (3) bilateral auditory rehabilitation with the patient's SP + the cHA, (4) unilateral auditory rehabilitation with the AMP only (no vestibular stimulation – CI-AMP), (5) bilateral auditory rehabilitation with the AMP + the cHA (CI-AMP + cHA), (6) unilateral auditory rehabilitation with the AMP + constant amplitude vestibular stimulation (50% of the dynamic range – CVI-AMP), (7) bilateral auditory rehabilitation with the AMP + constant amplitude vestibular stimulation + the cHA (CVI-AMP + cHA), and (8) unilateral auditory rehabilitation with the cHA + constant amplitude vestibular stimulation (VI-AMP + cHA). Subjective evaluations of sound quality with the AMP or the SP were also gathered.
Results: We observed significant improvements in auditory performance in unilateral and bilateral auditory rehabilitation conditions, with no significant difference between the AMP and the SP. Vestibular stimulation did not alter auditory performance significantly. Finally, the quality of the perceived sound was equivalent for both processors.
Conclusions: These promising results indicate that the AMP and the SP are equivalent regarding both auditory performance and subjective quality of the sound. Moreover, concurrent vestibular stimulation did

not significantly alter auditory performance demonstrating the feasibility of simultaneous electrical auditory and vestibular stimulation and thus potential full restoration of the complete inner ear function in patients who have completely lost both functions.

MO31. Magnetic Stimulation of the Cochlear Nerve

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Category: Auditory Prostheses

Background: Cochlear implants (CIs) strive to restore hearing to those with severe to profound hearing loss by artificially stimulating the auditory nerve. While most CI users can understand speech in a quiet environment, hearing that utilizes complex neural coding (e.g., appreciating music) has proved elusive, probably because of the inability of CIs to create narrow regions of spectral activation. Several novel approaches have recently shown promise for improving spatial selectivity, but substantial design differences from conventional CIs will necessitate much additional safety testing before clinical viability is established. Outside the cochlea, magnetic stimulation from small coils (micro-coils) has been shown to confine activation more narrowly than that from conventional micro-electrodes, raising the possibility that coil-based stimulation of the cochlea could improve the spectral resolution of CIs.

Methods: We delivered magnetic stimulation from micro-coils to multiple locations of the cochlea and measured the spread of activation utilizing a multi-electrode array inserted into the inferior colliculus; responses to magnetic stimulation were compared to analogous experiments with conventional micro-electrodes as well as to the responses to auditory monotones.

Results: The extent of activation with micro-coils was ~60% narrower than that from electric stimulation and largely similar to the spread arising from acoustic stimulation. The dynamic range of coils was more than three times larger than that of electrodes, further supporting a smaller spread of activation.

Conclusions: While much additional testing is required, these results support the notion that coil-based CIs can produce a larger number of independent spectral channels and may therefore improve functional performance. Further, because coil-based devices are structurally similar to existing CIs, fewer impediments to clinical translational are likely to arise.

MO32. Effect of Electrode Configuration on a Multipulse-Integration Measure of Cochlear Health

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Category: Auditory Prostheses

Background: It has been shown that psychophysical detection thresholds for cochlear implant (CI) stimulation decrease with increasing stimulus pulse rate. The steepness of the slopes of these multipulse integration (MPI) functions have been shown to be related to the density of spiral ganglion neurons (SGNs) in the vicinity of the implanted electrodes in guinea pigs. Thus, MPI slopes might be useful for identifying areas of greater and lesser nerve survival along the CI electrode array. It has been suggested that MPI functions obtained from tripolar electrode configurations might be better than those from monopolar configurations for predicting neural health near the electrodes. To test this idea, we compared MPI for monopolar and tripolar configurations and examined their relationship to SGN density near the stimulated electrodes.

Methods: Psychophysical detection thresholds were obtained from two groups of guinea pigs: 1) Implanted in a hearing ear (N = 19) and 2) Deafened by cochlear perfusion of neomycin into the scala tympani and then implanted (N = 10). Six of the deafened animals also received neurotrophin at the time of implantation, but SGN density in these cochleae was not significantly different from that in the group that received only neomycin, so all of the deafened animals were assessed as one group. After implantation and post-implant threshold stabilization, MPI functions were measured by obtaining detection thresholds for a fixed duration pulse train of 200 ms presented at 156, 313, and 625 pulses per second using monopolar and tripolar configurations. Following this and other functional testing, the animals were euthanized, and the cochleae were extracted for histological analysis.

Results: Consistent with previous studies, these results revealed that tripolar stimulation resulted in higher thresholds than those obtained with monopolar stimulation. However, the extent of decrease in thresholds as a function of pulse rate was similar for both electrode configurations. MPI slopes obtained with monopolar and tripolar configurations were weakly but significantly correlated with each other ($r2 = 0.23$; $p < 0.01$). Similar to previous studies, MPI was dependent on SGN density such that those with lower SGN density had shallower MPI slopes than those with higher SGN density. This relationship was not dependent on electrode configuration.
Conclusions: The results of this study suggest that there is some, but not complete, overlap in neural populations activated by the two electrode configurations resulting in small differences in their sensitivity to conditions near the stimulation sites. However, overall, the results suggest that MPI functions for both monopolar and tripolar configurations provide reasonable estimates of neural health near the stimulated electrodes.
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MO33. Open Board

MO34. Protection of Residual Hearing Upon Cochlear Implantation Through the Use of Near-Infrared Light in Guinea Pigs and Humans
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Category: Auditory Prostheses
Background: Electrode array insertion into the cochlea can initiate the loss of hair cells and spiral ganglion neurons in both animals and humans. It is assumed that direct mechanical injury, along with the expression of intracochlear inflammatory cascades, are detrimental to the survival of cochlear structures. Various approaches have been investigated that try to prevent or minimize such damage. One simple, local, and effective option is the perioperative application of near-infrared light (NIR). Specific wavelengths within the NIR-spec¬trum are known to influence cytochrome-c-oxidase activity, which leads in turn to a decrease of apoptotic and inflammatory mechanisms. Our group has previously shown that NIR can significantly decrease the auditory threshold shift if ap¬plied as a single pre-treatment, immediately before a noise trauma (Basta et al., 2020). The present study investigated the efficacy of a single NIR pre-treatment on cochlear structure and function upon cochlear implantation in guinea pigs and humans.
Methods: During a cochlear implant surgery, normal hearing adult guinea pigs had one cochlea pre-treated with NIR-light for 15 minutes. Immediately after NIR exposure, a specifically designed guinea pig electrode array, was inserted through a cochleostomy into the scala tympani of the first cochlear turn. The contralateral ear received a similar treatment (insertion) but was sham-exposed only.
Patients were implanted unilaterally with a HiRes 90k cochlea implant with or without (controls) a pre-treatment with NIR-light for 15 minutes.
Four weeks after implantation, frequency specific auditory brainstem response thresholds were determined on both sides in all implanted animals, whereas pure tone audiometry was performed in all patients. Impedance measures of electrode contacts were performed intraoperatively and four weeks after implantation.
Hair cell and spiral ganglion neuron densities were determined in histological cochlear samples of implanted guinea pigs. All data were compared between the two ears (with or without NIR pre-treatment) for each animal.
Results: The data demonstrated that a 15 min NIR pre-treatment can protect the residual hearing in both humans and animals. The protection amounted of approx. 20 dB. Furthermore, electrode impedances were decreased in NIR pre-treated patients. The protection of hair cells and spiral ganglion cells possibly contributed to hearing preservation, since both were significantly less reduced in NIR pre-treated guinea pig cochleae.
Conclusions: Our results suggest that a single NIR pre-treatment offers a very effective protection of cochlear structure and function during cochlear implantation. Decreased electrode impedances suggest an

inhibition of fibrotic tissue growth due to the NIR treatment. Future studies should investigate these effects in more detail.
This study was supported by Advanced Bionics GmbH, Hannover, Germany.
References: Basta et al., 2020, PeerJ 8:e9384

MO35. Getting More Auditory-Nerve Bang for Your Facial-Nerve Buck: Effects of Pulse Shape on Loudness and Facial-Nerve Activation in Cochlear-Implant Listeners

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Category: Auditory Prostheses
Background: Facial-nerve (FN) activation by cochlear-implant (CI) electrodes can require those electrodes to be re-programmed or turned off. We investigated the effects of two manipulations – increasing phase duration and using asymmetric pulses – that have been proposed to reduce FN effects. Both implicitly assume that the proposed manipulation reduces the current needed for the so-called Most Comfortable Loudness level (MCL), but produces a smaller or no reduction in the FN threshold ("FNT"), thereby increasing the MCL-FNT "headroom".
Methods: Three methods used pulse shapes that included symmetric cathodic-leading biphasic pulses with phase durations of 32 and 150 µs ("SYM32" and "SYM150"), pseudomonophasic pulses with 1st/2nd phase durations of 32/256 µs and with the first phase either anodic or cathodic ("PSA", "PSC"), and triphasic pulses with a 150-µs central phase flanked by two 75-µs flanking phases and with an anodic or cathodic central phase ("TPA", "TPC"). Method 1 involved 7 patients undergoing implantation of an Advanced Bionics (AB) CI, plus one MedEl patient undergoing re-implantation due to excessive FN activation. FNTs were estimated intra-operatively for single pulses using the clinical FN monitor; MCLs were obtained post-operatively using loudness scaling and 500-pps or 70-pps pulse trains. Method 2 measured MCLs with awake patients known to experience FN activation, and measured behavioural/electrophysiological FNTs for the same stimuli. Method 3 simulated MCLs by passing stimuli through a finite-element cochlear model combined with 1500 AN neurons based on Rattay's multi-compartment cable model (Brochier et al, 2021). The FNT was estimated using a multi-compartment cable model of a single FN neuron; input to this FN model was obtained from facial-nerve-canal recordings in cadaver heads.
Results: Method 1: No FN response was observed for the SYM32, PSA, PSC pulses at safe stimulus levels. FNTs for the TPA pulses were similar to or (usually) HIGHER than for TPC pulses but MCLs were 1.2 dB lower for TPA than TPC, and 1.7 dB LOWER for PSA than PSC, supporting the clinical use of anodic-dominant asymmetric pulses to minimise FN effects. MCLs for SYM150 were about 10 dB lower than SYM32; as SYM32 didn't activate the FN we can only estimate the minimum duration effect for FNTs, which was typically at least 8 dB. Data collection for method 2 is ongoing. Method 3 predicted greater headroom for PSA than for PSC pulses and for TPA than for TPC pulses, providing theoretical support for the use of anodic-dominant asymmetric pulses. However the model did not predict greater headroom for SYM150 than SYM32.
Conclusions: These preliminary data support the use of anodic-dominant asymmetric pulses to maximise the headroom between FNT and MCL, but so far provide no evidence that the widely adopted practice of increasing phase duration can increase headroom.

MO36. Psychophysical Tuning Curves as a Measure of Spectral Resolution in Children and Adults With Cochlear Implants

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Category: Auditory Prostheses
Background: Although cochlear implants (CIs) are highly successful neural prostheses for hearing restoration, speech perception outcomes are highly variable across individuals. One known source of variability in speech perception outcomes is the electrode-neuron interface (ENI), defined as the quality of the interface between individual electrodes and their target auditory neurons. The main factors contributing to the quality of the ENI are: 1) the distance between electrodes and the modiolus where the target neurons are housed, 2) the health and/or quantity of auditory neurons near each electrode, and 3) the tissue impedance likely determined by bone and tissue growth around the electrode array. Two measures that are

sensitive to ENI quality are auditory detection thresholds obtained using focused electrical fields and the sharpness of psychophysical tuning curves (PTCs).
In this study, we explored the perceptual consequences of poor ENIs in children and adults by quantifying the relationship between focused thresholds, PTCs, and vowel identification. We hypothesized that frequency selectivity in CI listeners is related to vowel identification: the better the frequency selectivity, the higher the vowel identification scores. As previous studies suggest that children have a higher density of healthy auditory neurons than adults, a second hypothesis is that children will have lower thresholds and sharper PTCs than adults.
Methods: Eight children (four bilateral recipients) and eight unilaterally implanted adults participated. All participants were implanted with Advanced Bionics devices. Focused thresholds were measured with a sweep procedure using a focused electrode configuration. PTC's were measured for one electrode near the middle of the array through a two-interval two-alternative forced choice procedure in children and a sweep procedure in adults. Vowel identification performance was assessed using ten /h-vowel-d/ words in both silence and noise. Multiple linear mixed effects models were constructed to test (a) whether focused thresholds differed between adults and children, (b) whether PTC sharpness differed between adults and children, and (c) whether PTC sharpness was associated with performance on vowel identification in noise as tested separately for children and adults.
Results: Although not statistically significant, children trended towards lower focused thresholds ($p = 0.085$) and steeper PTC slopes ($p=0.12$). However, steeper PTC slopes were significantly correlated with higher vowel identification in noise scores for both children and adults ($p=0.02$). Children also performed better on vowels in noise identification when controlling for the sharpness of tuning ($p=0.03$).
Conclusions: These findings show that PTC sharpness, a measure of spectral resolution, is positively correlated with vowel identification in noise. To our knowledge, this is the first study to report the relationship between PTC sharpness and speech perception scores in children with CIs. The use of vowels in noise, a spectrally challenging task, may be more revealing than previously used word or sentence recognition tasks.

MO37. Validating Loudness Summation as a Method of Assessing Peripheral Neural Masking in Cochlear Implants

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Category: Auditory Prostheses
Background: Psychophysical forward masking is typically used to assess temporal and spatial interactions between cochlear implant stimuli. However, the roles of peripheral and central mechanisms in psychophysical masking patterns cannot be dissociated. We propose an alternative psychophysical method for specifically assessing peripheral neural masking in cochlear implants. The proposed method is based on loudness summation, which refers to the overall loudness of different sounds presented together. It is known from acoustic hearing that loudness summation differs for tones that are separated by more than a critical band from those that are within a critical band and presumably interact at the basilar membrane. Extending those findings to cochlear implants, we evaluated the hypothesis that loudness summation of interleaved pulses depends on their interactions at the electrode-neural interface.
Methods: The first experiment evaluated loudness summation for pairs of interleaved pulses using a similar paradigm to McKay and McDermott (1998). Pulse train stimuli were constructed with a single pulse or a pair of pulses in each 50ms period. Two parameters were varied in the paired-pulse stimuli. First, electrode separation was varied by changing the cochlear location of the first pulse, while fixing the second pulse on an electrode in the middle of the array. Secondly, the time delay between the two pulses was varied, spanning the range between 0.25 and 20ms. Loudness summation was evaluated for each electrode pair by increasing the current of the single-pulse stimulus to achieve equal loudness to paired-pulse stimulus at fixed 1ms time delay. The effect of temporal separation on loudness was evaluated by loudness balancing paired-pulse stimuli of 1ms delay to stimuli with the same pulse pairs at different time delays.
The second experiment obtained electrically-evoked compound action potentials (ECAP) from the implant electrodes to directly evaluate peripheral neural masking. ECAP was measured for the same pulse pairs that were used in the previous experiment, i.e. same electrodes, current levels, and time delays. The effect of the first pulse (or masker) on the ECAP response to the following pulse (or probe) was used to quantify peripheral neural masking.

Results: The results showed that loudness evoked by a pair of pulses is affected by their temporal and spatial separations. The patterns of loudness versus spatial and temporal separation were consistent with interactions at the electrode-neuron interface. When the time delay between pulses was within the recovery period of the auditory nerve (typically <10ms), ECAP masking patterns explained loudness summation patterns for individual subjects. At longer time delays where peripheral interactions dissipated, loudness summation could only be explained by central temporal integration mechanisms.
Conclusions: The results support the potential of the proposed psychophysical method to assess peripheral neural masking in cochlear implant users.

MO38. Assessing Neural Health in Cochlear Implant Users With Residual Acoustic Hearing Using the Inter-Phase Gap Offset Effect

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Category: Auditory Prostheses
Background: Recently, it was suggested that neural health in cochlear implant (CI) users can be assessed by the ECAP inter-phase gap (IPG) offset effect (Brochier et al. 2021, https://www.doi.org/10.1007/s10162-020-00774-z), defined as the dB offset between the linear parts of ECAP amplitude growth functions for two stimuli differing only in IPG. We aimed to 1) examine whether the IPG offset effect reflects neural health in CI recipients with residual acoustic hearing, and 2) investigate the dependency of the IPG offset effect on hair cell integrity and intracochlear electrode impedances. We hypothesized that the IPG offset effect negatively correlates with the ECAP threshold and the preoperative pure-tone audiogram (PTA) if a higher IPG offset effect indeed represents better neural health.
Methods: Seventeen adult subjects with residual hearing at 500 Hz undergoing CI surgery at the University Hospital of Zurich were prospectively enrolled. ECAP thresholds, IPG offset effects, ECochG responses to 500Hz tone bursts, and monopolar electrical impedances were obtained at an apical, middle and basal electrode pair during and between four and twelve weeks after CI surgery.
PTAs were recorded within three weeks prior to surgery and approximately six weeks after surgery. Relationships between (changes in) ECAP threshold, IPG offset, impedance, PTA and ECochG amplitude assessed using linear regression analyses and t-tests. Lumped-element model simulations were conducted to better understand the influence of electrical impedances on the IPG offset effect.
Results: The IPG offset effect positively correlated with the ECAP threshold in intraoperative ($r = .36$, $p = .016$) and postoperative recordings ($r = .58$, $p = .00074$) and did not significantly correlate with the preoperative PTA ($p = 0.982$). The IPG offset effect showed a significant postoperative decrease in subjects with a postoperative ECochG amplitude drop ($p = 0.026$), but not in subjects without such a drop ($p = 0.32$). The change in impedance between intra- and postoperative recordings negatively correlated with the IPG offset effect change ($p = 0.0305$). The lumped-element model simulations revealed a relationship between electrode-tissue interface impedance and the IPG offset effect.
Conclusions: The hypothesized relationships between the IPG offset effect and the ECAP threshold or between the IPG offset effect and pre-operative acoustic hearing could not be confirmed in this study. However, the results revealed a dependency of the IPG offset effect on postoperative changes in electrical impedances. These findings would limit the method's usability for determining neural health in CI recipients with residual acoustic hearing.

MO39. Cochlear Implant Sound Coding Using a Model of the Auditory Nerve

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Category: Auditory Prostheses
Background: Cochlear implants can restore speech understanding in quiet, but the average performance is still below that of normal hearing subjects and decreases rapidly with background noise. Most commercial and research speech coding strategies focus on encoding and potentially enhancing features from frequency

bands, primarily the temporal envelope. The improvements in speech understanding that can be achieved with various modifications of such strategies have been found to be small and inconsistent. Seeber and Li (2022, proceedings of the ISH) proposed a new way of speech coding: a neural-model based sound coding approach which starts with a desired neural response and tries to approximate this response by altering the stimulation. The connection between stimulation pulses and neural response is made with a model of the electrically stimulated auditory nerve. The difficulty is the reversed operation: the output is known but the input is unknown. Seeber and Li demonstrated a proof of concept with a single neuron and one possible stimulation electrode. We present an extension of this approach that is able to derive a multi-electrode stimulation sequence that approximates the desired target neurogram.
Methods: Like Seeber and Li, we used the model by Takanen and Seeber (2022, Trends in Hearing) of a single electrically stimulated auditory neuron which is able to accurately model adaptation, facilitation, and refractoriness. In a first step, we extended the model to simulate multiple neurons stimulated by multiple electrodes using exponentially decaying current spread. The proposed algorithm then optimizes the electric pulse positions and amplitudes such that the neurogram is met as closely as possible. The amplitudes of all possible pulse positions over time are regarded separately in sequential order, while the pulses at different electrodes are optimized simultaneously. The algorithm was constrained such that the pulses at different electrodes do not overlap. Before the final placement of an electric pulse, it was tested if placing it in a later position would not be more beneficial. To develop, study, improve, and validate the algorithm, target neurograms were created by various means, for example, using continuous interleaved sampling together with the multi-neuron model.
Results: The algorithm was able to create an electrodogram resulting in a neurogram which closely approximates the target neurogram. Due to the many degrees of freedom and the non-linearity in the neural model, the electrodogram can differ from the original one.
Conclusions: We propose an algorithm creating an electrodogram such that a target neurogram is approximated by a model. If future studies show an improvement in speech understanding with these electrodograms, it can form the basis for the development of future cochlear implant coding strategies.
Support:ERC Starting Grant No. 716800 to M.D.

MO40. eCAP Simulation to Optimize Vestibular Implants

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Category: Auditory Prostheses
Background: To objectively assess the electrode-nerve interface, confirm the presence of excitable nerve tissue, provide intraoperative guidance, and facilitate programming of cochlear implants in infants and other patients who cannot cooperate with perceptual auditory testing, cochlear implant scientists and engineers developed systems for measuring electrically-evoked compound action potentials (eCAPs). Recently, this technique was used to evaluate the performance of vestibular implant. This study will create a computational model to simulate eCAP for this new technology in an effort to refine VI electrode design and placement by computational simulations.
Methods: A computational model of the inner ear capable electrical analysis was created from µMRI scans of a chinchilla head. The geometry of this model includes simplified recreations of the cochlea and vestibular system where perilymphatic and endolymphatic fluid spaces are distinct and all major sensory organs are present. The cerebellum will also be included. The capability of the model for electrical simulation of VI's will be assessed by conducting simulation of eCAP with both monophasic and biphasic stimuli through a dimensionally accurate implant. Comparison between data obtained animal experiments and simulation results will allow improvement of the model through a phenomenological approach. The material properties of the model will be modified until adequate agreement is met. Then, simulation will proceed to evaluation of different placements and designs of the vestibular electrodes.
Results: This study should yield information on the most optimal placement of vestibular implants in the inner ear of the chinchilla. Simulations which can recreate eCAP data obtained from experiments for the response of the cupulas and maculae based on speed of rotation and translation will be treated as most optimal when evaluating the model's initial performance. Comparison will then be made to experimental data on eye movement responses when refining VI placement and design.

Conclusions: eCAP simulation was performed to optimize the VI in a computational model of the chinchilla. The capacity to conduct eCAP simulation will help surgeons to place VI's in the best locations for patient outcomes. It should also inform further research into vestibular implants, determining what the next steps should be in design and software.

MO41. Barriers to Early Progress in Cochlear Implant Outcomes

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Category: Auditory Prostheses

Background: Cochlear implant (CI) outcomes are known to be variable and difficult to predict. We established a criterion of 90% sentence recognition with the implant in quiet at 1-month post-activation as the minimum expected score in the absence of limiting factors (Fraysse and James, IntechOpen 2020). We hypothesized that CI subjects with one or more 'bottom-up' or 'top-down' limiting factors would not achieve the 90% criterion.

Methods: A short battery of top-down and bottom-up tests was developed for the routine clinical evaluation of adult CI recipients in our center. Top-down tests were the Montreal Cognitive Assessment (MoCA), the Stroop test for executive function, and two subtests from the French ECLA-16+ reading battery to evaluate phonological awareness. Bottom-up tests were based on electrically evoked action potentials (ECAP) performed on a mid-basal and a mid-apical electrode contact: amplitude growth functions (AGF) for anodic- and cathodic-leading biphasic pulses, spread of excitation (SOE) and recovery functions. Sound processor data logs were used to measure time-on-air during the first month after CI activation.

Test battery results and sentence recognition scores at 1-month after activation were collected for 32 adult Nucleus CI recipients. Cognitive test results were compared to published normative data, using 1.65 standard deviations from the mean as a clinical threshold of significance for top-down limiting factors. Large differences in slopes for anodic- versus cathodic-leading AGFs, wide SOEs and long recovery periods signaled bottom-up limiting factors.

Results: CI subjects ranged in age from 26-87 years (median 70). The predominant (70%) etiology of deafness was unknown, followed by Meniere's disease (10%). Progressive hearing loss was reported in three quarters of cases.

13/32 subjects' scores met the 90%-correct 1-month sentence recognition criterion. Six subjects had below normal (<22/30) MoCA scores, five registered interference, and six poor phonological awareness. Across ECAP-based tests, responses could not be recorded in three subjects and two subjects had one or more limiting ECAP based feature for both apical and basal electrode contacts. Subjects with no limiting top-down factors and no limiting bottom-up factors covering both apical and basal areas were significantly more likely to achieve the 90% correct criterion (Fisher test, odds ratio 10.9, $p<0.005$). During the first month CI subjects were on air on average 12.2 (S.D. 2.6) hours per day with only three subjects less than 8 hours per day.

Conclusions: These preliminary results in a limited sample suggest that cognitive factors may more often be at play in limiting sentence recognition scores in quiet, with potentially cochlear-wide neural degeneration producing bottom-up limitations. Low scores were not associated with low total time on air. The current test battery may improve predictions of outcomes with CI as well as inform counselling and rehabilitation. Long-term data will be presented at the meeting.

MO42. Hebbian Learning Underlying Anticipated ITD Cue Reliability in the Barn Owl Auditory System

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Category: Binaural Hearing and Sound Localization

Background: All animals are faced with the challenging task of transforming natural sensory information into percepts that can drive adaptive behavior. Due to the inherently noisy nature of almost all sensory cues, it is crucial to select or emphasize those cues that are most meaningful and most reliable for mediating behavior. While meaningfulness often varies based on the task or immediate stimulus statistics, some aspects of sensory cue reliability for detection and coding are determined by prevalent physical and/or physiological conditions. In previous work from our lab, it was demonstrated that neurons in the barn owl's midbrain, specifically in the external nucleus of the inferior colliculus (ICX), which are sensitive to sound direction cues such as the interaural time (ITD) and level (ILD) differences, also exhibit frequency tuning

that matches anticipated ITD statistics. In other words, ICX neurons, which integrate across separate frequency channels from the immediate upstream nucleus, respond most strongly to those frequencies that are most reliably conveying ITD cues for their respective sound direction. More recent work has addressed the development of this frequency tuning by changing the physical properties underlying the cue reliability and found that the frequency tuning in ICX can indeed be changed in a way consistent to the relationship between frequency tuning and ITD cue reliability. This suggests that the frequency tuning observed in ICX is at least partially dependent on experienced reliability as opposed to being solely genetically predetermined.
Methods: In this modelling study, we test the hypothesis that experience-dependent plasticity leads to changes in frequency tuning based on the reliability of the ITD cue, which are predicted by a Hebbian learning model. This follows the idea that inputs from upstream neurons tuned to reliable frequencies are more likely to have a high covariance across longer time scales than those from neurons tuned to unreliable frequencies.
Results: Hence, the reliable frequency channels are more likely under a Hebbian learning rule to form stronger connections onto the ICX neurons than their unreliable counterparts. Noteworthy, this is a function of the sound direction and the physical properties of the head.
Conclusions: A rather simple learning rule like the one we propose here could explain both the formation of tuning patterns during development and plasticity in adult animals which some preliminary data has suggested and other similar studies have found before.

MO43. Open Board

MO44. Differences in Head and Pinna Morphology in Two Closely Related Species and Implications for Sound Localization Ability

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Category: Binaural Hearing and Sound Localization
Background: The ability to localize where sounds are coming from is pertinent to an animal's fitness, as it directly influences the ability to effectively communicate, forage, and avoid predators. In many mammalian species including rodents, the external ears (pinna) are the starting point of hearing by receiving sounds from the environment. The localization of these sounds is directly dependent on monoaural and binaural hearing ability in these animals. Binaural hearing ability of mammals relies heavily on interaural time differences (ITDs), which are the difference in the arrival time of a sound between two pinnae, and interaural level differences (ILDs), or the difference in sound level between the two pinnae. Therefore, head and pinna morphology has been shown to have direct impacts on binaural hearing and sound localization ability.
Methods: To further understand these impacts, we measured the dimensions of the head and pinna of over 900 preserved specimens of Peromyscus leucopus and Peromyscus maniculatus. Specimens were collected from all over the U.S. and were provided to us by the Collection of Vertebrates at Oklahoma State University. Our measurements included pinna size (length and width), distance between pinnae (inter pinna length), and distance from nose to pinna.
Results: Our preliminary data present differences in the dimensions of the pinnae across the two closely related species, which could be related to differences in binaural hearing as well. We also measured auditory brainstem responses (ABRs) in live-caught P. leucopus and P. maniculatus, in Oklahoma, to assess their hearing range and spatial hearing ability.
Conclusions: Together these results will help determine if pinna size is directly related to physiological measures of hearing ability in two closely related species.

MO45. Quantifying Impacts of Hearing Protection Devices on Sound Localization in Azimuth and Elevation: Toward Predictors of Performance

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Category: Binaural Hearing and Sound Localization
Background: Modern hearing protection devices (HPDs) effectively mitigate the risk of noise-induced hearing loss when used as intended, and some even preserve the audibility of low-to-moderate-intensity

sounds. However, HPDs also exert negative auditory perceptual side-effects that can limit usability in critical settings. For example, dozens of studies have shown that HPDs lead to large errors in sound source localization, including disorienting and potentially hazardous front-back confusions. Several studies have specifically linked such errors to HPD-induced distortions of the "spectral shape" of transmitted signals. Here we provide an update on a multi-site study designed to quantify patterns in sound localization errors across different classes of HPDs, including passive and active earplugs and earmuffs, toward validation of acoustic predictors of HPD performance.
Methods: At two independent study sites, human listeners localized brief (100 ms) broadband signals presented at 70 dBA from randomized source locations in a darkened hemianechoic chamber. Subjects completed the task with open ears (control) and during use of several passive and active earplug and earmuff-style HPDs. Sources spanned 360° in azimuth and -30° to +60° in elevation, enabling measurement, via wireless head-tracking, of two-dimensional localization error across a broad range of source locations.
Results: All HPDs led to an increase in sound source localization errors, notably including an increase in front-back confusions and large errors in elevation responses. Differences in the form and extent of errors across devices were also evident. Acoustic data obtained using both standardized test fixtures (manikins) and individualized measurements also demonstrate cross-device differences. Parallel quantification of behavioral and acoustic performance changes is intended to support prediction of HPD impacts on source localization.
Conclusions: The long-term goal of this effort is to develop and standardize novel acoustic performance metrics for HPDs that will support application-specific selection of existing HPDs and inform the design of new HPDs. Acoustic and behavioral measurements and refinements of predictive metrics are ongoing.

MO46. Bayesian or Bust: Effects of Stimulus Statistics on the Binaural Auditory Brainstem Response

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Category: Binaural Hearing and Sound Localization
Background: Modern theories of perception suggest that the brain maintains an internal perceptual model that is updated according to sensory input. Evidence of this kind of processing in binaural hearing has been demonstrated by behavior in humans, cortical electroencephalography in humans, and single-unit responses in the midbrain and brainstem of animals. That is, sound source localization is affected by preceding stimuli. The goal of this study was to assess whether populations of neurons in binaural brainstem adapt to the statistics of preceding stimuli. We hypothesized that an aberrant cue (i.e., change from expectation) would result in an increased response and a sharpening of tuning to binaural cues.
Methods: Auditory brainstem responses (ABRs) were measured in adult chinchillas. The binaural interaction component (BIC) of the ABR, thought to be derived from the lateral superior olive, was derived by taking the difference between the binaurally evoked ABR and the sum of monaurally evoked ABRs. Interaural timing differences (ITDs) were manipulated. In the standard condition, the ITD was chosen at random. In the experimental condition, one ITD was overrepresented. Stimuli were presented at 90 dB peak SPL via insert earphone and consisted of broadband or narrowband transients.
Results: In the standard condition, the BIC amplitude was modulated by ITD consistent with previous literature, showing a decrease as absolute ITD magnitude increased from 0 microseconds. The experimental condition showed inconsistent evidence of adaptation to stimulus statistics in a variety of stimulus conditions.
Conclusions: These results suggest that single-unit adaptation in the binaural brainstem may be more prominent than the population level assessed by ABRs. Clinically, these results may imply that the BIC can be measured using random or repeated presentation of ITD with consistent results.
Work was supported by T32 DC012280 awarded to University of Colorado Anschutz Medical Campus and R01 DC011555 awarded to DJT.

MO47. Duplex Perception Reveals Brainstem Auditory Representations Are Modulated by Listeners' Ongoing Percept for Speech

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Category: Binaural Hearing and Sound Localization

Background: So-called duplex speech stimuli with perceptually ambiguous spectrum to one ear and isolated low- vs. high-frequency third formant "chirp" to the opposite ear yield a coherent percept that supports their phonetic categorization. Critically, such dichotic sounds are only perceived categorically if speech cues are binaurally integrated.
Methods: Here, we used frequency-following responses (FFRs), scalp-recorded potentials reflecting phase-locked subcortical neural activity, to investigate brainstem responses to fused speech percepts and determine whether FFRs reflect binaural integration and category-level representations. To this end, we recorded FFRs to diotic and dichotic stop-consonants (/da/, /ga/) that either did or did not require binaural fusion to properly label along with perceptually ambiguous sounds without clear phonetic identity. Stimuli were presented using a novel clustered paradigm with variable interstimulus interval to maximize paradigm efficiency and recording of brainstem FFRs during active behavior.
Results: Behaviorally, listeners showed clear categorization of tokens when speech cues were split between ears confirming they were heard with a fused, phonetic percept. Neurally, we found FFRs were stronger for speech that was perceived categorically relative to category-ambiguous tokens but also differentiated phonetic categories for both diotically and dichotically presented speech sounds. Correlations between neural and behavioral data further showed FFR strength predicted the degree to which listeners labeled tokens as "da" vs. "ga".
Conclusions: The presence of binaurally integrated, category-level information in FFRs suggests human brainstem processing reflects a surprisingly abstract level of the speech code typically circumscribed to much later neocortical brain areas.

MO48. Contralateral Sound Field Attenuation Can Improve Speech-In-Noise Intelligibility for Bilateral Hearing-Aid Users

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Category: Binaural Hearing and Sound Localization
Background: We have recently proposed a binaural sound pre-processing method to attenuate sounds contralateral to each ear and shown that it can improve speech intelligibility for normal-hearing (NH) people in cocktail party listening situations (Lopez-Poveda et al., 2022, Hear Res 418:108469). The aim here was to evaluate if the benefit remains for hearing-impaired listeners when the method is combined with two independently functioning hearing aids, one per ear.
Methods: Twelve volunteers participated in the experiments (five of them with bilateral sensorineural hearing loss and seven NH listeners with simulated bilateral conductive hearing loss). Speech reception thresholds (SRTs) for sentences in competition with a source of steady, speech-shaped noise were measured in unilateral and bilateral listening, and for (target, masker) azimuthal angles of (0º, 0º), (270º, 45º), and (270º, 90º). Stimuli were processed through a pair of software-based multichannel, fast-acting wide dynamic range compressors, with and without binaural preprocessing.
Results: For spatially collocated target and masker sources at 0□ azimuth, the pre-processing did not affect SRTs. For spatially separated target and masker sources, pre-processing improved SRTs when listening bilaterally (improvements up to 10 dB) or unilaterally with the acoustically better ear (improvements up to 14 dB), while it worsened SRTs when listening unilaterally with the acoustically worse ear (decrement of up to 18 dB).
Conclusions: Contralateral sound field attenuation can improve speech-in-noise intelligibility for bilateral hearing-aid users. [Work supported by MED-EL GmbH, and the Spanish Ministry of Science and Innovation (grant PID2019-108985GB-I00), and the European Regional Development Fund]

MO49. SSD Speech Localization Testing

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Category: Binaural Hearing and Sound Localization
Background: Single-sided deafness (SSD) refers to the clinical scenario in which hearing loss in one ear is non-serviceable with traditional amplification due to profound degree or poor word recognition abilities. The traditional audiometric battery fails to adequately characterize the performance deficits experienced by individuals with SSD. Here, we propose a novel testing paradigm combining speech-in-noise and

localization while monitoring head position and ear-level acoustical cues in order to characterize behavioral adaptations and quantify resultant changes to binaural cues to better understand the impact of SSD on complex binaural task performance.
Methods: Eligible subjects include individuals with normal hearing and SSD. Subjects undergo testing in a hemi-anechoic chamber with 24 speakers aligned 15 degrees apart. The stimuli from the novel speech-in-noise localization task are the CID Everyday Sentences presented from a single speaker in the array. Background multi-talker babble is generated from Connected Speech Test sentences presented at random by four speakers. The SNR begins at zero and gradually increases until the subject reaches the presentation level at which they can reliably identify 100% of the target speech. Subjects are encouraged to move their head as they would naturally in everyday listening situations to optimize their listening ability. A head-worn position tracking system captures their real-time compensatory head movements. Simultaneously, in-ear measurements via probe tube microphones placed in the subjects' ear canals will capture the auditory input to each ear.
Results: Head movement patterns will be compared between subjects with normal hearing and those with SSD. The impact of head movement on binaural cues as characterized by ear-level measurements of the auditory input will be quantified according to ear-specific signal-to-noise ratios. We will also examine performance on sentence identification relative to spatial separation of signal and noise by comparing the level at which subjects can reliably repeat 50% of the sentences.
Conclusions: Compared with normal hearing individuals, those with SSD demonstrate maladaptive patterns of head movement that favor optimization of the signal-to-noise ratio in the better-hearing ear. We hypothesize that this novel paradigm will functionally differentiate individuals with normal hearing from those with SSD and provide a meaningful metric for evaluation of prosthetic device performance benefit for rehabilitation of SSD.

MO50. Cellular Basis for the Detection of Frequency Sweeps in Octopus Cells of the Cochlear Nucleus
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Category: Brainstem: Structure and Function
Background: Octopus cells in the posterior ventral cochlear nucleus encode sound with remarkable temporal precision and convey this precision to higher auditory nuclei. Low-threshold K conductances, evoked by excitatory synaptic input from auditory nerve fibers (ANFs), make octopus cells highly sensitive to the rate of rise of depolarization, and underlie their capacity to select for only the most coincident ANF inputs. Octopus cell dendrites are oriented roughly perpendicular to the tonotopic array of ANFs, with higher frequency inputs targeting progressively more distal dendritic regions. A previous modeling study has suggested that octopus cells may compensate for cochlear delay through differential dendritic filtering of EPSPs propagating to the soma from different dendritic locations. However, there is no experimental data to date on how the tonotopic position of an ANF influences synaptic timing at the soma and axon.
Methods: We made whole-cell patch recordings from octopus cells under IR-DIC optics in parasagittal brain slices of the cochlear nucleus of the mouse (P21-P42). In these slices we activated individual or small groups of ANFs systematically along the dorso-ventral axis in the AVCN, corresponding to high- and low frequency regions, and recorded synaptic responses in either current or voltage clamp. Cells were filled with 1% biocytin and either Alexa-488 or 568 for post-hoc morphological reconstruction using a Neurolucida system.
Results: Focal, minimal stimulation, of ANFs evokes synaptic potentials and currents with time courses that vary with the ANF topographic axis. Post-synaptic responses evoked by activating high frequency ANFs were slower than those generated by low frequency ANFs, which accords with a distal-to-proximal spread of high-to-low frequency ANF inputs on octopus cell dendrites. Synaptic rise times ranged from ~500-60µs respectively from the high to low frequency axis of ANFs. Decay times were highly variable with peak synaptic amplitude, suggesting differential activation of non-linear conductances at different dendritic distances from the soma. In ongoing work, we are examining the morphological bases of synaptic integration by individual octopus cells. Further, using a variety of stimulus paradigms, including variable stimulus currents, stimulus sweeps and Poisson trains, we are determining the kinetics of conductances that shape the temporally precise encoding of frequency modulation by octopus cells.
Conclusions: We conclude that ANF fiber inputs from high-frequency tonotopic positions undergo substantial dendritic delays as compared to more low frequency ANFs. These frequency dependent dendritic

delays may allow octopus cells to detect frequency modulations in sounds and/or compensate for cochlear delay.

MO51. Investigating the Effect of Selective Attention on Speech Encoding From Auditory Nerve to Cortex

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Category: Brainstem: Structure and Function

Background: The subcortical auditory system consists of an ascending pathway which processes and passes auditory information towards the cortex and an efferent pathway with at least as many projections whose function is much less clear. Selective attention is considered a possible role, since many of these projections originate in the cortex and cortical responses are robustly modulated by attention. However, there are many studies which find no effect of attention in subcortical responses, while some others report attentional effects as early as the cochlea. Nearly all of these studies have been limited to simple stimuli or consider only one stage of processing. Here we used several new tools, including stimuli, analyses, and electrodes, to assess attention-driven differences in the encoding of natural, connected speech at distinct stages of processing beginning at the auditory nerve and spanning the brainstem through cortex.

Methods: We developed an experiment to determine where attention effects are first visible by presenting competing, diotic audiobooks, instructing subjects to attend to one of them, and then computing the compound action potential (CAP), auditory brainstem response (ABR), and cortical temporal response function through deconvolution. To perform the deconvolution, we developed a new regressor based on modeled auditory nerve firing rates. The CAP is generated in the auditory nerve and was measured using an electrode placed on the tympanic membrane. The ABR indexes encoding in several subcortical areas and was recorded with a purpose-specific pre-amplifier. Cortical responses were measured with a standard array of 32 active EEG channels. All responses were recorded simultaneously. In addition to this experiment, which is ongoing, we also used our new regression techniques analyze a second dataset (unpublished, n=24) previously collected in an experiment with a similar design but using dichotic stimuli.

Results: Preliminary findings from our in-progress dataset show no differences in the amplitude or latency of the CAP or ABR, suggesting no subcortical effects of selective attention. The expected large cortical attentional effects are present. Additionally, our analysis of the dichotic speech dataset also shows no effect of attention until latencies corresponding to cortical generators.

Conclusions: We find no evidence that selective attention measurably affects subcortical encoding of speech. Because there was no effect in either the auditory nerve or later brainstem stages, we suggest that cochlear effects are also unlikely, given the expectation that they would propagate to later stages of the auditory system. We do, however, replicate the strong modulation of cortical responses seen in many prior studies—an indication that neural effects of attention, where present, are readily observable using our analyses. Thus, we find no evidence of a role for the efferent pathway in selective attention to speech.

MO52. Non-Linear Encoding of Sounds in the Auditory Brainstem

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Category: Brainstem: Structure and Function

Background: Sound perception is shaped along the auditory pathway. Understanding precisely how non-linear categorization of complex sounds such as speech arises requires the analysis of extensive data from every area of the auditory system. However, the first layers of computation are still poorly understood due to the difficulty of recording large number of neurons in the auditory brainstem areas.

Methods: Here, we used the length and recording capacity of Neuropixels 1.0 probes to record more than a thousand neurons from the cochlear nucleus (CN), superior olivary complex (SOC) and inferior colliculus (IC) regions each in head-fixed, awake mice listening passively to a set of 307 short (<500ms) artificial sounds and 10 long (30s) sounds from natural environments. The set of short sounds was designed to include sounds of increasing complexity: diverse categories of simple sounds, complex sounds (bird songs, music), as well as partial and complete reconstructions of the complex sounds.

Results: We compared the recorded responses with described physiology in anesthetized animals and typically found primary-like and onset response units. Thanks to the size of our dataset, we could apply population analysis to estimate the degree of non-linearity of auditory brainstem neural responses and the

relevant features extracted in each area. These findings will be combined with responses from a detailed biophysical cochlear model and 2-photon data acquired with the same paradigm in the auditory cortex to build a data constrained model of the auditory pathway from the cochlea up to the auditory cortex.
Conclusions: This model of cortical afferents could be used in hearing restoration devices targeting the auditory cortex.

MO53. Patch-Seq on Principal Neurons and Lateral Olivo-Cochlear Neurons in the Lateral Superior Olive (LSO)

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Category: Brainstem: Structure and Function
Background: The LSO is a conspicuous hub in the auditory brainstem. It is a heterogeneous nucleus because it contains neurons associated with the ascending or descending pathways. Principal LSO (pLSO) neurons, the major cell type, compare intensity differences between both ears and send information about the location of sound sources to higher brainstem centers. In contrast, LOC cells (lateral olivo-cochlear) send information to the periphery and appear to modulate nerve fiber activity in a reflex manner. Our study aimed to obtain a comprehensive overview of the genes expressed in each cell type and to reveal differences as well as similarities. Thus, we try to decipher the biophysical and genetic determinants of heterogeneous neuron populations. For this purpose, we applied patch-seq, a powerful multi-modal single-cell RNA-sequencing approach that combines electrophysiology with global transcriptomic analysis.
Methods: Patch-seq experiments were performed on brainstem slices of juvenile mice. Immunohistochemistry, pharmacology, and computational analysis were used to characterize the proteins encoded by interesting candidate genes. Differentially expressed genes (DEGs) were revealed at FoldChange ≥ 2 and a FalseDiscoveryRate ≤ 0.05. Besides DEGs, we also assessed ‘cluster specificity’ and ‘similarity’ of genes whose mean expression level was ≥ 4 transcripts per million (TPM) in at least one cluster.
Results: Our patch-seq sample comprised 86 neurons. For those, by far the highest expression levels were found for Ckb, Snurf, and Calm1 (> 23,000 TPM). In general, the highly expressed genes are consistent with high energy demands in LSO neurons. In an unbiased approach, we found two clusters of neurons. 332 DEGs were seen in cluster 1, and 73 genes in cluster 2. Subsequent analysis, mainly assessment of differences in the spiking pattern, attributed cluster 1 to pLSO neurons and cluster 2 to LOC cells (n = 56, 30). The clustering revealed via transcriptomics was confirmed by a principal component analysis of 16 electrophysiological parameters (e.g., input resistance, tau membrane). The top 3 DEGS for pLSO neurons were Spp1, Lpgat1 and Tenm2. For LOC cells, the top 3 DEGs were Calca, Ucn and Cal4a3. Concerning voltage- and ligand-gated ion channels (VLGICs), pLSO neurons showed DEGs or ‘cluster-specific’ genes for these proteins: Kv11.3, Cavγ5, KChIP1, GluA4, GluN2b, Kvβ3, Kv1.1, GABAARβ3. Corresponding genes for LOC cells were: Nav1.3, Cav2.3, Nav1.2, GABAARα1, nAChRα3. Whereas an average pLSO neuron expressed 14.5 genes, LOC cells expressed 12.3. Together, these results show clear differences in the gene expression profile for VLGICs. Top VLGIC genes demonstrating ‘similarity’ encode for Cav2,2, GABAARβ2, GABAARγ1 and TRPML1.
Conclusions: Collectively, our results show striking differences between ascending pLSO and descending LOC neurons. Nevertheless, there are also some similarities. Both features emphasize the role of these two major neuron types in information processing at an early level of the central auditory system.

MO54. Effects of Stimulus and Electrode Configurations on the Frequency-Following Response to a 100-Hz Tone

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Category: Brainstem: Structure and Function
Background: Frequency-following response (FFR) studies examine neural activity synchronized to the periodic or transient aspects of sound. However, there is no consensus on stimulus and electrode configurations or response characterizations. Here, we compared differences in signal and noise between single and linked reference electrodes. Next, we varied the number of stimulus presentations to characterize effects on signal to noise ratios. We also characterized responses from binaural and monaural contributions

at different intensity levels, and binaurally with the tone either in or out of phase between ears. These stimulus configurations allowed us to test whether the binaural FFR is a result of linear summation of left and right neural activity, or loudness summation, or additional binaural interactions. Finally, we propose a novel index to objectively quantify FFR periodicity and its utility in predicting behavioral thresholds.
Methods: Normal-hearing listeners were presented with a 170-ms, 100-Hz pure tone. Listeners heard 6000 trials per condition except for the stimulus presentation study. FFRs were recorded at Cz (active), Fpz (ground), A1/A2 (references). For experiment 1, the stimulus was presented at 80-dB SPL in two binaural (single and linked references) and four monaural conditions (single and linked references with right ear presentation, single and linked references with left ear presentation). For experiment 2, the tone was presented binaurally for six blocks of 500 trials per block. The third set of experiments included 80-dB SPL monaural, 90-dB SPL monaural, and 80-dB SPL diotic presentations, and an 80-dB SPL dichotic presentation with the right ear stimulus phase-shifted by 180-degrees compared to the left. To quantify FFR periodicity, fidelity to stimulus periodicity, and prediction of behavioral thresholds, we propose an index that is the dot product of the stimulus and response's autocorrelation.
Results: First, we found no difference across reference electrode configurations. Second, while there was no consistent effect on signal level, noise level was reduced as a sqrt function of trial number. We observed a ~2x increase in response for binaural compared to monaural presentations, providing evidence for contribution of linear summation to the FFR. Responses to increasing intensity level monaurally were not equivalent to the lower intensity binaural condition, suggesting that loudness summation is not reflected in the FFR. No evidence of interaural timing differences contributing to the FFR was found when comparing unshifted and phase-shifted conditions. Finally, compared with the RMS measure, our index improved FFR periodicity detection threshold by 4.5-dB SPL (35 for RMS, 30.5 for our index), surpassing the behavioral stimulus detection threshold (35.9-dB SPL).
Conclusions: We provide systematic evidence that FFR parameters related to reference configurations do not impact responses, while those related to the number of trials, ear presentation, and periodicity should be carefully considered regarding implications for FFR mechanics and characterization.

MO55. Group I mGluR-Triggered Temporally Patterned Spontaneous Synaptic Transmission in Mouse MNTB Neurons

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Category: Brainstem: Structure and Function
Background: Rhythmic action potentials are generated via intrinsic ionic mechanisms in pacemaking neurons, producing synaptic responses of regular inter-event intervals (IEIs) in their targets. In auditory processing, evoked temporally patterned activities are induced when neural responses timely lock to a certain phase of the sound stimuli. Spontaneous transmitter release, however, is a stochastic process, rending the prediction of the exact timing of the next synaptic event completely based on probability. Furthermore, neuromodulation mediated by metabotropic glutamate receptors (mGluRs) is uncommonly associated with patterned neural activities. Here, we report an intriguing phenomenon, in which activation of mGluRs triggered temporally patterned spontaneous transmission.
Methods: Brainstem slices were prepared from C57/B6 mice of either sex, and whole-cell voltage-clamp and cell-attached recordings were performed at 35 °C. A group I mGluR agonist 3,5-DHPG (200 μM) was bath-applied. Auto-correlation was performed to assess rhythmic activity.
Results: In a subpopulation of medial nucleus of the trapezoid body (MNTB) neurons, temporally patterned glycinergic sIPSCs and glutamatergic sEPSCs were elicited by activation of group I mGluRs with 3,5-DHPG (200 μM). Auto-correlation analyses revealed rhythmogenesis in these synaptic responses. Knockout of mGluR5 (one member of group I mGluRs) largely eliminated the effects of 3,5-DHPG on spontaneous synaptic transmission. Cell-attached recordings showed temporally patterned spikes evoked by 3,5-DHPG in potential presynaptic cells for synaptic inhibition and excitation onto MNTB. Finally, immunocytochemical studies identified presynaptic presence of mGluR5 and postsynaptic localization of mGluR1 (another member of group I mGluRs) within MNTB.
Conclusions: Our results imply a potential central mechanism underlying the generation of patterned spontaneous activity necessary for auditory circuit development.

MO56. Distinguishing Misophonia From Hyperacusis Using Auditory Brainstem Response

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Category: Brainstem: Structure and Function
Background: Misophonia is a newly described condition characterized by intense and excessive emotional responses to specific “trigger” sounds (e.g., chewing, tapping, sniffling). This condition is often classified within the larger category of Decreased Sound Tolerance (DST) disorders, which also includes hyperacusis (the perception of sounds as excessively or painfully loud to a broad range of moderately loud sounds). Few studies have examined either hyperacusis or misophonia using auditory brainstem responses (ABR). However, ABR may be a useful tool in this context, given the involvement of modified gain of central auditory neurons and the influence of nonauditory brain regions on the auditory pathways. Therefore, the current study aims to explore possible biomarkers of auditory function using ABR in hyperacusis and misophonia in normal hearing controls while ruling out retrocochlear disorder.
Methods: A total of 83 participants, ages 18 to 25 years were recruited to conduct a full audiological evaluation and ABR assessment. The participants (all with normal hearing thresholds) were categorized into four groups: 13 with misophonia alone, 12 with hyperacusis alone, 22 with misophonia and hyperacusis, and 35 with control subjects with neither condition. ABR waveforms were elicited by broad-band click stimuli presented at 80, 70, 50, and 30 dB nHL in both ears. Recording parameters included a bandpass filter of 100-3000 Hz; stimulus rate of 13.3 Hz; and 4000 averages for 80, 70 and 50 dB nHL; 6000 averages for 30 dB nHL to obtain clear, replicable waveforms. The highest intensity level was only performed below the subject’s loudness discomfort level (LDL). Stimulus levels were randomly changed in each participant. ABR waveform analysis included absolute latencies/amplitudes of wave I, III, and V, interpeak latencies as well as amplitude ratios of waves III/I and V/I.
Results: Both hyperacusis and misophonia alone groups showed enhanced wave I amplitude (indicating reduced auditory nerve activity) but no significant changes in wave V (reflecting input to the inferior colliculi) compared with the control group and well as the group with both conditions. Preliminary analysis of latencies revealed that the hyperacusis and misophonia group and the misophonia only group had delayed wave V latencies compared to the other two groups.
The III/I amplitude ratios were reduced in the misophonia-alone group, indicating reduced peripheral activity to central hyperactivity in misophonia. No significant V/I ratio was identified.
Conclusions: Our results highlight the feasibility of using ABRs to distinguish between hyperacusis and misophonia. They suggest a potential central auditory mechanism in misophonia and hyperacusis, perhaps involving processing at the lower brainstem level. This study is ongoing and as more data are collected, we will have a better idea about the involvement of the brainstem and midbrain in these conditions and their interactions with cortical centers of auditory processing.

MO57. The Lateral Paragigantocellular Nucleus (LPGi): A Third Source of Cholinergic Input to the Cochlear Nucleus

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Category: Brainstem: Structure and Function
Background: Acetylcholine has been implicated in modulation of temporal processing, neuronal excitability and plasticity in the cochlear nucleus (CN). Previous studies identified two sources of cholinergic input to the CN: (1) the superior olivary complex (SOC), and (2) the pontomesencephalic tegmentum (PMT) a source of cholinergic projections to many levels of the auditory system. Here, we show input to the CN from a third cholinergic source: the lateral paragigantocellular nucleus (LPGi), and we compare the patterns of input from each of the three sources.
Methods: To label cells making cholinergic projections to the CN, we injected retrograde tracers into the CN in C57BL/6J mice, then labeled tissue with immunostaining for vesicular acetylcholine transporter (VAChT) to identify cholinergic cells. To visualize cholinergic axons in the CN, we injected AAV carrying Cre-dependent fluorescent protein genes into each source in mixed background "normal-hearing" ChAT-Cre mice.
Results: In animals with retrograde tracer injections, we found VAChT+ retrogradely-labeled cells in the SOC, PMT, and LPGi, indicating cholinergic projections from all three regions into the CN. The LPGi typically had the fewest retrogradely-labeled cells, as well as the fewest VAChT+ retrogradely-labeled cells. When we selectively labeled the cholinergic pathways from each nucleus and examined the axons in the CN,

we observed unique but overlapping areas of input from each source. As previously described, inputs from the SOC were heaviest in the granule cell area (GRCA) between the ventral CN (VCN) and dorsal CN (DCN), and along the medial edge of the CN. We observed SOC input distributed throughout the layers of the DCN, and lighter SOC input to the core areas of the VCN. PMT inputs were also present across the GRCA and DCN layers. PMT inputs preferentially targeted more rostral parts of the VCN, and core areas of caudal VCN lacked PMT input. LPGi inputs were the sparsest of the three; light input from LPGi was present across all layers of the DCN, in the GRCA, and along the medial and lateral edges of the VCN. In our sample, the LPGi was the only cholinergic source that sent axons to the lateral edge of the VCN.
Conclusions: We conclude that, in addition to cholinergic inputs from the SOC and the PMT, the CN receives cholinergic inputs from the LPGi, a small medullary nucleus connected to multiple auditory nuclei. The three sources have overlapping but unique patterns of innervation in the CN. Inputs from the SOC are likely to carry auditory information relating to the olivocochlear reflex, while PMT and LPGi inputs might serve to modulate CN activity based on attention or arousal state, perhaps including a multisensory integrative function.
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MO58. Auditory Brainstem Responses Evoked by Running Speech in Preterm Infants

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Category: Brainstem: Structure and Function
Background: The auditory brainstem response (ABR) has typically been evoked by brief synthetic stimuli such as clicks and tonebursts to estimate audiometric thresholds and by short speech tokens to study speech processing. However, these stimuli bear little resemblance to natural sounds, the most important of which is connected speech. Recent studies have developed methods for measuring the ABR to running, natural speech, though these efforts have so far been limited to normal hearing adults. An index of subcortical encoding of natural speech in infants may be informative for future language outcomes. The goals of the study are to (1) determine which ABR waves can be evoked by running speech in preterm infants; and (2) how the latency and amplitude of ABR waves evoked by running speech compare to those evoked by clicks.
Methods: Thirteen preterm infants were tested at 33-36 weeks gestational age while they were cared for in the neonatal intensive care unit. Five minutes of clicks generated through a Poisson process and 30 minutes of broadband peaky speech (speech made as click-like as possible while preserving its spectral-temporal properties) from an audiobook narrated by a male were presented to one ear of the infant through ER-2 earphones. The ABRs were derived using cross-correlation for clicks and deconvolution for peaky speech. The input to the auditory system was the sequence of clicks for click stimuli, and the sequence of glottal pulses associated with the speech for the peaky speech stimuli, and the output was the EEG response recorded between electrodes placed at high forehead and mastoid of ipsilateral ear (ground at low forehead). The primary analysis was manual peak-picking by trained audiologists familiar with ABR measured in preterm infants to determine the amplitude and latency of response components.
Results: Observable waves were recorded from 12 of the 13 infants. Notable peaks and troughs were observable across the post-stimulus time frame consistent with ABR recorded in a traditional manner. ABR waves evoked by speech had overall lower amplitudes than those evoked by clicks, which was expected given studies in adults. The relationships between click and speech latencies varied depending on the wave: the negative trough around 4 ms was similar for clicks and speech, but Wave V latency was longer for speech than clicks.
Conclusions: The ABR derived using clicks and speech are measurable and present in preterm infants cared for in the NICU. The results suggest the possibility of exploring the contribution of subcortical speech processing to future language outcomes. [Research supported by the University of Rochester CTSA award number UL1 TR002001 from the National Center for Advancing Translational Sciences of the National Institutes of Health]

MO59. Interrogating Ensembles and Dynamics of the Cochlear Nucleus in Processing Ultrasonic Vocalizations in Awake Mice

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Category: Brainstem: Structure and Function

Background: Mice vocalize and communicate at 50-100 kHz, but mice showed relatively poor hearing sensitivity among such frequency range. Previous studies showed that higher characteristic frequency (CF) (>50 kHz) neurons were rarely recorded in the cochlear nucleus (CN), instead, dorsal CN (DCN) neurons with lower CF (<30 kHz) selectively responded to ultrasonic vocalizations (USVs). One prevailing explanation to such phenomenon is that the DCN neurons are actually evoked to the intermediate distortion products generates by the frequency jump of multi-components USVs. This hypothesis, however, cannot interpret how a neuron possesses distinct selectivity on pure tone and complex vocalizations.
Methods: Here we used multichannel recording of cochlear nucleus in awake 2-month female CBA/J mice to simultaneously measure multiple CN areas. Multi-unit activities (MUA) were recorded using 16 or 64 channel silicon probes, which were later sorted to single unit activities (SUA). Our aim is to find a local circuitry that may contribute to such selectivity through simultaneous measurement of multi neurons. By separating components of multi-components USVs, we tested whether component selectivity or intermediate distortion products contributes to USV perception in the level of CN. Boltzmann model was used to mimic cochlear nonlinearity and its input to CN. Linear prediction was made to predict response to USV based on pure tone receptive field.
Results: Preliminary results show that in awake mice, DCN responds heterogeneously to USVs, while VCN shows homogenous response to USVs that can be well predicted by their receptive field. In DCN, while large portion of neurons show CF below 50 kHz, some type III units with CF ~ 30kHz appear inhibitory response to USVs. Intriguingly, a few neurons are responsive to USVs while having CF below 30 kHz or no clear CF. The summation of MUA to decomposed USVs and the MUA to original USVs were similar, despite some neurons fire actively to the onset of USV components.
Conclusions: These results indicated that in mice, vocalization selectivity emerges as early as in DCN but not in VCN. Our data showed that frequency jump induced multi-components or intermediate DPs contribute little to DCN processing. Therefore, DCN selectivity deserves additional research. In the future, multichannel SUA interrogated from communication-deficient mouse models are necessities to address these puzzling questions.

MO60. Serotonergic Modulation of Medial Olivocochlear Neurons
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Category: Brainstem: Structure and Function
Background: Medial olivocochlear (MOC) efferent neurons are located in the superior olive of the brainstem and form a sound evoked feedback loop that inhibits cochlear amplification via suppression of OHC electromotility. Previous work has identified a functional role for MOC neurons in protection from acoustic trauma, and signal extraction in noisy environments. In addition to primary afferent input via T-stellate neurons of the cochlear nucleus, MOC neurons receive additional auditory input from the auditory cortex, inferior colliculus and medial nucleus of the trapezoid body and are putatively modulated by synaptic inputs from non-auditory brain regions. Given the proposed role of these neurons in context dependent tasks, we are interested in investigating the non-auditory modulation of MOC activity. Serotonin (5-HT) has been identified as a potential neuromodulator in central auditory circuits, however there is a scarcity of physiological data addressing the effect of 5HT on intrinsic response properties of MOC efferent neurons. Thus, the question of how serotonin affects hearing via effects on MOC neurons remains to be answered.
Methods: Here we use the previously characterized ChAT-IRES-Cre;tdTomato mouse model for identification of cholinergic MOC neurons. To anatomically demonstrate the existence of serotonergic terminals in close apposition to MOC efferent neurons we combined retrograde tracer injections into the cochlea with immunohistochemistry for anti-5-HT or anti-TPH2 (tryptophan hydroxylase 2, the rate limiting step in serotonin synthesis). To characterize the effect of this putative serotonergic synapse, in-vitro patch clamping methods were coupled with exogenous application of 100µM serotonin. To investigate endogenous release of 5-HT from pre-synaptic axons, we will use optogenetic stimulation of serotonergic neurons and terminals while recording post-synaptic responses in identified MOC neurons (ChAT-IRES-Cre; tdTomato; TPH2-ChR2-EYFP mice).
Results: Immunohistochemical data have validated the existence of serotonergic terminals in close apposition to both retrogradely-labeled and genetically-identified MOC neurons in mouse. During patch-clamp recordings from identified MOC neurons, application of 5-HT increased neuron excitability as measured by increased action potential firing rate and decreases in both rheobase and AP threshold,

indicating post-synaptic 5-HT receptors and suggesting the presence of a serotonergic synapse. Additionally, in the presence of serotonin, less stimulation is required to evoke a given action potential rate in MOC neurons. Experiments investigating activation of MOC neurons by endogenously released serotonin using optogenetic stimulation of serotonergic terminals are currently under way.
Conclusions: Our data demonstrates that serotonin plays a role in modulating MOC neuron excitability in vitro. Current experiments will enable us to confirm that endogenously released 5-HT from serotonergic neurons underlies this increase in MOC neuron excitability. These data will aid in our understanding of central auditory processing and how factors such as mood and attention are involved in modulating MOC responses in complex listening situations such as in the presence of background noise.

MO61. The Effect of Hearing Loss on Cognitive Function in Subjective Cognitive Decline

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Category: Clinical Otolaryngology and Pathology
Background: Subjective cognitive decline (SCD) is a self-reported cognitive decline without objective cognitive impairment. The relationship between audiometric hearing loss and cognitive function has not been reported in SCD. The purpose of this study was to investigate whether hearing loss affects cognition-related indexes in SCD individuals.
Methods: This is a cross-sectional study that used the baseline data of a multicenter cohort study that monitors clinical progression from SCD to dementia. Individuals aged ≥ 60 who reported cognitive decline but had no objective cognitive impairment on comprehensive neuropsychological tests were recruited. Participants were grouped into the normal hearing (NH) and bilateral hearing loss (HL) groups. The demographics, clinical characteristics, dementia biomarkers, global cognition, questionnaire scores, neuropsychological test scores, and segmental brain volumes from MRI were compared between the groups.
Results: Of a total of 120 participants, one hundred two had normal hearing (n = 57) or bilateral hearing loss (n = 45). There were no group differences in the demographic and clinical data except the age. The biomarkers, global cognition, and questionnaire scores were not different between the groups. The HL group performed worse (the z-score of -0.06) in the Stroop Color Word test than the NH group (0.27) (P = .025). Brain volumetric analysis revealed that the HL group had reduced gray matter volumes in four brain subregions: left temporal pole, left caudal middle frontal gyrus, left hippocampus, and right isthmus of cingulate gyrus.
Conclusions: In SCD, hearing loss exerted an adverse effect on cognitive function, primarily frontal executive function tested in the Stroop task. Hearing loss was also related with gray matter volume reductions in brain subregions, although causality needs further investigation. This study may provide evidence for a potential link between hearing and cognition in SCD, an emerging clinical entity.

MO62. Risk Factors for Bell's Palsy Based on the Korean National Health Insurance Service National Sample Cohort Data

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Category: Clinical Otolaryngology and Pathology
Background: The associations between hypertension, diabetes, and dyslipidemia with Bell’s palsy have been controversial and only a few studies have assessed risk factors for Bell’s palsy based on population-based data. The aim of the present study was to evaluate whether sociodemographic factors such as sex, age, residence, household income, and metabolic diseases such as hypertension, diabetes, and dyslipidemia were risk factors for Bell’s palsy using the National Health Insurance Service National Sample Cohort data of Korea.
Methods: Patients who visited an outpatient clinic more than twice or had more than one admission and received steroid medication under the International Classification of Diseases diagnostic codes for Bell’s palsy from 2006 to 2015 were defined as patients with Bell’s palsy in this study. The associations of sociodemographic factors and metabolic diseases with Bell’s palsy were analyzed with univariate and multivariate Cox proportional hazard regression models.

Results: There were 2,708 patients with Bell's palsy recorded from 2006 to 2015. The average annual incidence of Bell's palsy was 25.9 per 100,000 for 10 years. Multivariate Cox proportional hazard regression analysis indicated that significant risk factors for Bell's palsy were male sex (hazard ratio [HR] = 1.169, 95% confidence interval [CI] = 1.081 – 1.263), advanced age (HR = 1.730, 95% CI = 1.530 – 1.955 [30 – 39 years old]; HR = 2.153, 95% CI = 1.915 – 2.420 [40 – 49 years old]; HR = 2.554, 95% CI = 2.248 – 2.901 [50 – 59 years old]; HR = 2.801, 95% CI = 2.422 – 3.238 [60 – 69 years old]; HR = 2.663, 95% CI = 2.204 – 3.217 [70 – 79 years old]; HR = 2.278, 95% CI = 1.576 – 3.293 [80 years old or older]), residence in regions other than the capital and metropolitan cities (HR = 1.182, 95% CI = 1.067 – 1.309), hypertension (HR = 1.362, 95% CI = 1.208 – 1.535), and diabetes (HR = 1.579, 95% CI = 1.347 – 1.851).
Conclusions: Male sex, advanced age, residence in a location other than the capital and metropolitan cities, hypertension, and diabetes were significant risk factors for Bell's palsy based on the population-based data. This study is significant for patients and providers because we analyzed the relationships using a population-based database over a long-term follow-up period.

MO63. Renin Angiotensin System Inhibition and Decreased Incidence of Cochlear implantation: A Cross-Sectional Analysis Among Hypertensive Patients

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Category: Clinical Otolaryngology and Pathology
Background: Background: Up to 1 in 12 people worldwide is estimated to suffer from hearing loss, with 38 million Americans among them. Currently approved therapy for hearing loss in all its varieties is limited to sound amplification or surgically implanted cochlear devices. Age related hearing loss, or presbycusis, has been associated with cardiovascular disease, with hypertension as a predictor of faster rates of hearing decline. Impairments in cochlear microcirculation and perfusion have been identified in patients with presbycusis and in preclinical models of noise induced hearing loss. RAS targeted treatments have system-wide vasoprotective effects with the potential to protect microcirculation within the inner ear, such as in the stria vascularis and spiral ligament, and thus preserve hearing in clinical conditions with significant vasculopathic contributions, such as hypertension.
Methods: Methods: To establish the RAS as a therapeutic target, we have queried the NIH's AllofUs database to identify previously unexplored associations of RAS blockade and hearing loss among patients with hypertension. Two groups of hypertensive patients were compared: one wherein patients were treated with either angiotensin converting enzyme inhibitors or angiotensin receptor blockers (ACEi/ARB) and a second group of patients treated with calcium channel blockers or beta blockers (CCB/BB). These two groups were compared for frequency of diagnosis codes indicating cochlear implantation.
Results: Results: Our data from a sample of 33,609 hypertensive patients show a trend toward statistically significant association of RAS blockade with ACEi/ARBs and decreased frequency of cochlear implantation (n = 7 out of 10,980) versus treatment with CCB/BB (n = 22 out of 22,629; chi square 0.96, p = 0.16), with a relative risk reduction of 34%. There was no association of hypertensive treatment (ACEi/ARB vs CCB/BB) and frequency of diagnosis of SNHL.
Conclusions: Conclusions: These findings suggest that among hypertensive patients, treatment with RAS blockers may be associated with hearing preservation, as ACEi/ARB treatment tended toward reduction of risk of cochlear implantation by as much as one third, despite both groups having similar baseline frequencies of diagnoses of sensorineural hearing loss. This finding has significant clinical implications for selection of appropriate anti-hypertensive regimen among hypertensive patients with known hearing loss or those who are at risk for hearing loss.

MO64. 2D Measurements of the Angle of the Vestibular Aqueduct Using CT Imaging

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Category: Clinical Otolaryngology and Pathology
Background: Recently, two categories of Meniere's Disease—hypoplastic and degenerative—have been reported based on differences in pathology involving the vestibular aqueduct.1 Bachinger et al. developed software that measures the angle between the vestibular aqueduct proximal to the vestibule and the distal vestibular aqueduct on axial computed tomography (CT) scans and found differences in the vestibular aqueduct angle between the hypoplastic and degenerative categories of Meniere's Disease.2 Patients with

Meniere's Disease and a hypoplastic vestibular aqueduct had vestibular aqueduct angles >140°. Patients with Meniere's Disease and degenerative changes as well as normal adults had angles <120°. Having established that hypoplastic and degenerative Meniere's Disease categories can be diagnosed by a measured angle, they published additional work using the angle of the distal vestibular aqueduct (aexit) as a diagnostic measurement to define hypoplastic and degenerative Meniere's Disease categories and to form the basis for further study of those two groups.3 The purpose of this study is to examine whether the software developed by Bachinger et al. as a diagnostic tool for categories of Meniere's Disease can be applied to a large dataset of adult patients with varied otologic diagnoses.
Methods: Adult patients who underwent high resolution flat panel CT scans without intravenous contrast for otologic indications (n=301) were retrospectively reviewed. Measurements of the angle of the vestibular aqueduct were made in the axial plane aligned with the lateral semicircular canal. Software developed by Bachinger et al. to measure the angle of the distal vestibular aqueduct aexit was used. Non-parametric test statistics were used to compare angles of the vestibular aqueduct by medical diagnosis.
Results: 218 patients had angles aexit <120°, 76 patients had angles 120°< aexit >140°, and in 7 patients, aexit >140°. Only one of the 7 patients with aexit >140° had a diagnosis of Meniere's Disease and not in the ear with aexit >140°. None of these patients had Meniere's Disease like symptoms that were not attributable to another pathologic process. No significant differences in the aexit medians and interquartile ranges of patients with Meniere's Disease, Anatomic Semicircular Canal Dehiscence without superior semicircular canal dehiscence syndrome (SCDS), and SCDS were found ($p>0.05$).
Conclusions: Most of the vestibular aqueduct angles measured in this study fell into the Bachinger et al. category for normal adults and degenerative Meniere's Disease, aexit <120 degrees. However, a few patients had measurements that fit the Bachinger category for only hypoplastic Meniere's disease (aexit >140°), and none of these had a diagnosis of Meniere's in the ear that met aexit criteria for hypoplastic Meniere's. These results suggest that although generally effective, 2D measurements of the vestibular aqueduct may face limitations in a population of individuals with vestibular symptoms who undergo CT imaging.

MO65. Comparison of Endoscopic Myringoplasty With and Without Middle Ear Packing for Repairing Chronic Perforation

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Category: Clinical Otolaryngology and Pathology
Background: This study was performed to compare the efficacy of the endoscopic modified cartilage over-underlay technique with and without middle ear packing for repairing chronic tympanic membrane (TM) perforations.
Methods: A total of 70 cases of chronic TM perforation were randomly allocated to endoscopic cartilage over-underlay myringoplasty groups with (n = 35) and without (n = 35) middle ear packing (Fig.1). 0.3% ofloxacin eardrops (2-3 drops, twice daily) were applied daily for 4 weeks in no packing group. The external auditory canal was packed with gauze soaked in erythromycin ointment up to the tragus incision, which was not sutured. The graft success rate and hearing outcomes were compared between the two groups. In addition, neovascularization scores were subjectively obtained.
Results: At 12 months postoperatively, the difference in graft success rate between the packing (Fig.2) and no-packing groups was not significant (94.3% vs. 100.0%, P = 0.473). In addition, there were no significant differences between the two groups in the pre- or postoperative air–bone gap (ABG) (15.18 ± 2.73 vs. 15.07 ± 4.02, P = 0.623 and 8.63 ± 3.03 vs. 8.52 ± 4.50, P = 0.591) or mean ABG gain (6.56 ± 3.23 vs. 6.54 ± 2.83, P = 0.751). However, the average operating times were 43.6 ± 7.1 and 32.7 ± 2.1 minutes in the packing and no-packing groups, respectively (P < 0.001).
Conclusions: Surgical and hearing outcomes were comparable between patients with chronic TM perforation treated using the endoscopic over-underlay technique with and without middle ear packing. However, without packing, the procedure was less invasive and had a shorter operating time.

MO66. Patient Reported Hearing Loss in Facioscapulohumeral Muscular Dystrophy (FSHD)

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Mul[8], Perry Shieh[9], Elena Carraro[5], Leo Wang[10], Russel Butterfield[11], Nicholas Johnson[12], Maya Hatch[13], Jay Han[13], Michael McDermott[3], Rabi Tawil[3], Jeff Statland[4]
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Category: Clinical Otolaryngology and Pathology
Background: Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common forms of muscular dystrophy affecting more than 870,000 people worldwide. The most common form of FSHD, FSHD type 1, is caused by contraction of D4Z4 repeats in the subtelomeric region of chromosome 4 with a permissive allele. This leads to aberrant expression of the transcription factor DUX4. High frequency hearing loss is a systemic manifestation of FSHD which has been less well characterized. Previous studies have shown that hearing loss is more prevalent in FSHD patients with large D4Z4 repeat deletions. Our objective was to determine whether there is an association between patient reported hearing problems and FSHD outcome measures.
Methods: We analyzed data from a large international observational study run by the FSHD Clinical Trial Research Network which included patient reported data, motor assessments, and quality of life measurements.
Results: We included 219 patients in the analysis. 47 patients (21%) reported hearing problems. Univariate analysis did not find an association between patient reported hearing problems with sex, age of symptom onset, D4Z4 repeat size, disease severity or quality of life. Rates of hearing problems were the same in early onset and typical onset FSHD patients.
Conclusions: These data point to the importance of further characterizing hearing loss in FSHD and uncovering patient perspectives surround hearing loss.

MO67. 4-D Vocal Fold Imaging and Motion Reconstruction Using Optical Coherence Tomography
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Category: Clinical Otolaryngology and Pathology
Background: Laryngeal videostroboscopy, the current gold standard for diagnosing dysphonia, is limited to 2-dimensional vocal fold (VF) surface visualization without subepithelial visualization, leading to subjective diagnostic and therapeutic decisions. Optical Coherence Tomography (OCT) is a medical imaging technology that could meet the need for accurate evaluation and treatment of laryngeal disorders by allowing better measurement of the VF's multilayered structure and 3-dimensional mucosal wave during phonation. Previous OCT imaging studies reported challenges related to imaging speed, sub-Nyquist sampling of the human voice fundamental frequency (90-260 Hz), intolerance to trans-oral endoscopy, and motion artifact. We aim to demonstrate OCT's accuracy and effectiveness in laryngology by developing a clinically oriented hand-held probe and 4-D visualization scheme via direct Nyquist sampling of the VF microscale morphology and vibration in vivo. Our OCT system's imaging speed will make 4-D (x,y,z,t) reconstruction of the VF oscillation possible and allow observation of the 3-D morphology with high temporal resolution.
Methods: A custom hand-held swept-source OCT system was designed to match the unique demand for VF functional imaging. Depth of focus, resolution, variance in VF distance, and field of view were all considered. A laser swept at 200 kHz and centered at 1.31 μm could provide 15 μm axial resolution in tissue using a new multi-window approach. The lateral resolution was 106 μm with a maximum imaging depth of 12.6 mm. A healthy volunteer was imaged during vowel /i/ phonation. Stabilization was maintained by incorporating a chin rest mounted to a clamped optical breadboard and establishing a two-hand approach for holding the OCT hand-held device. To obtain volumetric data, multiple B-scans per glottal cycle were captured by scanning a MEMS mirror at 1.33 kHz on the fast axis and .33 Hz on the slow axis. A novel

algorithm that takes advantage of the cyclic nature of the mucosal wave was developed to reconstruct highly sampled volumetric images of one cycle.
Results: The fast-scanning OCT laryngoscope and reconstruction algorithm were evaluated by acquiring volumetric data from the VFs of a healthy volunteer during phonation. With the developed algorithm, the glottal cycle was reconstructed for each dataset, demonstrating that the periodic motion of the VFs can be reconstructed using the proposed scheme. In each reconstructed sequence, the vocal fold layers are visible throughout the sequence. Reconstruction of the glottal cycle was achieved with as few as 20 B-scans with the condition of negligible motion artifact during a constant phonation frequency.
Conclusions: The OCT laryngoscope system and developed reconstruction algorithm offer the potential for 4-D imaging of the phonating VFs in the clinic. 4-D reconstruction of VF vibration may enhance diagnostics and treatment of VF mucosal diseases. In the future, incorporating motion correction will help improve OCT data acquisition and reconstruction.

MO68. Vertigo in Pregnant Patients Attended at a Tertiary Center in Colombia

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Category: Clinical Otolaryngology and Pathology
Background: During pregnancy several physiologic, psychologic, and anatomic changes are widely reported, however, there is a lack of information about detailed symptoms that may affect hearing and balance such as vertigo, unsteadiness, and dizziness. Hormones may be involved in the presentation of vestibular disorders during pregnancy, and changes to proprioception, vision and to the ear would lead to altered quality of life and disability. To date, there are few studies reporting how vertigo is observed during pregnancy, its frequency, onset, and duration. Little is known about most common type of vertigo during pregnancy and the most affected gestation weeks. Herein, we described the epidemiologic and clinical characteristics of pregnant patients with vertigo in one tertiary center in Colombia.
Methods: A cross-sectional study done was conducted at Clinica Universitaria Bolivariana -an affiliated clinic to Universidad Pontificia Bolivariana between November 2021 to April 2022. 102 pregnant patients were interviewed at the Ob-Gyn Department. A questionnaire including demographic, obstetric and ENT history was performed. Questions were focused on onset, frequency, type of vertigo and dizziness handicap inventory. Descriptive statistics including IQR and relative and absolute frequencies were obtained for quantitative analysis.
Results: At Clinica Universitaria Bolivariana the prevalence for vertigo in pregnant females was 19.4%. 60% of patients reported frequent episodes of vertigo during the second trimester with a median of 3 (IQR 2-5). At least 40% of females presented vertigo in previous pregnancies, in these cases, no treatment was done. Pregnant females presented dizziness before vertigo episodes in 20% of the population studied. Postural changes were the most common trigger (70%) of vertigo, followed by food consumption (chocolates, pickles, tuna, ham, cheese, and Chinese food). The most common concomitant symptoms of vertigo were unsteadiness and headache (60%), and tinnitus and nausea (45%). Short episodes of vertigo were mostly seen in this population. No falls were reported for pregnant females.
Conclusions: Short episodes of vertigo triggered by postural changes and food consumption concomitant to symptoms such as unsteadiness and headache were mostly seen in females during their second trimester. These episodes may affect the quality of life of pregnant patients and further vestibular testing and follow-up is suggested for Ob-Gyn Physicians and Otolaryngologists.

MO69. Open Board

MO70. Laminin Stimulates Schwann Cell De-Differentiation via β1-Integrin Receptors to Facilitate Cellular Outgrowth in Spiral Ganglia Cultures

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Category: Development: Cellular/Systems
Background: Laminin is an extracellular matrix component that is known to act on various integrin receptors, composed of alpha and beta subunits, promoting cell motility, survival, and proliferation. Laminin

has been associated with increased neurite outgrowth in spiral ganglia cultures in vitro, which may have potential translational applications as a cochlear implant electrode coating. However, the mechanism of this cellular growth has not been fully characterized. Schwann cells, which can assume different states, likely play a role in promoting spiral ganglia neuronal extension. This study aims to understand the mechanism by which laminin stimulates spiral ganglia cellular migration and outgrowth to optimize future formulations of cochlear implant electrode biological coatings.
Methods: Adult rat Schwann cells were incubated with control media, laminin (10ug/ml), β1-Integrin antibody (1ug/ml), or both conditions together for 2 hours. Immunohistochemistry and capillary gel electrophoresis were subsequently performed to quantify glial fibrillary acidic protein (GFAP) expression, or Schwann cell de-differentiation, under these conditions. Then, adult rat Schwann cells and rat spiral ganglia were plated in two-well insert dishes. Migration assays were performed when cells were co-cultured with: (1) control (laminin base coating, 5ug/ml), (2) control + β1-integrin antibody (1ug/ml), (3) laminin (high density laminin coating, 1.1 mg/ml), and (4) laminin + β1-integrin antibody (1ug/ml). Immunohistochemistry staining for Tuj1 (neuronal cell marker), S100 (Schwann cell marker), and/or GFAP was performed. Confocal images were obtained, and Schwann cell migration and neurite extensions were quantified with ImageJ software (n=5 per condition).
Results: Application of β1-Integrin antibody to adult rat Schwann cells decreased laminin-induced GFAP expression. In migration assays, β1-Integrin antibody significantly decreased laminin-induced Schwann cell migration. These findings suggest that blockade of the β1-Integrin receptor may decrease Schwann cell dedifferentiation, and thus cellular movement. With primary cultures obtained from rat spiral ganglia, laminin significantly increased the number of neurite extensions into the gap toward laminin coating. Although β1-Integrin antibody decreased the mean number of laminin-induced neurite extensions, the difference in means was not significant. Interestingly, laminin coating also promoted alignment and survival of Schwann cells and neurons while treatment with β1-Integrin antibody was associated with more neuronal disorganization.
Conclusions: Laminin may induce Schwann cell de-differentiation and migration through activation of β1-Integrin receptors, which in turn initiates spiral ganglia neurite outgrowth. This mechanistic study highlights important pathways that may be targeted in future studies of biological coatings or medications that may be used with cochlear implantation to improve hearing outcomes. Additional studies optimizing laminin formulations for clinical use and investigating its effects on hearing outcomes in vivo are warranted.

MO71. Classification and Maturation of Astrocytes in the Medial Nucleus of the Trapezoid Body
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Category: Development: Cellular/Systems
Background: Several defining features of mature astrocytes have been identified using electron microscopy, including endfoot processes that contact blood vessels as an element of the blood-brain barrier, fingerlike projections that interpose between pre- and post-synaptic membranes to form the tripartite synapse, large mitochondrial length, and prevalent stacks of endoplasmic reticulum (ER). However, it has not been established when these defining characteristics are acquired in maturing astrocytes in the developing brain. During early brain development, the brain is growing, vascular networks are expanding, and competition occurs between neural inputs for synaptic targets. We investigated the change in astrocyte cellular structure during this period. The medial nucleus of the trapezoid body (MNTB) is a useful model system for studying neural circuit development due to the rapid maturation of a nearly homogenous neuronal population and its transition from multi-innervation to mono-innervation by the largest terminal in the mammalian brain, the calyx of Held (CH).
Methods: We utilized our unique developmental series of serial block-face scanning electron microscopy (SBEM) image volumes to observe the change in the astrocyte morphology. Seven criteria were chosen to evaluate the diversity of cell types and rated by three graders for all glial cells in the postnatal day (P)4 and P6 volumes. We then employed a random forest classifier to determine the utility of each criterion for cell type identification. To visualize the morphological features of astrocytes at an ultrastructural level during CH development, we have completed the first full reconstructions of astrocytes at P2, P4, P6, and P9.
Results: In all cells, we observed the ultrastructural features of large mitochondrial length and stacks of ER found in mature astrocytes. We noted several novel morphological features, including large mitochondrial

width, sheet-like vellus processes, and axonal ensheathment, that could be used for cell type identification. We are further evaluating the strongest criterion, mitochondria, as a classification by autosegmentating, using a U-net, all mitochondria within an astrocyte boundary. An initial comparison of the reconstructions reveals the presence of mature astrocytic features, such as endfeet and contacts with nerve terminals, from the earliest age. Our novel observation of the thin vellus processes covering large areas on MNTB cell bodies was found at all ages. Additionally, we observed these thin vellus processes on the CH after the onset of growth. We observed novel wrapping of large caliber axons by astrocytic processes at P4 and P6, before the onset of myelination at P9.
Conclusions: This study highlights the complex morphology of astrocytes while establishing the presence of mature features during development. By identifying novel ultrastructure morphological features, we have expanded the criteria for future cell identification. Furthermore, our developmental series of reconstructions provide insight into the elaborate process of astrocyte maturation.

MO72. erbB4 is Required for Normal Hearing

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Category: Development: Cellular/Systems
Background: During the development of the inner ear, cells undergo cycles of proliferation followed by differentiation to create the highly organized structures necessary for proper hearing. Defects in these processes can lead to hearing loss. The epidermal growth factor receptor family members erbB tyrosine kinases have been shown to regulate cellular proliferation, differentiation and programmed cell death. Specific spiral ganglion expression of a dominant negative form of ErbB4 (that can interact with other erbB receptors), has shown that ErbB signaling regulates myelin development in the auditory nerve as well as the survival of spiral ganglion neurons. But immunohistochemical analysis has shown that erbB4 has a more widespread expression in the inner ear with positive labelling reported in the hair cells, supporting cells, spiral ganglion, lateral wall, and stria vascularis (Zhang et al., 2002).
In this study, to understand the broader role played by ErbB4 in the inner ear development and function, we decided to work with a complete ErbB4 null model in which erbB4 expression is maintained only in the heart to avoid embryonic lethality (Tidcombe et al 2003).
Methods: We recorded auditory brainstem response (ABRs) and endocochlear potential (EP) in 1 month old ErbB4-/- HER4heart mice and their wildtype or heterozygous littermates. To analyze the role of ErbB4 in cell proliferation, we injected EDU at E16.5, P0 and P6 and we will characterize the level of proliferation of the mutant versus WT mice. We will also quantify the survival of the stria vascularis cells, hair cells, as well as the spiral ganglion neurons.
Results: ErbB4-/- HER4heart mice exhibit a higher hearing threshold compared to WT or Heterozygous littermates. We also noticed a slight increase in endocochlear potential, which could show that erbB4 is necessary either for proper function of the stria vascularis or for the survival of the organ of Corti cells. Our preliminary data suggest that ErbB4 plays a role in hair cell survival.
Conclusions: Further analyses are necessary to elucidate the specific role played by ErbB4 in inner ear development and function, but our preliminary data showed that the physiological defects detected in the full KO might arise from other cell types than previously reported.

MO73. Modulation of Spontaneous Neural Firing in the Prehearing Cochlea by SEMA5A

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Category: Development: Cellular/Systems
Background: In the pre-hearing cochlea, the ATP release from the cochlear epithelium and subsequent excitation of hair cells followed by glutamate release result in spontaneous firing of spiral ganglion neurons (SGNs). The spontaneous firing pattern during embryonic stages appear infrequent and uncoordinated, but become more frequent and coordinated by early postnatal stages. The molecular factors and mechanisms that initiate and refine the nature of spontaneous activity in SGNs are not well known.
Semaphorins, well known for their role in axon guidance, are secreted and membranous proteins that are reported to have a role in synaptogenesis and regulating neuronal membrane excitability. Several semaphorins are expressed in the developing cochlea and control different aspects of neural circuitry

development. In this study, we explored the role of semaphorin-5A (SEMA5A) in cochlear neural circuit development, and have discovered that it regulates SGN spontaneous activity.
Methods: In-situ hybridization was performed to determine the expression of Sema5a in the developing cochlea. To examine the effect of semaphorin on spontaneous activity, cochleae with Snap25-GCaMP6s-expressing SGNs from postnatal day 2 (P2) mice were cultured briefly then recorded by time lapse imaging in the presence of SEMA5A-Fc protein. The movies were recorded immediately and ten minutes after the application of the recombinant protein. A baseline activity was recorded prior to the application of the protein. The data were analyzed to compare the effects of SEMA5A on frequency and coordinated activity changes. Spontaneous activity in SGNs was also evaluated in Sema5a-/-;Snap25-GcAMP6s+ mice at P2. Immunostaining was performed to evaluate innervation patterning and activity dependent molecular markers in Sema5a-/- cochlear tissues.
Results: In-situ hybridization results show a wider distribution of Sema5a in SGNs and mesenchyme in developing cochlea at P0. SEMA5A-Fc protein had dramatic effects on the spontaneous firing of SGNs of cochlear explant immediately after application: the area of activity, frequency, and coordinated events were all significantly reduced compared to baseline data, whereas application of control IgG had no effect. Although the activity resumed after ten minutes (in constant presence of SEMA5A-Fc) with increased active area, the overall frequency and coordinated events were comparable with the baseline activity. Analysis of cochlear explants from Sema5a-/-;Snap25-GcAMP6s+ animals showed an increased fluorescence level of Calcium signal compared to wild-type samples. Preliminarily, we observed increased fluorescence level of activated CREB in SGNs and cochlear epithelium of Sema5a-/- samples further suggesting an increase in spontaneous activity in Sema5a-/- animals. Supporting these findings, we also observed reduced terminal branches of type I SGNs in Sema5a-/- suggesting a homeostatic mechanism.
Conclusions: Our data indicate the possible role of SEMA5A in regulating spontaneous activity in SGNs during early cochlear development. In ongoing work, we are investigating the potential mechanisms by which SEMA5A achieves this.

MO74. Anatomical and Physiological Development of Octopus Cells Before and After Hearing Onset in Mice

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Category: Development: Cellular/Systems
Background: Octopus cells (OCs) in the posteroventral cochlear nucleus (PVCN) are known for their sensitivity to the temporal components of sound, and for their large dendrites which spread perpendicularly across tonotopically arranged auditory nerve fibers (ANFs). During development, form and function must be refined together to specialize OCs for speed. To understand how the OC circuit develops, we paired data about changes in electrophysiological properties with the parallel refinement of OC dendrites.
While OCs reach full soma size at ~P12 in mice and in kittens, immature OCs have growth cones and filopodia emanating from dendrites. However, little information exists about the development of dendritic morphology with respect to the onset of hearing, and whether sound-evoked activity is necessary for the acquisition of adult morphology. The influence of sound-evoked activity on physiological maturation is also unknown. Adult OCs encode precisely timed inputs from ANFs in part due to hyperpolarization-activated conductances and low-voltage depolarization-activated potassium conductances. These conductances contribute to low input resistances (5-10MΩ) and fast time constants (200μs). In the developing mouse, these conductances have not been thoroughly characterized, although in vivo recordings in kittens show immature firing during early development.
Methods: OCs in the mouse (P6-18) were sparsely labeled using a Thy1-YFP-H line. We made 100μm parasagittal sections of the OC area and collected confocal images of optically cleared tissue. Reconstructions of OC dendrites and synaptic inputs were made in Imaris to quantify morphological changes in soma size, dendritic diameter, number of branch points, and synaptic density. We also targeted OCs for in vitro whole-cell current clamp recordings. We examined passive membrane properties over development by quantifying membrane time constants and input resistances. Changes in spike properties over development were primarily visualized using phase plots and quantified with measurements of height and half-width.
Results: The onset of hearing is correlated with anatomical and physiological changes in OCs in the PVCN. Pre-hearing OCs have full-sized somas and stereotypical unipolar dendritic orientations. However, unlike

mature OCs, the terminal dendrites have more complex dendritic branching and are covered in filopodia. These results support the hypothesis that there is dendritic pruning after hearing onset. Immature OCs (P6-P11) show surprisingly fast membrane properties. In addition, both spontaneous and evoked synaptic inputs from auditory nerve fibers have similar temporal dynamics as inputs recorded in adults (P35-50). Ongoing experiments will examine the refinement of ANF collaterals and changes in the density and location of glycinergic synaptic inputs.
Conclusions: Although the morphological and electrophysiological properties of adult OCs are well known, when these adult-like properties emerge is not well understood. Our anatomical reconstructions and electrophysiological recordings, collected from both pre- and post-hearing mice, provide insights into the role of auditory experience on development and refinement of the temporally precise OC circuit.

MO75. The Atypical Cadherin Fat3 is Required for Proper Hearing

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Category: Development: Cellular/Systems
Background: Fat4 and other tissue polarity proteins play a central role in the proper alignment of hair cells in the Organ of Corti. Previously, we showed that Fat3, another member of the Fat family of proteins known for their effects on tissue polarity in classical epithelial systems, also influences neural circuit polarity. Fat3 contributes to proper laminar organization of the vertebrate retina – and thus the proper flow of visual information – by regulating cell morphology and migration, as well as synapse formation and localization of amacrine cells. Our preliminary studies also show that in the absence of Fat3 vision is impaired in mice. This effect on the visual system seems to be a consequence of loss of Fat3 in bipolar cells, which show reduced ribbon synapses with photoreceptors in the mutants, possibly due to a change in the localization of synaptic components. In support of this idea, the Fat3 intracellular domain binds to multiple synapse scaffold proteins, including PSD95. Here, we aim to determine whether a similar sensory function is played by Fat3 in the auditory system and whether the ribbon synapses of the inner hair cells and the spiral ganglion neurons can serve as a model to study synapse localization.
Methods: To corroborate the expression and localization of Fat3 in the inner ear, we performed immunohistochemistry in wild type tissue and tissue from animals where the Fat3 protein is fused to GFP. We assessed the sense of hearing by performing Auditory Brainstem Response (ABR) tests in wild type and Fat3-deficient mice. To evaluate synapse formation and localization, we stained whole-mount cochlea preparations for synaptic proteins and detected the signal by confocal microscopy.
Results: Our immunohistochemical assays show that Fat3 is expressed in spiral ganglion neurons during early postnatal development. Upon removal of the Fat3 gene from mice, the ABR tests show that the wave I amplitude is significantly diminished. To understand the cellular origin of this response, we analyzed ribbon synapse formation and localization using whole-mount cochlea preparations. Our stainings of pre- and post-synaptic proteins (GluA2 and CtBP2, respectively) show that synapses form in Fat3 mutants. We are currently analyzing the synaptic localization pattern in more detail.
Conclusions: Our results show that the atypical cadherin Fat3 is expressed by spiral ganglion neurons that form ribbon synapses with inner hair cells. In addition, Fat3 is required for proper auditory function in mice. The connections that form between spiral ganglion neurons and inner hair cells provide a good system to study synapse formation and localization after perturbation of gene expression.

MO76. Ebf1 Suppresses the Proliferation of the Cochlear Prosensory Domain and Forms the Scala Tympani and Spiral Limbus during the Development of the Inner Ear

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Category: Development: Cellular/Systems
Background: Early B-cell factor 1 (Ebf1) is a member of the Ebf family, a basic helix-loop-helix (bHLH) transcription factor, which is highly conserved in all metazoans. Ebf1 is essential in differentiating various tissues, including B-cells and adipocytes, and is a tumor suppressor. Although the development of the inner ear depends on various bHLH transcription factors, previously reported bHLH transcription factors do not explain the whole mechanisms of inner ear development. Our in silico analysis of the single cell RNAseq data suggested that Ebf1 is expressed in the prosensory domain of the developmental inner ear, especially in

the cochlea (Yamamoto R et al., 2021). Therefore, we decided to investigate the function of Ebf1 during inner ear development.
Methods: We examined the spatiotemporal expression pattern of Ebf1 using in situ hybridization (ISH) and immunofluorescence (IF), the expression level of Ebf1 using quantitative RT-PCR (qPCR), and the inner ear morphology of Ebf1-deleted mice. ISH, IF, and qPCR studies were performed in ICR mice from embryonic day9.5 (E9.5) to post-natal day0 (P0). We used conventional knockout mice (Ebf1-/-) and Foxg1-mediated conditional knockout mice of Ebf1 (Foxg1cre; Ebf1fl/fl) as Ebf1-deficient mice. Morphological analyses of Ebf1-knockout mice included the cochlear length measurement, hair and supporting cell number count, the evaluation of various differentiation markers, and cell proliferation and apoptosis assays. EdU and cleaved caspase 3 were used to perform cell proliferation and apoptosis assays, respectively.
Results: The Ebf1 expression started at the ventral and lateral side of the otocyst at E10.5 and continued in the progenitor cells of the cochlea, macula, and crista and the spiral ganglion. In addition, the expression of Ebf1 was observed in the mesenchyme surrounding the inner ear epithelia from E9.5. The expression level of Ebf1 in the inner ear is maximized at E13.5 when the cochlear prosensory domain is established. Ebf1 deletion results in a slight decrease in cochlear length and incomplete formation of the spiral limbus and scala tympani at E18.5 cochlea. The development of the scala tympani in Foxg1cre; Ebf1fl/fl mice was normal, suggesting the involvement of mesenchymal Ebf1 in the scala tympani formation. More prominently, an increase in the cochlear hair and supporting cell numbers and an expansion of Kölliker's organ were observed. In the E13.5 cochlea of Ebf1-/-, cell proliferation was enhanced in the cochlear prosensory domain, although apoptosis was not inhibited, indicating that Ebf1 controls the proliferation of the cochlear prosensory domain. The timing of Atoh1 expression in the cochlea was delayed in Ebf1-/-.
Conclusions: The spatiotemporal expression of Ebf1 and morphology of Ebf1 deficient embryonic cochleae suggested that Ebf1 suppresses the proliferation of the cochlear prosensory domain and contributes to the formation of scala tympani and spiral limbs.

MO77. The Role of SHANK2 in the Patterning Stereociliary Bundles in Cochlear Hair Cells

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Category: Development: Cellular/Systems
Background: Auditory hair cells of the mammalian cochlea have a staircase arrangement of the stereocilia filled with actin filament on the apical surface. During development, the kinocilium, the primary cilium of hair cells, moves from the center to the lateral pole. And the V-shaped stereociliary bundles of outer hair cells have a vertex pointing to the kinocilium. This machinery is referred to as cell-intrinsic polarity. The establishment of the intrinsic polarity is regulated by GPSM2 and Gαi, which are located in the lateral domains of the apical surface of hair cells. However, the potential players in the medial domain remain largely unknown. In addition, the role of asymmetrical hair bundles in hearing function is difficult to determine because proteins regulating intrinsic polarity also play other roles such as stereocilia elongation. SHANK2 is a multidomain-scaffolding protein implicated in the structural and functional coordination of multiprotein complexes in the brain. Recently, SHANK2 has been shown to interact with aPKC and play a critical role in the tight junction formation in epithelial cells. We investigated the role of SHANK2 in stereociliary patterning using mouse genetics.
Methods: Temporal and spatial expression patterns of Shank2 were analyzed using in situ hybridization. Proteins related to intrinsic polarity and hair cell orientation were determined using immunofluorescence. Hair bundle morphology was observed using scanning electron microscopy. The hearing function is assessed by measuring auditory brainstem responses and distortion product otoacoustic emissions.
Results: Shank2 mRNAs are expressed in the developing hair cells and spiral ganglion neurons. In the hair cells, SHANK2 proteins are restricted to the medial apical surface. Shank2-/- mice have disorganized hair bundles with normal localization of kinocilium at the lateral pole. Shank2-/- mice suffer from progressive hearing loss, especially at mid-high frequencies. To analyze the cause of hearing loss in Shank2-/- mice, we compared auditory phenotypes of Shank2-/- with hair cell-specific (Gfi1-Cre; Shank2lox/lox) or spiral ganglion neuron (SGN)-specific (Bhlhe22-Cre; Shank2lox/lox) conditional knockout mice. We observed that Gfi1-Cre; Shank2lox/lox mice exhibited almost identical phenotypes of hearing loss and hair bundle defects as Shank2-/- mice. In contrast, stereociliary morphologies and hearing function were unaffected in Bhlhe22-Cre; Shank2lox/lox mice. To understand the molecular mechanisms by which SHANK2 contributes to shaping hair bundles, we conducted yeast-two-hybrid screening with cochlear cDNA libraries

using SHANK2 as a bait and identified several candidate binding partners of SHANK2 in the cochlea. We are currently investigating the role of these candidates in hair bundle patterning using cochlear explant culture.
Conclusions: These results suggest that SHANK2 localized on the medial apical surface of cochlear hair cells plays a crucial role in stereociliary bundle patterning and is essential for hearing function.
Supported by the BK 21 FOUR Project for Medical Science, Yonsei University College of Medicine

MO78. Determining the Roles of Individual Wnt Ligands in Planar Cell Polarity in the Developing Cochlea in Silico and in Vivo

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Category: Development: Cellular/Systems
Background: The planar cell polarity (PCP) pathway organizes cells in tissue planes, utilizing a set of core PCP proteins such as Dvl2 and Fzd6. Hair cells in the cochlea are precisely oriented with hair bundles aligned radially, serving as a prime example where proper development requires the PCP pathway. We previously showed that PCP during cochlear development, including cochlear extension, stereocilia orientation and core PCP protein polarization, is regulated by Wnt secretion from the embryonic cochlear epithelium. However, the specific Wnt ligands contributing to cochlear PCP are still unknown. To test the hypothesis that cochlear PCP requires specific Wnts, we analyzed single-cell RNA-sequencing (scRNA-seq) data and evaluated the role of candidate Wnts with conditional KO (cKO) mice.
Methods: We used the CellChat algorithm (Jin et al., 2021) to analyze publicly available scRNA-seq data from embryonic day (E)14.5 and E16.5 cochlear epithelium tissues (Kolla et al. 2020). This method quantitatively infers and analyzes intercellular communication networks to rank Wnt members predicted to interact with Wnt receptors in the organ of Corti. cKO mice were generated by crossing Emx2-Cre mice with floxed mice for the target Wnts, including Wnt4, Wnt5a and Wnt7b. To assess for abnormalities of cochlear PCP, we analyzed the following at E18.5: 1) cochlear length, 2) hair cell orientation, and 3) polarized expression of Dvl2 and Fzd6 in HCs.
Results: The analysis with the CellChat algorithm inferred that Wnt4, Wnt5a and Wnt7b are most actively used as ligands for Wnt signaling within the developing cochlear epithelium. In cochlea from Wnt4, Wnt5a and Wnt7b cKO mice, we did not detect any significant change in cochlear length or HC rotation compared to control cochleae (unpaired t-test; n=3-5). Polarized expression of Dvl2 and Fzd6 was detected in Wnt4, Wnt5a and Wnt7b cKO mouse cochlea. Together these data suggest that Wnt4, Wnt5a and Wnt7b individually was not required for PCP during cochlear development.
Conclusions: Computational analysis using existing datasets predicted that Wnt4, Wnt5a and Wnt7b are the primary candidate Wnts in the cochlear epithelium regulating cochlear PCP. However, Wnt4, Wnt5a and Wnt7b cKO mouse cochlea showed no abnormal PCP phenotypes. Ongoing experiments involve analysis of double cKO mice for the candidate Wnts.

MO79. Defining the Human Otic Progenitor Niche

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Category: Development: Human Subjects
Background: Inner ear development involves the assembly of cells from a variety of different embryological origins. There is an unmet need for developing a human inner ear model in vitro to mimic both the complex structure and the function of the inner ear outside of the body. Because the human inner ear is difficult to biopsy, a major challenge is to faithfully recapitulate the embryonic development of the inner ear from hPSCs. However, our limited understanding of the microenvironmental niche in which human otic progenitors emerge and develop is a key barrier to progress. This project builds on our previous discovery of a 3D culture system that uses hPSCs to generate inner ear (otic) organoids containing sensory epithelium, neurons, and mesenchymal cells.
Methods: The current inner ear organoid culture protocol contains three stages. In Stage-I, we induce inner ear progenitors by modulating TGF, BMP, and FGF signaling. In Stage-II, the premature otic organoids (otic vesicles) appear after WNT activation. In Stage-III, the organoids mature into multi-chambered cysts

containing sensory and non-sensory epithelium surrounded by mesenchyme. The mature inner ear vesicles usually reside inside solid aggregates, giving rise to technical difficulties in imaging and closely monitoring the developmental process. We constructed new reporter cell lines and novel platforms to better visualize the inner ear organoids, as a tool to understand inner ear developmental biology in a real-time fashion. Drawing upon recent single-cell genomic studies in our lab, we have gained deeper insight into the early progenitors that form otic organoids. We identified a founding population of otic placode/vesicle-like epithelial cells, which are defined by the expression of TBX2, PAX2/8, SOX2, and OC90. However, the off-target cell population, such as epidermis and mesenchymal cells still took a large proportion of the single-cell RNA-Seq clusters. We aimed to characterize a series of surface markers, which could be used to isolate these critical progenitor cell populations for sub-culture and expansion. Another approach was increasing the yield of premature otic vesicles during the early stage of differentiation. We have tested a series of additional treatments to stimulate the expansion of inner ear organoids.
Results: Here, we will present our progress toward a better platform for monitoring the growth of inner ear organoids. We will demonstrate the result of isolating human otic epithelial and mesenchymal progenitor cells using FACS- or MACS-based approaches. In addition, we will outline a test screen to determine the responsiveness of these cells to additional otic patterning factors.
Conclusions: The goal of this project is to elucidate the critical microenvironmental cues that contribute to the expansion, specification, and maturation of otic progenitor cells. Long-term, this defined model of the inner ear will be integral to pre-clinical studies for therapies to treat congenital deafness and balance-related disorders.

MO80. The Development of Temporal Processing and Language Abilities Face Increased Risks From Early, Chronic Otitis Media

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Category: Development: Human Subjects
Background: A controversial issue in pediatric otolaryngology concerns how aggressively to treat otitis media, especially when middle-ear effusion is present. Potential impacts on both auditory (largely temporal processing) and language development have been examined, with mixed results. This study explored those potential impacts from a novel theoretical perspective. Two possible mechanisms of effect were considered: (1) Delayed language development as a consequence of early otitis media could arise from diminished opportunity for hearing the ambient language, due simply to temporarily raised thresholds. (2) Delayed language development could arise due to delayed development of foundational auditory skills, particularly temporal processing, that may interfere with children's abilities to recover critical acoustic detail. If the former, no strong relationship would be expected between language and auditory measures. If the latter, strong relationships would be predicted, and it could be expected that phonological sensitivity would be most strongly impacted because of its enhanced reliance on acoustic detail, compared to vocabulary acquisition; the early lexicon consists of rather holistically structured representations.
Methods: 113 children between the ages of 5 years; 0 months and 10 years, 11 months participated: 68 children had three or fewer episodes of otitis media before 3 years; 0 months of age and 45 children had six or more episodes. Temporal modulation detection thresholds were obtained at two modulation rates (16 and 64 Hz) using transformed up-down procedures. Two language measures were obtained: vocabulary and phonological sensitivity. Age at time of testing was considered to examine developmental changes in both auditory and language measures. Statistical analyses included: t-tests to assess group differences for the four dependent measures (two auditory; two language); correlational analysis to examine pairwise relationships among all variables; and partial correlational analysis to examine how temporal processing abilities contribute to vocabulary knowledge and phonological sensitivity when age is held constant.
Results: Significant group differences were observed for all four dependent measures (auditory and language) with Cohen's ds between 0.41 and 0.61. Correlational analyses revealed strong relationships between each of the two auditory measures and each of the two language measures. When controlling for age, the relationship between temporal modulation detection at 16 Hz and phonological sensitivity remained strongest, especially for children with histories of otitis media, $r = -.48$.
Conclusions: Early, chronic otitis media poses a risk to language development through the first decade of life, especially where phonological sensitivity is concerned. These effects appear due to a combination of diminished opportunity for hearing the ambient language and delayed development of critical auditory skills.

A two-pronged approach to intervention is warranted, designed to mitigate delays in auditory development and support language acquisition.

MO81. Preliminary Screen to Determine Transduction Efficiencies of Various AAVs in Human Inner Ear Organoids

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Category: Gene Therapy

Background: Adeno-associated viruses (AAVs) have become the preferred method for delivering small (< 4.6 kb) genes of interest to specific cell types for both in vivo and in vitro applications. While many gene therapy studies for hearing loss and deafness, especially those in mice, have evaluated a variety of capsid and promoter designs to achieve high transduction efficiencies of murine hair cells and neurons, there has been little work in characterizing the transduction efficiency of these AAV capsids and promoters in human tissue. The inner ear organoid model, bearing human hair cells and neurons of the inner ear derived from human induced pluripotent stem cells, provides a key platform for the study of AAV transduction efficiency in human tissue.

Methods: After generating inner ear organoids from the WA25 cell line, we evaluated a variety of AAV subtypes (AAV1/2/5/8/9) and synthetic AAV vectors (AAV9-PHP.B, AAV9-PHP.S) encoding either green or red fluorescent reporter proteins. The reporter proteins were driven by various promoters (CAG, CMV, or Synapsin). In a preliminary proof-of-concept study, we bathed organoids in equal titers of virus-containing media for 24 hours after induction of otic progenitor tissues during directed differentiation, and then examined the tissues one week after viral treatment. We hypothesized that early transduction of our 3D organoid tissues will allow for adequate penetration depth of viral media to transduce otic progenitor cells that will carry the gene of interest to its differentiated daughter cells.

Results: In this preliminary screen, we find synthetic AAV vectors bearing constitutively expressing promoters such as CAG or CMV are able to broadly transduce many cells of the inner ear organoid, with the intensity of CMV transduction higher than that observed with CAG promoters. There appears to be restricted expression of reporter fluorescence when using neuron specific promoters, but the specificity and transduction efficiency of neurons has not yet been quantified. We have yet to quantify hair cell specific transduction efficiency, but we plan to continue this work in the intervening months.

Conclusions: Alongside the clear therapeutic benefit of delivering genes of interest to their proper cellular targets in the goal of treating patients suffering genetic or acquired hearing loss, there are many basic science applications that will benefit from this work. We hope to expand these results to include transduction efficiency studies in mature organoids and developing organoids. We plan to apply these preliminary results to accelerate our basic science and translational inquiries through delivery of AAVs carrying genetically encoded calcium indicators or proteins of interest for gene therapy evaluation, and aim to have additional preliminary work ready to present at the 2023 ARO MidWinter Meeting.

MO82. A Novel AAV Variant for Selective and Efficient Gene Delivery to the Mammalian Inner Ear Supporting Cells

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Category: Gene Therapy

Background: Recent studies have found adeno-associated viruses (AAV) as promising vectors targeting both inner hair cells and outer hair cells. Some AAVs, such as Anc80L65, DJ and AAV2.7m8, also transduce supporting cells with varying degrees of efficiency. However, their infectivity is limited in the undamaged ear to a narrow neonatal period and only a subset of supporting cells were shown to be transduced via these vectors. AAV-ie and its most recently studied variant, AAV-ie-K558R, transduce a broad variety of cells in the inner ear, including supporting cells, and thus lack selectivity. Here we investigate ShH10, a novel AAV variant with high tropism towards mammalian supporting cells, which are the putative target for hair cell regeneration and the cell type affected in many cases of congenital deafness.

Methods: Each vector of interest (ShH10, Anc80L65, DJ and AAV2.7m8) was delivered to the cochleae of P1 wildtype mice through posterior semi-circular canal (PSCC) injections. Two promoters, ubiquitous CMV and supporting cell-specific GFAP, were used to drive reporter gene expression. Sixty-two hours post-

operatively, the animals were euthanized and their cochleae and utricles were dissected and immunostained to quantitatively assess the prevalence and localization of reporter gene expression.
Results: Our analysis revealed that in the neonatal cochlea and utricle, ShH10 AAV transduces supporting cells with markedly higher selectivity and efficiency compared to the other tested AAVs. We observed a base-to-apex gradient in the rate of supporting cell transduction, with the greatest transduction occurring at the base.
Conclusions: Our data shows that ShH10 is a promising novel AAV serotype for supporting cell-specific gene delivery in the neonatal mammalian inner ear.

MO83. Nonclinical Pharmacology, Biodistribution, and Safety Studies Supporting the Clinical Development of DB-OTO (AAV1-Myo15-hOTOFv5) for Hearing Loss Due to Genetic Otoferlin Protein Deficiency

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Category: Gene Therapy
Background: Otoferlin is a calcium sensor protein expressed in the inner hair cells and is important for proper synaptic transmission between inner hair cells and the afferent fibers of the spiral ganglion. Biallelic loss of function mutations in the OTOF gene lead to congenital severe-to-profound sensorineural hearing loss in both humans and in mice.
DB-OTO is an adeno-associated virus (AAV)-based dual-vector gene therapy designed to provide durable hearing to individuals with profound congenital hearing loss caused by mutations of the otoferlin gene.
Methods: Good Laboratory Practice (GLP) studies were performed to support the conduct of human clinical trials of DB-OTO. OTOF-deficient mice and wild type non-human primates (NHPs) were used to characterize the pharmacology, toxicity, and biodistribution of DB-OTO after inner ear delivery. In the experiments involving NHPs, we modeled the surgical approach and delivery of DB-OTO that is being used in the first-in-human clinical trial
Results: We have previously shown that DB-OTO can instate hearing function in OTOFQ828X/Q828X mutant mice as measured by ABR, that it can be successfully delivered to the NHP ear via round window injection with lateral semi-circular canal fenestration, and that using a cell-specific promoter is key to its durable effect.
Using qRT-PCR, we found that hOTOF mRNA transcript levels peaked 4 weeks after DB-OTO injection in mice and 6 weeks after DB-OTO injection in NHPs, plateauing thereafter. A similar time course was observed for ABR improvements post DB-OTO injection in the congenitally deaf OTOFQ828X/Q828X mutant mice. We followed OTOFQ828X/Q828X mice for 8 months post-administration of DB-OTO and observed stability of the instated ABR throughout that period at clinically relevant doses.
In GLP studies, there were no adverse DB-OTO-related findings in otic or non-otic tissues across any evaluation in OTOFQ828X/Q828X mice (5-7-week-old and post-natal day 14-16) or healthy NHPs. We assessed the distribution of vector genomes outside of the ear following DB-OTO injection in NHPs. Vector shedding and levels of systemic escape into tissues in NHPs were limited.
The proposed volumetric dose scaling was directly proportional to the total cochlea perilymph volumes of mouse, NHP, and humans. Comparable vector genome DNA and hOTOF mRNA levels in mouse and monkey GLP studies confirmed the validity of this volumetric scaling approach for dose adjustments of DB-OTO between species.
The presence of pre-existing neutralizing antibodies were not associated with transgene expression levels in the ear or safety post DB-OTO injection, suggesting that there may be limited impact of systemic pre-existing neutralizing antibodies on local administration of AAV gene therapies to the inner ear.
Conclusions: Together, these data supported and informed our plans for the first-in-human clinical translation of DB-OTO.

MO84. Local Delivery of Adenine Base Editors Targeting the USH1C c.216G>A Mutation Responsible for Usher Syndrome Type 1C

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Category: Gene Therapy
Background: Usher syndrome (USH) is characterized by the concurrent loss of hearing, balance, and vision. There are no treatments to prevent or slow the progression of the disease. Previously, we demonstrated two novel genetic treatment strategies that partially restore hearing, balance, and vision in an USH Type 1C (USH1C) mouse model harboring the human USH1C c.216G>A mutation. One strategy uses antisense therapy to correct aberrant splicing; the other is gene replacement therapy using adeno-associated viral (AAV) vectors. USH1C mice treated one time with antisense or gene therapy showed significant improvements in hearing, vestibular, and visual function for approximately 3 months. We aim to extend the duration of therapeutic benefits using gene editing approaches.
Methods: HEK293T cells were cotransfected with plasmids expressing adenine base editors (ABEs), candidate gRNAs, and an Ush1c 216A minigene. RFLP and Sanger sequencing were used to quantitate editing at the 216A mutation and a control site. To confirm the spit-intein trans-splicing approach to deliver large base editors, we designed AAV-ITR plasmids that express the N- or C-terminal halves of the EGFP gene. These plasmids were cotransfected into HEK293T cells to quantitate green fluorescence. In parallel, AAV.PHP.B-CMV-GFP and AAV.PHP.B-CMV-mCherry vectors were delivered to postnatal day 2 wild type mice via semicircular canal injection, and cochlea harvested 2- and 4-weeks post-treatment to quantitate dual transduction of hair cells by immunohistochemistry (IHC). We then created dual AAV-ITR plasmids expressing N- and C-terminal halves of our candidate ABEs with corresponding gRNAs. Once 216A>G editing is confirmed in HEK293T cells, plasmids will be assembled into AAV.PHP.B vectors for in vivo testing.
Results: HEK293T cells transfected with our candidate ABEs showed 216A>G editing up to 46% and control-site A>G editing up to 65% with minimal off-target effects. Additionally, cotransfection of pAAV-EGFP_N-terminal and pAAV-EGFP_C-terminal plasmids into HEK293T cells resulted in robust green fluorescence, validating our split-intein design. These will be assembled into AAV.PHP.B vectors for in vivo testing. Coinjection of AAV.PHP.B-CMV-GFP and AAV.PHP.B-CMV-mCherry vectors showed double-positive hair cells at both 2- and 4-weeks post-treatment. Finally, split pAAV-ABE_N-terminal and pAAV-ABE_C-terminal plasmids were cotransfected into HEK293T cells, resulting in 216A>G editing up to 22% with minimal OTEs. The best-performing candidates will be assembled into AAV.PHP.B vectors for future therapeutic testing in mice.
Conclusions: These results demonstrate the potential for gene editing strategies to correct the human 216A mutation at the chromosomal level for long-term improvements in hearing and balance function in USH1C patients.

MO85. Development and In-Vitro Optimization of Allele-Specific Antisense Oligonucleotides for the Treatment of Progressive, Adult-Onset Hearing Loss Type DFNA21

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Category: Gene Therapy
Background: A 12-nucleotide deletion in RIPOR2 segregates with dominantly inherited hearing loss in families with DFNA21. Young family members with a hearing-impaired parent therefore know that they are at risk to also become hearing impaired. Allele frequencies of the RIPOR2 variant indicate that DFNA21 is likely to be the most common type of hereditary hearing loss in the Netherlands and Northwest Europe, with respectively 13,000 and 30,000 individuals at risk to develop DFNA21. There are strong indications that the deletion in RIPOR2 acts via a non-haploinsufficiency disease mechanism. We therefore hypothesize that inhibiting the synthesis of mutant RIPOR2 proteins, by selectively degrading the (pre)-mRNA transcribed from the mutant allele, can alleviate the negative consequences of mutant RIPOR2 on auditory function.
Methods: In this study, gapmer antisense oligonucleotides (ASOs) were designed to specifically target mutant RIPOR2 transcripts for degradation by the endogenous RNase H1 enzyme. The molecular efficacy of the ASOs was validated in DFNA21 patient-derived fibroblasts and in HEK293T cells.
Results: We identified a lead ASO molecule that was able to significantly reduce mutant RIPOR2 transcript levels, whilst leaving the level of wildtype RIPOR2 mRNA intact. Additionally, western blot analyses showed that the decrease in mutant RIPOR2 transcripts leads to a marked decrease in mutant protein

synthesis. This study furthermore revealed an RNase H1-independent mechanism of ASO action: steric hindering of mRNA translation likely also contributes to the decrease in protein levels. We found that this process (also) affects wildtype mRNA translational in a dose-dependent manner.
Key feature of ASO technology is the ability to chemically modify each nucleotide to alter e.g. mRNA binding affinity or nuclease resistance. By chemically modifying the lead ASO, we were able to reduce the binding affinity for wildtype RIPOR2 mRNA whilst retaining the effects on mutant RIPOR2 transcripts.
Conclusions: With the proven safety of the ASO chemistry in man, exemplified by the market approval of several ASO treatments for other human diseases, and the rapid advancements in inner ear drug delivery, our in-vitro studies indicate that ASOs offer a promising treatment modality for DFNA21.

MO86. Dual AAV-Mediated Gene Replacement Therapy Improves the Vestibular Function in a Mouse Model of USH1B

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Category: Gene Therapy
Background: Usher syndrome is the most common cause of deafness-blindness in the world. Usher syndrome type 1B (USH1B) is caused by mutations in MYO7A. Patients with USH1B experience deafness, blindness, and vestibular dysfunction. In this study, we applied dual AAV-mediated gene therapy to a mouse model of USH1B, the shaker-1 (Myo7a4626SB/4626SB) mouse. The shaker-1 mouse has a nonsense mutation in Myo7a. It is profoundly deaf throughout life, and it has significant vestibular dysfunction, similar to patients with USH1B.
Methods: Neonatal (P0 to P5) shaker-1 (Myo7a4626SB/4626SB) mutant mice were used in this study. Due to the ~6.7 kb of the Myo7a cDNA, the dual AAV approach was used for gene delivery via the posterior semicircular canal. Auditory brainstem response (ABR) was used to assess auditory function. Vestibular evoked potential (VsEP) and circling behavior recording were used to assess vestibular function. Immunohistochemistry was used to evaluate viral transduction efficiency, sensory hair cell viability, and stereocilia morphology.
Results: The shaker-1 mice are profoundly deaf due to significant stereocilia disorganization and rapidly progressive cochlear hair cell loss. Even though vestibular hair cells do not undergo degeneration in shaker-1 mice, they also exhibit significant vestibular dysfunction, due to stereocilia disorganization of the vestibular hair cells as well. When Myo7a cDNA was delivered to shaker-1 inner ears using the dual AAV approach, cochlear hair cell survival was improved. However, stereocilia organization and auditory function were not improved. In the vestibular system, dual AAV-mediated Myo7a delivery caused an improvement in stereocilia organization. In addition, the treated shaker-1 mice also had improved vestibular function, reflected by reduced circling behavior and improved VsEP thresholds.
Conclusions: Our results showed that dual AAV gene therapy was able to improve the vestibular function in the shaker-1 mutant mice.

MO87. Transcriptomic Differences Between Sox2-Positive Supporting Cells From Adult Mouse Cochlear and Vestibular Sensory Epithelia

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Category: Genetics A: Genomics and Gene Regulation
Background: The sensory epithelia of the mammalian inner ear is comprised of mechanosensitive hair cells and non-sensory supporting cells. In non-mammalian vertebrates, supporting cells can promptly replace the hair cells when they become damaged or destroyed. In the mammalian cochlea, this ability of supporting cells to transdifferentiate into hair cells is present in neonates before rapidly being lost within a few days

after birth. Interestingly, adult vestibular supporting cells still retain some capacity to transdifferentiate into hair cells. The underlying mechanism for this difference between cochlear and vestibular supporting cells in adult mammals remains unexplained. We use single-cell RNA-sequencing to examine and compare the transcriptomes of Sox2-positive cochlear and vestibular supporting cells from adult mice to identify molecular differences between these cells and elucidate the driving factors of tissue regeneration.
Methods: 10x Genomics platform was used for single-cell gene expression analysis. Sensory epithelia were obtained from cochleae and vestibule (including crista) of P70 CBA/J mice. 35 mice were used for 5 biological replicates. Our Cell Ranger output data was imported into R, where we used the Seurat package (v4.1.1) for the bulk of the processing, analysis, and visualization. RNAScope in situ hybridization and immunostaining techniques were used for gene expression validation.
Results: Our results show numerous differentially expressed genes (DEGs) between adult vestibular and cochlear supporting cells (VSCs and CSCs, respectively). Among the highly expressed genes in VSCs were Bricd5, Sparcl1, Otog, and Otoa. Our gene ontology and functional analysis reveal enrichment of such processes as tissue morphogenesis, inner ear development, and cilium arrangement in VSCs, suggesting these cells are more equipped with genes important in tissue regeneration and hair cell program. We also compare our data to previously published scRNA-seq datasets of neonatal (P1) mouse supporting cells, finding genes that appear to be expressed in P1 VSCs and CSCs and P70 VSCs, but whose expression is lost in P70 CSCs. Some of these genes, including Sparcl1, Dkk3, Aldoc, Mgst3, and Ush1c, are among the top DEGs we found between adult VSCs and CSCs.
Conclusions: Transcriptomic analysis is an important tool in resolving the complex identities of inner ear cell types, including their biological responses and transient states. The distinct variations in gene expression we observed among supporting cells from different inner ear tissues and age groups provide insight into the molecular drivers of supporting cell-hair cell conversion in adult mammals. Our work is an important component of auditory and vestibular research to eliminate permanent hearing loss and improve quality of life in humans (Supported by R01 DC016807 from the NIDCD/NIH).

MO88. A Gene Regulatory Landscape and Network That Drives Hair Cell Regeneration in Zebrafish
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Category: Genetics A: Genomics and Gene Regulation
Background: Sensory hair cells in the vertebrate ear are responsible for hearing through the transduction of vibrations into nerve impulses. Unfortunately, hair cells do not regenerate when damaged or killed, leading to permanent hearing loss. Zebrafish and mammalian sensory hair cells are functionally and genetically homologous, however zebrafish hair cells rapidly regenerate after damage and turnover during homeostasis. Previous studies in our lab have characterized the transcriptional changes in the lateral line in a fine time scale using scRNA-seq, and we identified three core modules that drive the regeneration of hair cells. A key missing perspective, however, is the epigenetic and regulatory landscape during regeneration that direct these transcriptional changes.
Methods: To understand the genetic program driving hair cell regeneration, we completed an ATAC-seq and ChIP-seq time course of the epigenetic regulatory environment and combined this information with existing scRNA-seq data. Histone modifications assayed include H3K4me3, which marks active promoters, and H3K27ac, which marks both active promoters and distal enhancers. We used bioinformatic tools to find the most active and dynamic regulatory regions in the genome. Additionally, we performed DNA motif searches to identify highly enriched transcription factor binding sites to reveal a possible genetic code at different stages of regeneration. Finally, we explored the role of individual enhancers by both cloning them driving a GFP reporter and deleting them to assay their necessity in gene expression and hair cell development and regeneration.
Results: We found that chromatin accessibility and regulatory histone marks rapidly change matching the expression dynamics of genes during regeneration. Using hierarchical clustering, we found that co-regulated enhancers form ten “regulatory groups” across time. Motif enrichment analysis reveals that each enhancer group is defined by a unique core set of transcription factors necessary at steps of regeneration/regulatory modules in the time series. Early injury/stress response genes, such as fos and jun have enriched binding sites in enhancers of hair cell regeneration genes. Thus, there is a direct regulatory link between the injury response form dying hair cells and the genes necessary to regenerate new hair cells. We identified specific enhancers from the ten regulatory groups and show that they drive reporter gene expression. Functional

analyses show that their deletion causes hair cell regeneration defects, demonstrating that these regulatory links identified are essential for hair cells. We further mutated key genes identified in the GRN, such as cbx7a and prdm1a, and observed drastic hair cell development and regeneration deficiencies in these mutants.
Conclusions: Our new epigenetic data has allowed us to build a GRN to describe hair cell regeneration. By understanding the regulatory landscape and how links and binding motifs have broken or evolved between zebrafish and mammals, our data provide key insight and gene targets for hair cell regeneration in mammals.

MO89. Elucidating the Molecular Biology of Age-related Hearing Impairment Using Omics Analyses of GWAS Data

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Category: Genetics A: Genomics and Gene Regulation
Background: Age-related hearing loss (ARHL) has an important genetic predisposing component and its estimated heritability is ~50%. Genome-Wide Association Studies (GWAS) have identified, to date, few dozens of common SNPs that are associated with ARHL. However, functional interpretation of GWAS results poses a main genomic challenge since the vast majority of 'risk SNPs' map to the noncoding part of the genome. Accumulating evidence indicate that interference with transcriptional regulation is a main mechanism of action of these genetic variants. The overreaching goal of our study is to improve our understanding of the molecular pathogenesis of ARHL based on integrative analysis of ARHL GWAS results and transcriptomic and epigenomic data generated from inner ear tissues.
Methods: Using multiple RNA-seq, ATAC-seq and ChIP-seq datasets from murine cochleae at several developmental stages, generated by both bulk and single-cell experiments, we applied complementary bioinformatics approaches to detect (1) genes, (2) cell types (3) genomic regions (4) molecular pathways and (5) developmental trajectories that are enriched for ARHL risk signal. Among these methods, we applied statistical tests for heritability enrichment in different genomic annotations, correlation analyses between GWAS-based gene risk scores and expression profiles across cell types and over trajectories, as well as network-based analysis for detection of dense functional modules that are associated with ARHL risk.
Results: Using the methods described, we first delineated key developmental trajectories and transcriptional programs that determine cell fate in the inner ear, and then identified sets of regulatory elements (the 'regulome') that control the main cell types in the cochlea. Intersecting GWAS signals for hearing loss and the inner-ear regulomes and gene expression profiles, we identified and prioritize ARHL risk genes, biological processes, molecular pathways and putative causal SNPs.
Conclusions: Our integrative analysis of transcriptomic, epigenomic and GWAS data shed light on developmental trajectories, cell types, molecular pathways and target genes that are involved in the pathogenesis of age-related hearing impairment. These results could guide the search for novel therapeutics and preventative treatments that alleviate ARHL.

MO90. Molecular Analysis of Cell Guidance in Mouse Embryonic Stem Cell-Derived Inner Ear Organoids

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Category: Genetics A: Genomics and Gene Regulation
Background: Many head sensory organs originate from cranial sensory placodes that are specified during early embryonic development. These placodes, or ectoderm thickenings on the embryo's surface, are designated by exposures to varying levels of morphogenic factors such as BMPs, WNTs, and FGFs. In recent years, researchers have exploited our understanding of the spatiotemporal activity of these signaling factors to guide stem cells in vitro through placode formation and into sensory cell types. Using successive exposures to BMP, FGF, and WNT, combined with modulation of TGFß signaling, DeJonge et al., 2016 developed a protocol to induce the formation of otic placode and otic sensory epithelia from mouse embryonic stem cells (mESCs). These inner ear organoids represent a potentially limitless source of otic tissue for studying inner ear development, function, as well as response to ototoxic and regenerative medicines. However, unknown factors within the guided portions of otic induction may contribute to the

variability of existing guidance protocols. Here we have used 10X Genomics scRNA-seq to investigate the steps of otic placode induction and inner ear sensory cell formation within the organoid environment.
Methods: Inner ear organoids were generated using Fbxo2-Venus-Hygromycin-Cre (VHC) mESCs following established protocols, with minor modifications. Organoids were dissociated in biological duplicate at 6 developmental time points: day (d) 3, d4, d8, d11, d16, and d21. Cells were processed for scRNA-seq using 10X Genomics Chromium Single Cell 3' Reagents v3.1 and aiming for a capture of 10,000 cells per replicate. After sequencing, Cell Ranger was used for read mapping and quantification. Datasets were further analyzed using Seurat.
Results: Correct cell guidance was assessed using marker gene expression for definitive ectoderm (DE) at d3, non-neural ectoderm (NNE) at d4, pre-placodal ectoderm (PPE) at d8, otic vesicle (OV) at d11, and sensory epithelia (SE) and hair cells (HC) at d16 and d21. A subset of cells at d3 expressed Cdh1 and Sox2, denoting them as DE. At d4, NNE could be identified by co-expression of Cdh1 and Tfap2a. At d8, PPE cells expressed Cdh1, Sox2, Gata3, and some cells began expressing the OV marker Pax8. At d11, OV cells could be further distinguished by co-expression of Sox2 and Jag1, as well as low levels of the OV marker Fbxo2. Finally, at d16 and d21, the development of inner ear SE was evident by the presence of HCs, which co-expressed Atoh1, Gfi1, and Myo7a.
Conclusions: We present a comprehensive scRNA-seq dataset of sensory tissue formation in inner ear organoids, from definitive ectoderm to sensory epithelia. This dataset represents a valuable resource for identifying additional signaling factors that contribute to the success or failure of otic induction.

MO91. Clinical and Molecular Characteristics of Patients With Craniofacial Malformations due to Syndromic and Non-Syndromic Etiologics

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Category: Genetics B: General
Background: Craniofacial malformations account for approximately 15% of the congenital disabilities in the newborn population of the southern region of Colombia. However, due to the lack of knowledge of these pathologies, there is a detrimental delay between clinical and molecular diagnoses, leading to severe delays in management and treatment. Previous European studies have demonstrated delays of five years between clinical and molecular confirmation of the diagnosis. These delays generate a loss in the rehabilitation potential, increase morbidity, and multiple biopsychological sequelae in patients and caregivers. We aim to understand the clinical characteristics of patients with craniofacial malformations, determine the most frequent genomic loci associated with the development of these disorders in our population, and describe the duration between clinical to molecular diagnosis in this cohort.
Methods: This is a retrospective cohort of 330 patients who were identified as related to the ICD-10 code describing a craniofacial malformation and were seen in different outpatient facilities of our hospital (Fundacion Valle del Lili). A total of 145 were identified with craniofacial malformation, and 23 patients underwent molecular studies such as; G-band karyotyping, array CGH, NGS sequencing, and whole exome sequencing. The differential time between clinical diagnosis and molecular testing was calculated using Kaplan Meier survival analysis.
Results: Of the 145 patients in the study, 42/145 (29%) showed a syndromic phenotype, and 103/145 (71%) isolated craniofacial abnormalities. The median age of this cohort was 10 years old (IQR 6.6 to 18.8 years old); 85 (58.6%) patients were male, and 60 (41.4%) were females. The most frequent craniofacial malformations were; cleft lip, macrocephaly, microcephaly, craniosynostosis, and microtia. These craniofacial abnormalities were highly associated with gastroesophageal reflux, seizures, asthma, growth delay, cognitive deficits, and behavioral disorders. Only 15.8% (23) of the cohort presented molecular diagnosis. Various craniofacial syndromes were identified, including Cowden syndrome, Muenke syndrome, CHARGE syndrome, Joubert syndrome, and others. Different variants in the following genes were identified; PTEN, OTC, EFTUD2, ACVR1, AMER1, FGFR3, RPS6KA3, CHD7, KATNIP, PTPN11, EFNB1, STK11, COCH, FGFR2, MITF, L1CAM, ANKS6, and AHDC1. In 13% of the cases, we could not identify the molecular diagnoses. Variants of uncertain significance (VUS) in several genes, including TP63, STAMBP, OCLN, and FRMPD4, were also identified.

Conclusions: The present study identified novel variants associated with the development of craniofacial malformations and some of the most relevant genes associated with the normal development of craniofacial features. In addition, we identified a delay in the molecular diagnosis of the patients with craniofacial malformation at 13 months (IQR 6 to 34.3 months), which might be detrimental to the management and follow-up of this population.

MO92. Molecular Basis of Partial Deafness

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Category: Genetics B: General

Background: Loss of high-frequency hearing is called partial deafness (PD). To improve the hearing ability of PD patients, two major approaches are used i.e. electric (cochlear implant) and electric-acoustic (cochlear implant and hearing aid) stimulation. From a clinical point of view, it is important to what extent natural hearing is preserved after the cochlear implantation procedure and whether its possible loss is related to the natural progression of the disease. So far, it is well-accepted that genetic factors play an important role in the development of hearing loss (HL), but much less is known about the molecular basis of PD.

Methods: The study included 40 patients with PD and divided them into two groups, i.e. a group of patients with normal hearing in the low and mid frequency range (PDT-EC, n=20) and a group of patients with mild to severe hearing loss in these frequencies (PDT-EAS, n=20). All patients were negative for DFNB1 locus pathogenic variants, the m.1555A> G variant of the MT-RNR1 mitochondrial gene and had no environmental HL risk factors. Genomic DNA was isolated from peripheral blood or buccal swabs of available family members. In all probands targeted next-generation sequencing (NGS) using a multi-gene panel (237 genes) was performed and followed by bioinformatics analysis. Family segregation analysis were performed using standard Sanger sequencing.

Results: The cause of PD was identified in 35% of the studied patients (14/40). In the PDT-EC group, probably causative variants were identified in 20% (4/20) and in the PDT-EAS group in 50% (10/20) of patients. The majority of identified variants were located in known genes involved in the development of both autosomal recessive and dominant forms of PD, but as many as half of them (9/15) were novel, previously unreported in the context of the disease. Among the contributors to PDT-EC we identified OSBPL2 and SYNE4, two relatively new hereditary hearing loss genes with a low publication profile.

Conclusions: Our study revealed that, for all PD patients, a postlingual HL more severe in the low-frequency range is associated with a higher detection rate of causative variants. Isolating a genetic cause of PD is important in terms of prognosis, therapeutic effectiveness, and risk of recurrence.

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MO93. The Genetic and Phenotypic Landscapes of Tecta-Related Hearing Loss

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Category: Genetics B: General

Background: Genetic variants in TECTA have been implicated in both autosomal dominant (AD, DFNA8/12) and autosomal recessive (AR, DFNB21) non-syndromic hearing loss (NSHL). Although the severity of TECTA-related hearing loss is variable across the mutational spectrum, studies of human cohorts have not identified clear genotype-phenotype correlations. The goal of our study is to investigate genotype-phenotype correlations and to better understand the distinct molecular mechanisms underlying TECTA-related hearing loss.

Methods: We used targeted genomic enrichment and massively parallel sequencing to screen up to 224 deafness-associated genes (OtoSCOPETM v9) in a large multiethnic cohort. Bioinformatic analysis was performed using a customized pipeline, and identified genetic variants were discussed in the context of clinical and family history data and classified following guidelines of the American College of Medical

Genetics and Genomics/Association for Molecular Pathology for hearing loss. We also used the Deafness Variation Database to collate all pathogenic variants previously reported and expertly curate them.
Results: Of the 6172 probands tested on OtoSCOPETM, 2626 (43%) had an identifiable genetic cause. Variants in TECTA made up 3% of total positive cases. The majority (90%) of our TECTA cohort had ADNSHL while approximately 10% had ARNSHL. AD variants were overwhelmingly missense, with just 3 splice variants predicted to cause in-frame exon skipping. AR variants were loss of function (LOF) including nonsense, frameshift indels, and large deletions. Interestingly, we identified eight missense variants that could potentially be the cause of DFNB21 hearing loss—seven of which were in the zonadhesin (ZA) domain.
A comparison of domain location of both causative AD and potentially causative AR missense variants revealed that all variants in the zona pellucida (ZP) domain are associated with ADNSHL while variants in the ZA domain are implicated in both ADNSHL and ARNSHL. We reviewed and expertly curated all reported pathogenic variants in TECTA, which resulted in reclassification of a significant number of variants. Similar to previous reports, we showed that DFNA8/12-related hearing loss ranged from mild-to-severe while DFNB21-related hearing loss was notably more severe ranging from moderate-to-profound.
Conclusions: These results draw a more refined picture of the complex mutational and phenotypic spectrum of TECTA-related hearing loss. Knowledge of mechanisms underlying genotype-phenotype variability is a prerequisite to improved variant classification and patient care.
Acknowledgements: This work was supported in part by NIDCD R01s DC003544, DC002842 and DC012049 to RJHS.

MO94. Otitis Media in Down Syndrome is Associated With Altered Nasopharyngeal and Middle Ear Microbiota

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Category: Genetics B: General
Background: Otitis media (OM) is a leading cause of hearing loss and is the most frequently diagnosed disease in young children. For OM, pediatric patients with Down syndrome (DS) demonstrate higher incidence rates, greater severity, and worse outcomes. However, no studies to date have investigated the bacterial profiles of children with DS and OM.
Methods: We aimed to assess the diversity and composition of middle ear (ME) and nasopharyngeal (NP) microbiotas of pediatric OM patients with DS (n=11) compared with those without DS (n=84). We profiled microbiota in these patients by broad-range 16S rRNA gene sequencing and analyzed the sequence data for diversity indices and relative abundance of individual taxa.
Results: Individuals with DS demonstrated significantly ($p<0.05$) increased biodiversity in their ME and NP microbiotas after adjusting for age, sex, and batch effects. In the ME, several taxa were enriched in OM patient with DS, including Actinobacillus (FDR-adjusted $P<0.001$), Corynebacterium (FDR-adjusted $P<0.001$), Prevotella (FDR-adjusted $p=0.02$), Anaerococcus (FDR-adjusted $p=0.01$), Family-XI-Incertae-Sedis (FDR-adjusted $p=0.007$), Peptoniphilus (FDR-adjusted $p<0.001$), Proteus (FDR-adjusted $p=0.02$), Finegoldia (FDR-adjusted $p<0.001$) and Campylobacter (FDR-adjusted $p=0.009$). Conversely, Alloiococcus (FDR-adjusted $p<0.001$)was enriched in OM patients without DS. In the NP, Eikenella (FDR-adjusted $p=0.01$) had significantly increased relative abundance in individuals with DS.
Conclusions: In children with OM, DS is associated with increased biodiversity and higher relative abundance of multiple bacterial taxa, many of which were previously identified as potential otopathogens in both the ME and the NP, suggesting that dysbiosis of these sites contributes to OM susceptibility in children with DS. These findings increase our knowledge of how DS influences regulation of the mucosal microbiota and contributes to OM pathology.

MO95. Characterisation of a New Mouse Allele of Cdh23 With Progressive Hearing Loss

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Category: Genetics B: General

Background: Cdh23 is an extremely large gene consisting of 69 exons and spanning approximately 350kb in the mouse. It encodes a 365kDa single-pass transmembrane protein which, along with Pcdh15, forms the hair cell tip-links required for mechanoelectrical transduction (MET) channel opening upon stereocilia bundle deflection during normal hearing. Mutations in the CDH23 gene in humans have been found to underlie Usher syndrome type 1D (USH1D), nonsyndromic autosomal recessive deafness DFNB12, and have been implicated in age-related hearing loss. As in humans, a variety of phenotypes have been attributed to mouse Cdh23 alleles; ranging from congenital deafness with balance defects (e.g. waltzer alleles; v<2J>, v<6J>, and v<alb>) to early progressive hearing loss with no balance defects (e.g. the salsa allele). Furthermore, the ahl locus is also within the Cdh23 gene, assigned to a single nucleotide polymorphism (753G>A) in exon 7, and results in late-onset high frequency hearing loss from approximately 9 months of age. Here, we characterise a new tm2a allele of Cdh23 which we hope will serve as a good model for early-onset progressive hearing loss underlined by hair cell defects. The tm2a allele is a 'knockout-first' design with a LacZ-containing transcription disruption cassette between exons 9 and 10. Since this tm2a allele was generated in a Cdh23<ahl> background (C57BL/6N), and thus is always present with the point mutation of the Cdh23<ahl> allele, we will refer to it as the Cdh23<tm2a-ahl> allele. The wild-type Cdh23 allele used was a repaired version of ahl on a C57BL/6N genetic background, C57BL/6NTac-Cdh23<ahl+em3H>/H, which we refer to as Cdh23+.
Methods: Auditory-evoked brainstem response (ABR) recordings have been carried out at frequencies ranging from 3-42kHz on Cdh23+/+, Cdh23<tm2a-ahl>/+, Cdh23<ahl>/<ahl>, Cdh23<tm2a-ahl>/<ahl> and Cdh23<tm2a-ahl>/<tm2a-ahl> mice at P21, P28, P56, and P98.
Results: The Cdh23<tm2a-ahl>/<tm2a-ahl> mice exhibited early-onset, progressive hearing loss indicated by significantly increased ABR thresholds across all frequencies tested compared to littermate controls from P28. Hearing impairment at high frequencies begins as early as P21, progressing to almost complete deafness by P56. No balance defects have been observed in Cdh23<tm2a-ahl>/<tm2a-ahl> mice up to P98, implying normal vestibular function. Cdh23+/+ and Cdh23<tm2a-ahl>/+ mice exhibited normal hearing with comparable ABR thresholds. Interestingly, the Cdh23<tm2a-ahl>/<ahl> mice have raised ABR thresholds at 36kHz and 42kHz by P28, suggesting a shift in the age-related hearing loss caused by the Cdh23<ahl> allele to a younger age when present with Cdh23<tm2a-ahl>.
Conclusions: Cdh23<tm2a-ahl> is a recessive allele which causes early-onset progressive hearing loss without balance defects. The early high-frequency hearing loss of Cdh23<tm2a-ahl>/<ahl> compound heterozygotes warrants further investigation as it could provide insight into mechanisms underpinning age-related hearing loss. Overall, we believe this new mouse Cdh23 allele to be a valuable model to study sensorineural hearing loss caused by hair cell defects.

MO96. A2ml1-Knockout Mouse as a Potential Model of Chronic Otitis Media

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Category: Genetics B: General
Background: Inflammation and infection of the middle ear, known as otitis media (OM), is a leading cause of hearing loss and the most frequently diagnosed disease in children worldwide. Traditionally, mouse models for OM rely on inducing acute infection through inoculation of the middle ear, e.g. with the human otopathogen non-typeable Haemophilus influenzae (NTHi), with very few genetic models with spontaneous or chronic OM. A2ML1 variants, including loss-of-function variants, are associated with susceptibility to OM in humans, but no animal model has been reported for OM. Here, we report our middle ear findings in a mouse line with a CRISPR-induced knockout (KO) of A2ml1.
Methods: Mice were X-rayed prior to harvest to determine if there are craniofacial or skeletal abnormalities. Tissue from mouse middle ears, as well as other upper respiratory mucosal tissues, were harvested. The harvested middle ear bullae were examined under microscope for phenotypic indications of OM. RNA samples isolated from middle ear tissue were assayed for expression of genes correlated with A2ML1 expression in humans.
Results: There were no significant craniofacial differences by genotype, however skeletal abnormalities that are concordant with phenotypes observed in A2ML1-related Noonan-like syndrome in humans were identified. Otomicroscopic findings in mice heterozygous for the A2ml1-KO are frequently indicative of otitis media, with tympanic membrane perforations or thickening, as well as cases of middle ear effusion or fluid. Gene expression studies are in progress and will be reported.

Conclusions: Thus far, our preliminary results in this A2ml1-KO mouse line indicate spontaneous occurrence of OM in these mice without the need for NTHi inoculation.

MO97. Diffuse Cochlear Damage in Shaker-1 Mice: Potential Role of Oxidative Stress

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Category: Genetics B: General

Background: Usher 1B Syndrome is an autosomal recessive inherited disorder that presents with progressive sensorineural hearing loss, vestibular dysfunction, and tunnel vision within the first decade of life. The majority of cases are caused by mutations in MYO7A, a gene which encodes for an actin-binding motor protein found in inner and outer hair cells (HC) important for the movement of intracellular proteins along the actin filaments of HC stereocilia. Shaker-1 is a mouse model for Usher 1B with a MYO7A mutation causing hearing loss, circling behavior, and head tossing phenotype. While Myosin VIIA is only expressed in inner and outer HCs, Shaker-1 mice have unexpected diffuse cochlear damage including loss of spiral ganglion neurons and thinning of the stria vascularis. The underlying mechanism of this diffuse cochlear damage is unknown. In this study, we investigate an oxidative stress mechanism for diffuse cochlear damage in Shaker-1 mice.

Methods: Wild type (WT), heterozygous (HT), and homozygous mutant (HM) Shaker-1 mice were sacrificed at varying ages (2 weeks, 1 month, 3 months, 6 months, and 1 year). Cochlea were harvested and placed in ethylenediaminetretraacetic acid (EDTA) overnight. Cochlea were dissected by microtome and prepared for immunohistochemistry (IHC). After blocking for 1 hour, primary antibodies were applied for 48 hours using the following concentrations: Annexin-5 (Ann-5) 1:200, 4-Hydroxynonenal (4-HNE) 1:50, and 3-Nitrotyrosine (3-NT) 1:50. Secondary antibody was applied for 1 hour at 1:1000 concentration. Imaging was performed with confocal microscopy.

Results: Markers for apoptosis (Ann-5) and oxidative stress (4-HNE, 3-NT) were present in HT and HM mice but absent in WT mice. Ann-5 was most strongly expressed in inner and outer HCs at all ages. Notably, 4-HNE and 3-NT expression was found in inner and outer HCs, supporting cells, and the spiral ligament as early as 2 weeks in both HT and HM mice with continued expression at 1 month and 3 months of age. By 6 months, 4-HNE and 3-NT was strongly expressed throughout the spiral ligament.

Conclusions: Shaker-1 HT and HM mice show increased expression of Ann-5, 4-HNE, and 3-NT diffusely throughout the cochlea as compared to WT mice, suggesting that oxidate stress may play a role in diffuse cochlear damage.

MO98. SOX11 and CHD7 Act in the Same Gene Regulatory Network to Promote Inner Ear Development

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Category: Genetics B: General

Background: Development of the inner ear depends on precise spatiotemporal orchestration of gene expression via transcriptional networks in the cell nucleus. SOX4 and SOX11, SoxC transcription factors belonging to the SRY-related high-mobility-group (HMG) box family, are both required for proper development of hair cells in the inner ear, but their roles in formation of inner ear vestibular structures have not been fully defined. Individuals with SOX11-related Coffin-Siris syndrome exhibit variable hearing loss and growth delays. These clinical features overlap with CHARGE syndrome caused by pathogenic variants in CHD7, encoding an ATP-dependent chromatin remodeler and key regulator of neurogenesis. Given that SOX11 is a known genetic target of CHD7 in neural stem cells, we tested the hypothesis that SOX4, SOX11, and CHD7 function in a common genetic regulatory network during inner ear vestibular development.

Methods: We generated mice with germline or inner ear specific conditional heterozygous or homozygous loss of Sox4, Sox11, or Chd7 (Sox11-/-, Pax2Cre;Sox11flox/flox, Pax2Cre;Sox4flox/+;Sox11flox/flox, Pax2Cre;Sox4flox/flox, and Chd7Gt/+;Sox11+/-). We examined embryonic inner ears from these mice for structural abnormalities using paint-filling and assayed for changes in gene expression using qRT-PCR,

immunofluorescence and in situ hybridization. We assayed embryonic inner ear cell proliferation and apoptosis using BrdU incorporation and anti-Caspase3, respectively.
Results: Abnormalities in the semicircular canals and endolymphatic duct were observed in ears with germline or conditional loss of Sox11 (Sox11-/-, Pax2Cre;Sox11flox/flox) and in ears with heterozygous loss of Sox11 and Chd7 (Chd7Gt/+;Sox11+/-). In contrast, ears with loss of Sox4 (Pax2Cre;Sox4flox/flox) exhibited only enlargement of the common crus and endolymphatic duct. Complete loss of Sox11 resulted in delayed formation of the canal fusion plate, likely related to increased cell proliferation in the surrounding mesenchyme since apoptosis and Laminin expression in the fusion plate basement membrane were normal. Loss of Sox11 had no effect on Chd7 expression in the E10.5 inner ear, while loss of Chd7 significantly reduced Sox11 expression. Interestingly, expression of Bmp4, a marker of the presumptive lateral crista ampullaris, was also disrupted in Sox11-/- inner ears.
Conclusions: These conditional and germline mutant mouse analyses uncovered novel requirements for the SoxC transcription factors in semicircular canal and endolymphatic duct formation via regulation of cell proliferation. We also identified a common gene regulatory pathway including Chd7, Sox11, and Bmp4 that is critical for vestibular development. These data support a role for Sox11 as genetically downstream of Chd7 and provide a basis for designing therapeutic strategies to treat hearing and balance disorders.
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MO99. Gene-Editing Induced Rabbit Model for USH3A Mimics Human USH3A Progressive Hearing Loss

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Category: Genetics B: General
Background: Research in deafness genetics has identified many pathogenic variants in genes associated with Usher Syndrome. Autosomal recessive pathogenic variants in CLRN1 (clarin 1) cause Usher syndrome type 3A (USH3A), characterized by slowly progressing hearing loss with post-lingual onset. USH3A is relatively rare in most populations but in some, such as Ashkenazi Jews and Finns, it represents up to 40% of USH cases. Animal models are needed to design therapies that reduce or prevent the inner ear pathologies associated with genetic deafness. In mouse models for USH3A, hearing loss is early onset and severe, and hair cells degenerate prior to cochlear maturation. As such, these mice cannot be used for therapies that require hair cells as targets. Here we describe experiments to characterize a novel rabbit model for hearing loss in USH3A.
Methods: The generation of the rabbit was described at ARO in 2022 (Yang et al., podium session #13). Briefly, CRISPR/Cas9 mediated rabbit genome editing was used. ABRs were measured in anesthetized rabbits using TDT equipment. Tones were delivered through an earbud in the ear canal. Stimulus presentation was 15 ms tone bursts, with 1 ms rise/fall times, presented 10 per second at 4, 12 and 16 kHz. Age-matched wild-type rabbits served as controls. For histology, bullae were removed and opened to expose cochleae. The stapes was removed and fresh 4% paraformaldehyde was perfused locally. For light microscopy sections, bullae were transferred into 5% EDTA with 0.25% glutaraldehyde added to soften the bone. Then cochleae were processed for JB-4 embedding followed by sectioning with a glass knife. For gene transfer experiments, AAV vectors with a GFP reporter were injected into the perilymph. To assess reporter gene expression, bullae were lightly decalcified, dissected into organ of Corti segments, stained for GFP and F-actin and viewed in epi-fluorescence.
Results: ABR thresholds were elevated in 3-month-old CLRN1 rabbits compared to wild types, but the hearing loss was moderate. However, by 14 months of age, no hearing could be recorded at all tested frequencies. Histology showed that the severe hearing loss was accompanied by loss of hair cells. Injecting AAV.GFP into the perilymph of wild-type ears resulted in transgene expression in sensory hair cells.
Conclusions: We present a newly developed USH3A rabbit model exhibiting progressive loss of hearing over many months after birth. The degradation of thresholds between the ages of 3 months and 14 months presents a window for testing therapeutics including gene transfer approaches. The decline in thresholds is accompanied by loss of hair cells. AAV-based gene transfer into hair cells is possible, providing a feasible route for testing phenotypic rescue.

MO100. Ramifications of POU4F3 Variants Associated With Autosomal Dominant Hearing Loss in Various Molecular Aspects

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Category: Genetics B: General
Background: POU4F3, a member of the POU family of transcription factors, encodes a member of the POU family of transcription factors with two highly conserved POU domains: a POU-specific domain and a POU homeodomain. The function of POU4F3 as a transcriptional regulator depends on the intact function of both POU domains. In humans, POU4F3 is one of the most common autosomal-dominant deafness-associated genes, variants of which cause post-lingual onset, progressive NSHL.
Methods: Exome sequencing was used to identify four novel POU4F3 variants (c.564dupA: p.Ala189Serfs*26, c.743T>C:p.Leu248Pro, c.879C>G:p.Phe293Leu, and c.952G>A:p.Val318Met), and diverse aspects of the molecular consequences of their protein structures, expression, subcellular localization, and transcriptional activity were investigated.
Results: All the mutant POU4F3 compromised protein stability, probably by impairing the DNA-binding ability, and significantly reduced the transcriptional activity required to regulate the downstream target gene expression. The expression of three mutant proteins, encoded by missense variants, was also reduced compared to the wild-type protein, suggesting that the mutant proteins may be unstable and vulnerable to degradation. Remarkably, all the POU4F3 variants had distinct subcellular localization patterns. A mutant protein carrying p.Ala189Serfs*26, in which both mono- and bi-partite nuclear localization signals (NLSs) were disrupted, demonstrated abnormal subcellular localization. Furthermore, we identified and validated the altered expression of 14 downstream target genes associated with inner ear development using patient-derived lymphoblastoid cell lines. There was a significant moderate correlation of the expression profile between patient-derived cells and the cochlear hair cells, which provided a breakthrough for cases where the collection of human cochlear samples for transcriptome studies was unfeasible.
Conclusions: This study expanded the genotypic spectrum of POU4F3 in DFNA15, and further refined the previously proposed molecular mechanisms underlying POU4F3-associated DFNA15.

MO101. Localization of Transmembrane Protein 135 in Mouse Cochlea

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Category: Genetics B: General
Background: Transmembrane protein 135 (TMEM135) is a member of the TMEM family. This gene encodes a 52 kDa protein with five transmembrane domains that share similarities with the translocase of inner mitochondrial membrane 17 (TIM17) family, a group of proteins involved in protein import into the mitochondrial matrix. TMEM135 was originally identified as a longevity-factor in C. elegans. In humans, TMEM135 is highly expressed in the brain tissues. In mice, TMEM135 is localized to the mitochondrial constriction sites and involved in mitochondrial division, and a point mutation in Tmem135 (FUN025) causes age-dependent retinal degeneration. In the current study, we investigated the effects of the Tmem135 mutation on auditory function using wild-type (WT) and Tmem135 homozygous mutant (FUN025) mice that were backcrossed onto the CBA/CaJ strain for 4 generations, and the localization of TMEM135 in the cochlea of WT and CBA/CaJ mice.
Methods: Auditory brainstem response (ABR) thresholds were measured at 8, 16, 32, 48, and 64 kHz, and distortion product otoacoustic emission (DPOAE) amplitudes were measured at 8, 16, and 32 kHz in WT and FUN025 mice at 3-4 months of age. Cochlear whole mount tissues from 1-3-month-old WT and CBA/CaJ mice were stained for TMEM135 (anti-TMEM135), mitochondria (anti-VDAC, voltage dependent anion channel), hair cells (anti-MYO7A, myosin VIIA), and or nuclei (DAPI, 4′,6-diamidino-2-phenylindole). Maximum intensity projections of confocal z-stacks were obtained.
Results: FUN025 mice displayed significantly elevated ABR thresholds at 8, 16, 48, and 64 kHz and decreased DPOAE amplitudes at 8 and 16 kHz. To investigate how the FUN025 mutation causes hearing loss, we examined the localization of TMEM135 in the cochlear whole mounts of mice. Using super-resolution structured illumination microscopy (SIM) and the validated TMEM135 antibody, we found that TMEM135 signal was prominent in the outer hair cells (OHCs), but no or weak signal was detected in the inner ear hair cells (IHCs), supporting cells, auditory nerve fibers, spiral ganglion neurons, or spiral ligament. In the OHCs, TMEM135 was positioned along the subsurface cisternae, which are thought to be a

specialized system of the smooth endoplasmic reticulum (ER), the major storage site for calcium, and colocalized with mitochondrial VDAC, a conserved component of the ER-mitochondria contact sites (ERMCSs) that regulate the transfer of calcium from the ER to the mitochondrial matrix in eukaryotic cells.
Conclusions: These results suggest that TMEM135 may interact with ERMCSs in OHCs. We are currently investigating the role of TMEM135 in calcium trafficking and homeostasis in OHCs using mouse auditory House Ear Institute-Organ of Corti 1 (HEI-OC1) cells, and CBA/CaJ and FUN025 mice.

MO102. Novel Candidate Genes for Cholesteatoma in Chronic Otitis Media

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Category: Genetics B: General
Background: Cholesteatoma is a rare and benign disease, but its propensity to cause erosive damage through uninhibited growth can be detrimental to hearing and even cause fatal consequences. The exact mechanism of pathogenesis remains unelucidated despite multiple existing theories, but familial and syndromic incidences of cholesteatoma suggest a possible genetic component in at least a subset of patients. In this study, we aimed to identify rare DNA variants in patients with cholesteatoma.
Methods: In a previous study, isolated RNA from the middle ear tissues of six patients with cholesteatoma was submitted for bulk mRNA sequencing and analyzed for differentially expressed genes (DEGs). From the same six patients, salivary DNA was extracted and submitted for exome sequencing. The results were aligned to the hg38 reference sequence to generate .bam files. Variant calling produced .vcf files, which were annotated using ANNOVAR. Annotated variants were selected for: (1) occurrence within previously identified DEGs from the middle ear samples; (2) a minor allele frequency (MAF) □0.001 in public genome databases; (3) predicted as deleterious in bioinformatic analyses; and (4) passing quality control (QC) steps, including confirmation within Integrative Genomics Viewer (IGV). The resulting candidate genes were reviewed for differences in expression levels using available RNA-sequence data. NetworkAnalyst was used to determine the subnetworks inter-connecting the candidate genes, and the enriched pathways (FDR-adjusted $p<0.05$) coinciding with those from prior analysis performed using DEGs.
Results: Exome sequencing of the DNA samples yielded 59,313 variants, of which 5,078 had MAF<0.001. Of these rare variants, 510 were predicted to be deleterious; 84 were splice or frameshift variants and 426 were missense variants. Fifty-two of these variants occurred within previously established DEGs. The presence of resulting variants was confirmed in at least one sample using IGV. There were 12 candidate genes with rare deleterious variants, all identified in one pediatric patient. No variants meeting our selection criteria were found in the five adult patients. Network analysis of the 12 candidate genes yielded 20 significant pathways, including endocytosis (FDR-adj-p=3.0x10-17) and intracellular protein transport (FDR-adj-p=1.9x10-8). Seventeen cellular processes coincided with those resulting from DEGs.
Conclusions: We identified 12 novel candidate genes for cholesteatoma, supported by the DEGs from the middle ear tissues of the same patients. We hypothesize that these genes may be a part of key signaling pathways in mucosal response to middle ear inflammation. The occurrence of multiple rare variants may contribute to an earlier onset of cholesteatoma formation in chronic otitis media. Identification of these genes and pathways not only enriches our understanding of cholesteatoma but can potentially lead to new prevention and treatment strategies for otitis media.

MO103. The Outer-Hair-Cell Hair Bundle Does Not Respond Uniformly to Stimulation

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Category: Hair Cells: Anatomy and Physiology
Background: Outer-hair-cell hair bundles (OHBs) are required for hearing and their dysfunction causes hearing loss. Although we know OHBs convert sound-induced forces into receptor currents, we have a poor understanding of how their morphology regulates their receptor currents. An OHB comprises columns and rows of actin-packed stereocilia pivoting at the hair-cell apex. In a column, stereocilia of increasing height are linked by gating springs that gate mechanoelectrical transduction (MET) channels, through which the receptor current flows. Rows are defined by stereocilia of similar height. Sound-induced stimulus forces on the tallest row of stereocilia (row 1) deflect the OHB stereocilia, modulating the gating-spring forces that

open and close the MET channels in the shorter rows (rows 2 and 3). This varies the MET channel currents, which sum to produce the oscillating receptor current.
The prevailing view of an OHB is that its columns are nearly parallel, its stereocilium deflections and gating-spring forces are similar, causing its MET channel currents to be almost identical. We hypothesize that these assumptions about the uniformity of columns, stereocilium deflections, gating-spring forces, and MET channel currents are far from true. Consequently, the receptor current is smaller than it would be for an OHB with parallel columns.
Methods: To determine the angles between OHB columns, we used STED super resolution microscopy to image the stereocilium pivot positions in the hair-cell apex of P10 rat outer hair cells. The pivots were labeled by antibodies against the stereocilium rootlet proteins TRIOBP-4/5. We combined these data with morphological and mechanical data from the literature to build computational models of an OHB from the rat cochlear apex (approximately 5 kHz characteristic frequency). Using the models, we calculated stereocilium deflections, gating-spring forces, MET channel currents, and receptor currents in response to oscillatory stimuli. For comparison, we also calculated the same quantities for models with parallel OHB columns.
Results: OHB columns were not parallel. At their pivot positions, the angle between columns was up to 67°. In response to spatially-uniform stimulus forces on row 1 stereocilia, stereocilium deflections differed within columns and within rows, gating-spring forces were far from uniform across the OHB, and each row 2 and 3 MET current was different. As a result, the receptor current was much smaller than that of an OHB with parallel columns.
Conclusions: We quantified a previously neglected aspect of OHB morphology, the relative orientation of its columns, and found that it was critically important for OHB function. Nonparallel OHB columns cause nonuniformity in an OHB's responses to stimulation, which decrease its receptor current. We predict that changes in the relative orientation of an OHB's columns owing to mutations would cause OHB dysfunction and hearing loss.

MO104. Expression and Localization of Tubby-Like Proteins Tulp 2, 3 and 4 in the Mouse Organ of Corti

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Category: Hair Cells: Anatomy and Physiology
Background: Tubby-related proteins (TULPs) are phospholipid-binding adaptor proteins that are involved in directed protein import into primary cilia in diverse of tissues including kidney and brain. Mice defective in the tubby protein exhibit an obese and diabetic phenotype associated with retinopathy and hearing loss. While most of these pathologies have been explained by defects in ciliary function in neurons or photoreceptors, respectively, in the inner ear, tubby was recently shown to be localized to stereocilia tips of outer hair cells (OHCs), rather than to primary cilia or hair cell kinocilia. In OHCs, tubby is required for the assembly or maintenance of the protein complexes, that connect hair bundels to the tectorial memebrane and that link adjacent sterocilia. Accordingly, hearing loss in tubby mice results from loss of OHC function. Given the non-canonical function of tubby in the cochlea, we wondered whether other members of the TULP protein family may be involved in ciliary function in the cochlea.
Methods: In this study, we examined cellular and subcellular localization of TULPs 2, 3 and 4 in the developing and mature organ of Corti of the mouse using immunohistochemistry.
Results: At postnatal day 1 (P1) TULP2 was expressed in non-sensory supporting cells, where it localized to the base of the primary cilium. In adult mice, TULP2 was found in inner and outer hair cells, where it was mostly restricted to the actin rich cuticular plate. TULP3 was localized to cochlear and vestibular hair cell kinocilia within the first days after birth. Moreover, from P3 prominent TULP3 immunolabeling was detected in the microtubule bundles of non-sensory Pillar (PCs) and Deiters cells (DC). This localization pattern became more prominent with subsequent postnatal maturation. TULP3 additionally localized to the cuticular plate of OHCs at P20. In contrast, TULP4 immunolabeling did not reveal a ciliary localization but localization to the cuticular plate of inner and outer hair cells as well as in postsynaptic neurons at early postnatal age (P1). In adult mice only the expression in cochlear neurons remained.

Conclusions: Taken together, the protein expression and localization pattern of TULP 2, 3 and 4 in the organ of Corti during postnatal development suggests potential roles in hearing for all this protein family.

MO105. Otoprotective Ion-Channel Modulators Augment the Production of Lysotracker-Positive Cytoplasmic Bodies That Sequester Fluorescent Gentamicin

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Category: Hair Cells: Anatomy and Physiology

Background: Aminoglycoside antibiotics are used in clinical practice but can cause the loss of sensory hair cells, resulting in permanent sensorineural hearing loss and balance disorders. These ototoxic compounds are thought to enter hair cells via the mechano-electrical transducer (MET) channels, as well as by endocytosis. Once intracellular, irrespective of entry route, aminoglycosides are sequestered by lysosomes. Slower delivery to this organelle increases aminoglycoside toxicity (Hailey et al., 2017). In a screen of 160 ion-channel modulators, we identified 13 compounds that protect hair cells from aminoglycoside damage (Kenyon et al., 2017). Several of these compounds offered protection against aminoglycoside-induced toxicity in the absence of MET-channel block. We therefore sought to investigate if these compounds increased accumulation of fluorescently conjugated aminoglycosides to the lysosomes.

Methods: Mouse cochlear hair cells were treated with 6 of the previously identified otoprotectants for 48h and probed for irregular cytosolic characteristics by live differential interference contrast (DIC) microscopy. A subset of these, 13222 (Ifenprodil), 13154 (PNU 37883) and 13097 (XE-991), were then treated with Lysotracker green, both in the presence and absence of gentamicin conjugated to Texas Red (GTTR) and live-imaged using confocal microscopy. Ultrastructural analysis by TEM and correlative light and electron microscopy (CLEM) were also utilized to determine cytological changes in hair cells caused by long-term exposure to GTTR.

Results: Large granular structures were observed by DIC in hair cells treated with otoprotective compounds 13222, 13154 and 13142 for 48h, but not in those exposed to 13097, 13143 and 13170. These structures labelled with Lysotracker green suggesting they are lysosomal. Cultures incubated with compounds 13222 and 13154 for 24h followed by GTTR for a further 2h revealed that these large Lysotracker-positive structures co-localised with GTTR. In contrast, in DMSO-treated cells, only small Lysotracker-positive puncta were observed that occasionally co-localised with GTTR. The appearance of the large Lysotracker-positive inclusions in response to 13222 or 13154 treatment was reversible following washout of the compounds. Interestingly, in the absence of compound, treatment of cochlear cultures with GTTR for an extended period (24h) resulted in the formation of bright punctae, visible by DIC as granular structures. These structures were determined, by TEM and CLEM, to be cytoplasmic, lipid-rich membranous whorls.

Conclusions: Otoprotective MET-channel blockers 13222 and 13154 caused reversable enlargement of Lysotracker-positive structures that sequestered GTTR, while compound 13097, which does not block MET currents, did not generate these structures. In addition, long-term exposure of cultures to GTTR also causes the formation of similar membranous inclusions. These findings raise the possibility of lysosomal adaptation in hair cells, triggered either by the presence of putative lysosomotropic compounds or MET channel block, as a protective mechanism in sensory hair cells.

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MO106. A Machine Learning Based Segmentation of Inner Ear Hair Cell Stereocilia on Scanning Electron Micrographs

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Category: Hair Cells: Anatomy and Physiology

Background: Hair cells – the sensory cells of the inner ear – on their apical surface carry a bundle of precisely arranged actin-filled microvilli-like projections of increasing height called stereocilia. Mature cochlear hair cell bundles typically have three rows of stereocilia. This regular and highly precise organization is critical for proper hair-cell mechanotransduction function, thus stereocilia bundle morphology deficits often lead to a hearing loss phenotype. There is a growing amount of evidence showing differential distribution of key proteins between rows of stereocilia, aiding to the understanding of their functional heterogeneity. Stereocilia bundle morphology is often compromised in knockout mouse models

of human deafness genes and following various exogenous treatments, such as acoustic trauma. When affected, the bundle integrity tends to degrade over time, with stereocilia from different rows degrading at different rates. Quantification of cochlear hair cell stereocilia in scanning electron microscopy (SEM) images can contribute to our understanding of their pathophysiology. However, manually processing these images is time-intensive and requires significant attention to detail. This creates a limitation in the amount of imaging data that can be reasonably processed, in some cases resulting in qualitative reporting on stereocilia bundle morphology. To alleviate this burden, we have created an outer hair cell (OHC) stereocilia segmentation dataset and trained a deep learning model to quantify tall middle and short row stereocilia in SEM images of OHC bundles.
Methods: SEM images of OHC stereocilia bundles were collected at ~×20'000 magnification from adult mouse Organs of Corti (P20-P180). Stereocilia were manually segmented in the VGG image annotator (a free browser-based tool) and assigned a classification based on their row identity (tall, middle, or short). We used 70% of the manually annotated images to train a deep learning model based on the Mask-RCNN 2D segmentation architecture, while the remaining images were used to validate the accuracy of the model. To facilitate the use of this model by the research community, we created a graphical user interface which loads the image, evaluates it using the trained deep learning model, and exports the output.
Results: Thus far we have annotated 118 SEM images of hair bundles with an average of 30.5 tall, 27.1 middle, and 21.8 short stereocilia per image for a total of 9386 stereocilia: 3602 tall, 3201 middle, and 2583 short row stereocilia. We trained the Mask-RCNN model for 5000 epochs and achieved a F1 score of 0.88 with an average mask intersection over union of 77%.
Conclusions: Our model allows for accurate and fast stereocilia segmentation, classification and quantification in SEM images, offering a significant advancement in automated stereocilia bundle analysis. This model is available as an open-source tool for the research community.

MO107. Mice With Hair Cell-Specific down Expression of Otoferlin by CRISPR-Cas9 Technology in Vivo Shows Hearing Loss Associated With Ribbon Synapse Degeneration

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Category: Hair Cells: Anatomy and Physiology
Background: Mutations in the OTOF gene, encoding otoferlin, cause profound nonsyndromic recessive deafness, DFNB9. This large multi-C2 protein is an essential calcium-sensor triggering synaptic vesicle release (exocytosis) at the inner hair cell ribbon synapses. Remarkably, this protein has also been found to be essential for the maintenance and survival of the hair cell ribbon synapses by mechanisms that remain unknown. To decipher the role of otoferlin in this later process, we created a mouse model in which the hair cell expression of otoferlin was down modulated at various levels along the cochlear partition at late postnatal stages. The consequences on hearing thresholds and the maintenance of the hair cell ribbon synapses will be studied.
Methods: H11LSL-Cas9 CRISPR/Cas9 knock-in mice, which have Cre recombinase-dependent expression of CRISPR associated protein 9 (cas9) endonuclease directed by a CAG promoter (The Jackson Laboratory), were crossed with Myo15-Cre+/+ mice (Myo15atm1.1(cre)Ugds) to obtain offspring mice with a specific cas9 expression in hair cells. In these anesthetized P1–P3 offspring mice, a recombinant adeno-associated virus (AAV2/8) carrying a sequence encoding for otoferlin-specific gRNAs followed by a GFP sequence was microinjected in the cochlea through the round window membrane. Control mice were injected with AAV2/8 containing nonspecific random gRNAs or injected in mice that did not express cas9. The virus containing the otoferlin-specific gRNAs and control random gRNAs were packaged and titrated by Penn Medicine Vector Core. Auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) were recorded in anesthetized mice at P30 by using the BioSigRZ and RZ6 system of Tucker-Davies Technologies. Otoferlin and synaptic ribbons were specifically visualized under high resolution confocal immunofluorescence microscopy.
Results: H11LSL-Cas9 CRISPR/Cas9-Myo15-Cre+/- mice injected with AAV containing Otof-sepcific gRNAs displayed a mean increase of 35 dB in tone and click ABR thesholds as compared to control mice ($p<0.001$) while DPOAEs remained normal. In these mice, the mean rate of hair cell AAV transduction of the reporter gene GFP (and the associated gRNAs) was evaluated under fluorescence microscopy at 82% in IHCs and 36 % in OHCs. Remarkably, in these Otof-gRNAs injected mice, we found a mosaic decrease in

the otoferlin expression in IHCs along the cochlear partition. We could establish a good correlation between the expression level of protein otoferlin and the number of synaptic ribbons per IHCs ($r=0.76$ $p<0.001$). The size of the IHCs were also positively correlated to the level of otoferlin expression ($r=0.59$ $p<0.01$).
Conclusions: We demonstrate as a proof of concept that CRISPR-Cas9 gene editing works in hair cells in vivo. By this technology we could down modulate the expression level of otoferlin in postnatal hair cells and show that this protein is indeed essential for the maintenance of the synaptic ribbons.

MO108. Ultrasonic Measures of Prestin (SLC26a5) Charge Movements in Membrane Patches

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Category: Hair Cells: Anatomy and Physiology
Background: Gale and Ashmore (1997) characterized NLC as low-pass in membrane patches at the voltage of peak NLC (Vh). Contrarily, subsequent measures of electromotility (eM) suggested ultrasonic behavior (Frank et al., 1999). Those eM measures were made at resting potentials far removed from Vh. In fact, eM is low pass at Vh, similar to NLC (Santos-Sacchi and Tan, 2018). Dong et al. (2000) suggested that kinetics of thermal noise defines prestin's limiting speed, and that the frequency cut-off is much higher than that of voltage-driven, admittance-based measures of NLC. That observation violates the fluctuation-dissipation theorem, which posits that voltage-driven, admittance-based noise estimates and thermally-driven noise at equilibrium are the same (Nyquist, 1928; Callen and Greene, 1952; Lauger, 1978). Here we reinvestigate.
Methods: OHC complex NLC (cNLC) was measured in macro-patches under voltage clamp as previously described (Santos-Sacchi et al., 2021), except we now sampled currents at 1 MHz (16 bit NI-USB 6356; National Instruments) using an Axon 200B amplifier (capacitive feedback mode) with Bessel filter set at 100 kHz. In order to measure admittance-based and stationary noise concurrently, NLC and Nyquist noise spectra were simultaneously assessed with voltage chirps; thermal noise was assessed in the absence of AC voltage perturbation, all within the same voltage-clamp protocol. Methodology was confirmed with electrical models.
Results: Prestin NLC is a complex quantity, cNLC (Santos-Sacchi et al., 2021), separable into real and imaginary components. Below a particular frequency (Fis, see below), there is a decrease in the positive real component accompanied by an increase in the negative imaginary component, most notable at Vh. Here we have extended our bandwidth beyond our previous 30 kHz bandwidth (Santos-Sacchi and Tan, 2020), which enables us to discern the peak and subsequent reduction in the negative imaginary component as frequency increases. Our measures provide Abs(cNLC) values that are larger in the ultrasonic region than those of Gale and Ashmore (1997). While the characteristic cut-off frequency (Fis) of Abs(cNLC), determined from the intersection frequency of real and imaginary components of cNLC are low-pass at around 19 kHz, the high frequency roll-off shows a 20.5 dB drop at 120 kHz. The frequency cut-off of admittance-based Nyquist noise matches that of NLC. In measuring thermal-driven noise, we are faced with confounding noise sources, including amplifier headstage noise, RF noise and 1/f noise. We successfully overcome these and show that frequency responses of admittance-based (Nyquist) noise and thermal-driven noise correspond, indicating equivalent frequency cut-offs.
Conclusions: Our data indicate that prestin performance in the ultrasonic range is greater than previously thought, but the characteristic frequency cut-offs provided by cNLC, Nyquist admittance-based noise and thermally-driven noise correspondingly provide evidence for low-pass behavior in prestin. Is this roll-off in kinetics compatible with cochlear amplification at ultrasonic frequencies?

MO109. MYO15A Function in Stereocilia Requires Light-Chain Binding via the IQ3 Domain

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Category: Hair Cells: Anatomy and Physiology
Background: The hair cell sensory organelle is comprised of organized rows of membrane protrusions called stereocilia. Many deafness-associated genes are involved in stereocilia growth and patterning. One common example is MYO15A, with over 200 pathogenic variants identified at the DFNB3 locus. MYO15A is the motor component of the Elongation Complex (EC), a group of proteins that promote actin

incorporation to form the tallest stereocilia and establish graded height architecture across rows. Here we propose that the centriolar protein Centrin-2 (CETN2) is a new EC member and a light-chain which binds the third IQ domain (IQ3) of MYO15A. We further investigate how light-chain binding to IQ3 regulates MYO15A localization and consequent effects on hearing.
Methods: We generated mouse strains carrying a constitutive deletion in Cetn2 or Cetn3 and a strain with a missense mutation in the MYO15A IQ3 region, Myo15a-R1940W. We used an Egfp-Cetn2 transgenic strain to track CETN2 localization. Cochlear hair cells were imaged with scanning electron microscopy and confocal microscopy to analyze stereocilia morphology and localization of EC proteins. Auditory brainstem responses (ABRs) were measured to assess hearing in response to a range of pure tones or broadband stimuli. Myo15a constructs were electroporated in cultured Egfp-Cetn2 cochleae.
Results: CETN2 is unexpectedly enriched at stereocilia tips and relies on MYO15A for its localization. MYO15A's IQ3 domain is critical for association with CETN2, suggesting that CETN2 acts as a MYO15A light-chain in vivo. Cetn2 knock-out mice display elevated ABR thresholds but have overtly normal stereocilia, including normal localization of other EC proteins.
We next investigated CETN3, a CETN2 paralog also detected at stereocilia tips. Like Cetn2 mutants, Cetn2; Cetn3 double mutants have elevated ABR thresholds but still lack overt stereocilia defects. To investigate whether additional, redundant light-chains can compensate for the loss of CETN2/CETN3, we tested a converse R1940W mutation in MYO15A's IQ3 domain based on a human variant. The human ortholog R1956W was linked to deafness in a patient also carrying a MYO15A motor domain missense variant in trans. In cultured cochleae, MYO15A-R1940W does not bring excess EGFP-CETN2 to stereocilia tips, unlike wild-type MYO15A. Myo15a-R1940W homozygotes display high-frequency hearing loss in ABR profiles. Additionally, MYO15A enrichment at stereocilia tips is variably impaired along the tonotopic axis in Myo15a-R1940W/shaker-2 compound heterozygous neonates. Ongoing work will test whether Myo15a-R1940W homozygotes recapitulate this phenotype and identify the consequences of Myo15a-R1940W mutation on stereocilia development.
Conclusions: Our data suggest a novel role for CETN2 in hair cells as an EC member and a first identified endogenous MYO15A light-chain. We show that the IQ3 domain is important for MYO15A localization and for auditory function at high frequencies. Together, these results uncover new regulation of a classic deafness protein known for decades to be integral to hair cell function.

MO110. Loss of the Ferritin Light-Chain Leads to Auditory Deficit
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Category: Hair Cells: Anatomy and Physiology
Background: Hearing relies on the recruitment of two populations of cochlear sensory cells. While the pool of outer hair cells amplifies incoming sound-stimulation to achieve exquisite frequency selectivity, the inner hair cells convert the mechanical pressure into release of glutamate onto the auditory nerve fibers. Hair cell active zones harbor a synaptic ribbon, an electron-dense organelle that tethers a monolayer of glutamate-filled vesicles.
Methods: We first used a two-hybrid screen to isolate interacting partners of RIBEYE, the major component of the synaptic ribbon. Then, we examined the phenotype of the knock-out mouse corresponding to the protein of interest.
Results: We identified the ferritin light-chain (Ftl1), known to store iron and hence to prevent hydroxyl radicals formation, as an interacting partner of RIBEYE, the major component of the ribbon body. In the Ftl1 knock-out mouse, we found-out a threshold shift in 20% of the homozygous mice associated with the loss of the distortion product of otoacoustic emissions, as a proxy of the OHC's activity. Consistently, light and electron microscopy show a massive degeneration of the OHCs. In addition, we found-out splayed hair bundle in IHCs in the fraction of Ftl1 knock-out mice with threshold shift. Although the loss of Ftl1 did not change the number and size of the hair cell synaptic ribbons, we found-out a larger proportion of hair cells with reduced calcium current in the Ftl1 knock-out mice with hearing loss.
Conclusions: Given that ferritin stores ferric iron to prevent the generation of hydroxyl radical through the Fenton's reaction, we propose that ferritin may protect to some extent hair cells and notably the ribbon synapse against iron-induced hydroxyl radicals.

MO111. The MOC Efferent System Acts as a Control Parameter for Outer Hair Cell Dynamic Stability

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Category: Hair Cells: Anatomy and Physiology

Background: Outer hair cell (OHC) electro-mechanical power conversion is optimized at the characteristic frequency (CF) when key biophysical parameters set the operating point at the edge of a dynamic instability, the critical point. We have shown previously that the ratio of the passive RC corner frequency to CF is one of three cardinal control parameters that set the critical point. Modulation of the RC corner by the medial olivocochlear (MOC) efferent system therefore provides the mammalian central nervous system with powerful control over OHC amplification and stability. Here, we examine potential mechanisms underlying MOC modulation of spontaneous otoacoustic emissions (SOAEs) and power amplification when the OHC operates near the critical point.

Methods: We used a single compartment nonlinear model of a piezoelectric OHC coupled to a passive spring-mass damper load to examine the effect of MOC activity on OHC dynamic stability, limit cycle oscillations and power output. The cochlear load consisted of mass, stiffness and viscous drag acting on both the apical and basal ends of the cell. The system was driven by a force applied to the apical mass, representing sound-induced pressure in the cochlea. Amplification in this model is powered by the mechano-electrical transduction (MET) current entering the cell, which is gated in proportion to cell displacement. The cochlear load was treated as linear, but the MET current and the piezoelectric coefficient both include saturating nonlinearities. Dependence of the critical bifurcation point on MOC activity (basolateral conductance) was found in closed form using the linearized equations. The nonlinear equations were solved for conditions near the critical point using the fifth-order Runge-Kutta-Fehlberg method to examine how increasing or decreasing MOC activity alters OHC function.

Results: Results support the hypothesis that tonic MOC activity poises the OHC on the edge of a dynamic instability, which is the point of highest power output. Further increasing MOC efferent firing pushes the RC corner frequency above the optimum and reduces power output, eventually into a parameter space where the OHC consumes power rather than outputting power. This control parameter mechanism is likely responsible for the dramatic reduction of mechanical vibrations and reduction in spiral ganglion neuron sensitivity caused by activation of the MOC system. MOC function as a control parameter is also consistent with the influence of contralateral acoustic stimulation on spontaneous otoacoustic emissions (SOAEs).

Conclusions: Function as a dynamical control parameter gives the MOC system exquisite control over cochlear amplification, with the ability to influence OHC power output well beyond what would be expected from a simple change in receptor potential modulation alone. Results further predict activation of the MOC system will reduce SOAEs and shift the frequency up, consistent with findings during acoustic stimulation of the contralateral ear.

MO112. A Novel Population of Short Actin Filaments at Stereocilia Tips Contribute to Stereocilia Widening

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Category: Hair Cells: Anatomy and Physiology

Background: Stereocilia have a core of parallel actin filaments (F-actin) oriented so only the fast-growing barbed ends are localized to stereocilia tips while the pointed ends are found at the stereocilia base. F-actin in the stereocilia core is highly stable, but actin at stereocilia tips turns over more rapidly. Our preliminary data show there is also a separate population of short actin filaments at stereocilia tips. Our hypothesis is that Myosin-3 (MYO3) uses tip filaments to widen stereocilia.

Methods: We identified tip filaments by probing permeabilized postnatal mouse cochlear tissue with purified, exogenous His-tropomodulin1 (His-TMOD1) protein. Tropomodulins are well-characterized proteins that bind pointed ends of actin filaments, but not barbed ends. To determine if MYO3 uses tip filaments we assessed tip filament levels in Myo3a/b mutant hair cells, as well as in MYO3A overexpressing inner hair cells. Actin incorporation in stereocilia was assessed by super-resolution microscopy and by monitoring EGFP-actin in live cells.

Results: His-TMOD1 labeled the tips of stereocilia in all rows before postnatal day 6 (P6), with labeling declining until P9. Tip filament levels were highest when stereocilia were widening, suggesting they may contribute to this aspect of stereocilia growth. MYO3A and MYO3B localize to stereocilia tips and contribute to stereocilia widening, suggesting these myosins could stabilize tip filaments and recruit them to the stereocilia core. Correspondingly, mutating Myo3a/b decreased tip filament levels. Overexpressing EGFP-MYO3A dramatically increased tip filament levels and also increased stereocilia tip width. Overexpressing EGFP-actin also increased stereocilia width. Super-resolution imaging of fixed samples showed that the newly expressed EGFP-actin signal surrounded the stable, preexisting F-actin core. Live-cell imaging revealed that overexpressed EGFP-actin initially localized to stereocilia tips, but then extended down the stereocilia shaft over time. Together, these data suggest that stereocilia widening begins at stereocilia tips when MYO3 recruits tip filaments, which subsequently contribute to creating new actin filaments around the stereocilia core.
Conclusions: Our data show that F-actin at stereocilia tips has both barbed and pointed ends when only barbed ends would be expected based on current models of stereocilia structure. We propose that there is a population of short actin filaments at stereocilia tips, which we call tip filaments. These tip filaments contribute to a MYO3-dependent tip-down widening mechanism.

MO113. Kif1aa-Based Microtubule Transport Maintains Synaptic Vesicle Populations in Hair Cells
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Category: Hair Cells: Anatomy and Physiology
Background: Sensory hair cells utilize specialized ribbon synapses to reliably transmit sensory information to the brain. Ribbon synapses have high rates of spontaneous vesicle release and function without fatigue. To sustain this level of release, a continuous supply of synaptic vesicles must be trafficked to the presynapse. In neurons, the motor protein Kif1a has been shown to transport synaptic vesicles along microtubules to the presynapse. Whether Kinesin-mediated transport delivers synaptic vesicles along microtubules to the hair cell presynapses is not known. Recent work in mice and zebrafish suggests that Kif1a is expressed in hair cells. Therefore, we hypothesize that Kif1a is necessary for synaptic vesicle transport in hair cells.
Methods: To study the role of microtubules and Kif1a in hair cells we study the zebrafish lateral-line system. For our analyses we have created CRISPR-Cas9 kif1aa zebrafish mutants to examine the role of Kif1aa in hair cells. We have also used pharmacology to destabilize (nocodazole) microtubules. To visualize synaptic vesicles, we used immunohistochemistry against Rab3 and Vglut3, markers of synaptic vesicle populations. In addition, we used the vital dye Lysotracker to visualize synaptic vesicles in vivo. We have also assayed the behavioral output of hair cell systems in kif1aa mutants by monitoring the acoustic startle response. Lastly, we used transmission electron microscopy to quantify the number of vesicles at the synapse in kif1aa mutants.
Results: Our immunohistochemistry show an enrichment of Vglut3 and Rab3 label at the base of wild-type hair cells, near the presynapses. This enrichment is absent in kif1aa mutants. Using Lysotracker we also observed an enrichment of synaptic vesicles at the base of wild-type hair cells – this enrichment was also absent in kif1aa mutants. Furthermore, after microtubules were destabilized using nocodazole, a similar loss of Lysotracker enrichment was observed. Using TEM, we found significantly fewer synaptic vesicles tethered at the ribbon and within 200nm from the ribbon in kif1aa mutants compared to wild-type siblings. To assess the impact of loss of synaptic vesicle enrichment we assessed acoustic startle responses. Our behavioral results indicate that despite a depleted vesicle population there is no significant difference in startle response between kif1aa mutants and their wild-type siblings at three levels of stimulus intensity or in a habituation assay using a five second inter-stimulus interval.
Conclusions: Our results indicate that Kif1aa and microtubules are required to enrich synaptic vesicles at the base of sensory hair cells. In the future we plan to use functional imaging and electrophysiology to assess hair cell synapse function in kif1aa mutants. Overall, this work will reveal how synaptic vesicles are transported to and maintained at the hair cell synapses. This work will shed light on the significance of synaptic vesicle pools for proper hearing and balance.

MO114. Short Term Exposure to Ship Noise Alters Dopamine Synthesis in the Auditory Midbrain and the Auditory Efferent System of Opsanus Tau (Oyster Toadfish)

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Category: Hearing Loss: Consequences and Adaptation
Background: Research in vertebrates shows that exposure to noise can cause temporary and even permanent hearing loss. All vertebrates have auditory efferents which include cholinergic neurons in the hindbrain that regulate activity of the inner ear through inhibition; little is known about the functional significance of auditory efferents in general, and even less about the subtype of dopamine auditory efferents. In midshipman fish, forebrain dopamine efferents innervate the saccule, the main endorgan of hearing, as well as cholinergic auditory efferents in the hindbrain; in females, innervation of both targets change with reproductive state and a release of dopaminergic inhibition of the saccule is proposed to enhance detection of the advertisement call of males in the reproductive season. The oyster toadfish (Opsanus tau), a close relative of midshipman, is an excellent model species to examine the effects of anthropogenic noise on brain and behavior because they rely on acoustic signals for mate attraction and social interactions. Importantly, these fish thrive in noise-rich environments such as waterways surrounding New York City and therefore may express neural and physiological adaptations to communicate in such environments. We hypothesized that toadfish exposed to anthropogenic noise will exhibit 1) an increase in the stress hormone, cortisol and 2) changes in dopamine synthesis and release as measured by phosphorylation of tyrosine hydroxylase (pTH), the rate-limiting enzyme in catecholaminergic synthesis, throughout the auditory system.
Methods: We exposed oyster toadfish to recordings of continuous ship engine noise or ambient sound for 30 minutes. We collected blood to examine cortisol levels by ELISA and used immunohistochemical techniques and fluorescent microscopy to measure pTH immunoreactivity (-ir) in neurons of the dopaminergic auditory efferent system and their terminals in the inner ear (saccule) and on cholinergic efferent neurons in the hindbrain. We also measured pTH-ir in the auditory midbrain and thalamus.
Results: We found no difference in cortisol levels between conditions, suggesting this type of noise may not be sufficient enough to produce a stress response in oyster toadfish. However, significant changes in pTH-ir innervation of cholinergic auditory efferent neurons, the auditory midbrain, and in dopaminergic cells in the diencephalon were found in the noise condition.
Conclusions: Our results suggest that in the presence of noise, an increase in dopamine synthesis in the forebrain may regulate activity of the inner ear both directly and indirectly, through modulation of the cholinergic auditory efferent system. Additionally, changes in pTH-ir activity in auditory midbrain may function as a filter for other relevant stimuli. Overall, multi-layered neuromodulatory regulation of the auditory system, starting at the inner ear, along with a reduced stress response to noise may serve as important adaptations for reproductive-related communication and survival in noisy urban environments.

MO115. A Novel Mutation in the Fera Domain of Otoferlin Causes Age-Dependent Progressive Hearing Impairment

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Category: Hearing Loss: Consequences and Adaptation
Background: Otoferlin protein is essential for hearing because of its crucial role in vesicle release and replenishment at the inner hair cell (IHCs) ribbon synapse. Mutations in OTOF, the gene encoding otoferlin, lead to autosomal recessive prelingual deafness, DFNB9, an auditory synaptopathy. Otoferlin consists of six C2 domains and a phospholipid binding FerA domain, the function of which is still unclear.
Methods: We created a new mutant mouse line, Otof-p.KL>M, carrying a three base pair deletion in the phospholipid binding domain, leading to replacement of the lysine 824 and leucine 825 by a methionine. Immunohistochemistry was used to evaluate otoferlin expression levels and degeneration of IHC and OHC in homozygous Otof-p.KL>M and wild type mice littermates. Whole-cell patch clamp was used to evaluate IHC exocytosis. Hearing function was assessed by auditory brain stem responses and distortion-product otoacoustic emissions at different ages (4-weeks, 6-month, 12-month). Gap detection experiments in the Intellicage were used to behaviorally assess temporal properties of synaptic transmission at the IHCs ribbon synapse.

Results: Mutant IHCs showed a progressive attenuation in otoferlin fluorescence at 6 and 12 months respectively. Immunohistochemical analyses revealed no OHC loss in the mutant mice and only mild IHC loss. There was no significant difference in IHC Ca2+ currents and exocytosis to short depolarizations. However, long-term depolarization (100 ms) resulted in less exocytosis in Otof-p.KL>M mice. ABR measurements in the homozygous Otof-p.KL>M mice indicated a significant reduction in wave I amplitudes in all age groups and a progressive elevation of thresholds. DPOAE were unaltered. The mutant mice exhibited elevated gap detection thresholds in comparison to control mice.
Conclusions: Otof-p.KL>M mice are the first mouse model for DFNB9 carrying a mutation in the phospholipid-binding FerA domain. Mutant mice exhibit reduced otoferlin expression and a moderate age-dependent progressive hearing impairment despite preserved active cochlear amplification. The impairment of sustained vesicle release and of gap detection in Otof-p.KL>M mice are consistent with a progressive hearing impairment phenotype seen in some otoferlin patients and with auditory fatigue observed in patients but also other Otof mutant mice.

MO116. Differences in Neural Correlates of Auditory Working Memory Between Cochlear Implant Users and Normal Hearing Controls

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Category: Hearing Loss: Consequences and Adaptation
Background: A common concern for individuals with severe-to-profound hearing loss fitted with cochlear implants (CIs) is difficulty following conversations in noisy environments. Previous literature has alluded to cognitive resources related to attention and working memory as a factor explaining some the variability associated with speech in noise perception. However, the neural basis for this relationship is not fully understood.
Methods: In this study, we investigated behavioural and neural correlates of auditory working memory in 14 CI users and normal hearing (NH) controls using high-density electroencephalogram (EEG) while participants completed an N-back task consisting of two conditions, 0-back and 2-back. While 0-back measured speech perception ability, the 2-back measured cognitive ability through working memory and attention. The auditory stimuli presented for each condition and trial was ten double-digit numbers (DDN).
Results: Behavioural results suggest no differences between groups in both conditions but in both groups, participants performed better on the 0-back than the 2-back. Although no behavioural differences were found between groups, differences were observed in sensory and neural oscillatory activity. CI users, overall, showed decreased evoked responses (P1, N1, and P2) to digits compared to NH and showed differences in alpha/beta and beta activations throughout the encoding and retaining of digits into memory. Importantly, the degree of auditory evoked potentials and oscillatory power were significantly correlated to speech perception in noise in CI users and NH.
Conclusions: These results show neural differences in both bottom-up (encoding) and top-down (attention and working memory) processes in CI users which may contribute to difficulties speech communication.

MO117. Open Board

MO118. Early Hearing Loss in 5xFAD Transgenic Mouse Model of Alzheimer's Disease

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Category: Hearing Loss: Consequences and Adaptation
Background: Previous studies have implicated hearing loss in Alzheimer’s disease (AD). In fact, clinical studies have reported that patients diagnosed with AD have shortfalls in auditory processing, speech perception and interpretation. The development of 5xFAD transgenic mice has enabled better characterization of hearing deficits in AD, as these mice express distinct mutant proteins [3 amyloid precursor peptide (APP) mutations and 2 presenilin mutations] identified with AD in human genes. Therefore, this study was aimed to determine whether hearing deficits (elevated thresholds and cochlear

pathology) may serve as early biomarkers of AD in 5xFAD transgenic mice. The transgenic (C57BL6 and 5xFAD) mice, each having equal number of males and females (N= 12/group) were purchase from Jackson Laboratories and raised at the SIU School of Medicine Laboratory Animal Care facility.
Methods: Auditory brainstem response (ABR) threshold was determined in both animal types using Frequent Intelligent Hearing System (Miami FL) and repeated at an interval of approximately 4 weeks over a 7-month period. The animals were then euthanized and the cochleae were harvested for further immunohistochemical analysis.
Results: The ABR thresholds of C57BL6 mice were relatively stable up to 20 weeks of age, but showed slight elevations at 24 weeks and even greater elevations at 28 weeks. ABR thresholds at a frequency of 8 kHz were significantly ($p < 0.001$) higher in 5xFAD compared to the wild type (C57BL6) at 12, 20, 24 and 28 weeks. A similar trend was observed at a frequency of 16 kHz where the ABR values were significantly ($p < 0.01$) higher in the 5xFAD transgenic mice compared to the wild-type mice. At 32kHz, the ABR values were significantly ($p< 0.001$) higher in 5xFAD compared to C57BL6 mice at 12 and 24 weeks but not at other time points. We also observed significant time-dependent reductions in Wave 1 amplitudes in both C57BL6 mice and 5xFAD, which precede deficits in ABR thresholds. Time-dependent reductions in amplitudes in 5xFAD were more rapid than in C57BL6 mice. Both C57BL6 and 5xFAD mice showed age-dependent hearing deficits. However, the hearing deficits preceded and were more pronounced in 5xFAD mice, starting at 12 weeks and extending to 28 weeks. Whole mount preparations obtained from these mice at 28 weeks show statistically significant loss of outer hair cells in the basal and middle turns of the cochlea in the 5XFAD mice at 28 weeks, compared to age-matched C57BL6 mice.
Conclusions: These data indicate significant early deficits in hearing in the 5XFAD mouse model which could precede the onset of memory deficits.

MO119. Effects of Sensorineural Hearing Loss on Auditory Discrimination of Natural Soundscapes
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Category: Hearing Loss: Consequences and Adaptation
Background: "Natural soundscapes" correspond to complex arrangements of biological and geophysical sounds shaped by (natural) habitat-specific sound propagation effects. The capacity to build a "perceptual soundscape" should be useful for mapping the close environment, navigating, assessing resources and danger, or building a sense of place and time. However, despite the high adaptive and psychological value of processing natural soundscapes, very little is known about the auditory cues and mechanisms at work. This is true not only for typical (normal) hearing but also for all forms of hearing disorders. This is quite surprising given the numerous benefits of exposure to natural sounds, such as improved health and cognitive outcomes, positive affects and decreased stress and annoyance. The goal of the present study was to assess natural soundscape discrimination for listeners with bilateral mild-to-severe sensorineural hearing loss between 0.25 and 8 kHz.
Methods: The ability to discriminate natural soundscapes was measured in 15 hearing-impaired (HI) listeners with bilateral, mild to severe sensorineural hearing loss and 15 normal-hearing (NH) controls. The NH and HI listeners were tested using a database of acoustic recordings from a temperate terrestrial biome: the biosphere reserve of the Sequoia National Park in the USA (Krause et al., 2011). The recordings were made in four distinct habitats - a riparian forest, a meadow, a chaparral and a grassland - during four seasons and four periods of the day. Soundscape discrimination was measured using a three-interval (forced choice) oddity paradigm and the method of constant stimuli. On each trial, sequences of 2-second recordings varying the habitat, season and period of the day were presented diotically at a nominal SPL of 60 dB except for one HI listener who was tested at a nominal SPL of 80 dB.
Results: Discrimination scores were above chance level for both groups, but they were poorer for HI than NH listeners. On average, the scores of HI listeners were relatively well accounted for by those of NH listeners tested with stimuli spectrally shaped to match the frequency-dependent reduction in audibility of individual HI listeners. However, the scores of HI listeners were not significantly correlated with pure-tone audiometric thresholds and age.
Conclusions: These results indicate that the ability to discriminate natural soundscapes associated with changes in habitat, season and period of the day is degraded but it is not abolished. The deficits of the HI

listeners are partly accounted for by reduced audibility. Supra-threshold auditory deficits and individual listening strategies may also explain differences between NH and HI listeners.

MO120. The Role of Early Mechanotransduction in Development of the Auditory System

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Category: Inner Ear: Anatomy and Physiology

Background: In the developing auditory system, spontaneous bursts of electrical activity, initiated by a group of supporting cells in the cochlea, propagate from the periphery to sound processing circuits in the brain. In rodents, this spontaneous activity emerges before birth at around embryonic day 16. It lasts until the onset of the hearing (~ postnatal day 12), providing a prolonged time window for activity-dependent circuit refinement and maturation. Interestingly, intrinsically generated spontaneous activity is not the only activation mode available to the hair cells during this postnatal period. Even though the ear canals are filled with fluid and connective tissue and unable to transmit sound-induced vibrations to the cochlea, the mechanotransduction complex at the apical surface of the hair cells is already assembled and responsive to mechanical stimuli. Remarkably, supporting cells in the developing cochlea undergo dramatic changes in shape during spontaneous events, as a result of osmotic shrinkage, which can physically displace hair cells. These findings raise the possibility that supporting cell movements may amplify hair cell depolarization by triggering hair cell movement, bundle deflection and opening of mechanotransduction (MET) channels. However, the role of morphological events associated with spontaneous activity and the early presence of the mechanotransduction apparatus is still unclear. It is postulated that the morphological events could act as the mechanical force inducing mechanotransduction in place of acoustic signals from the external environment before the opening of the ear canal.

Methods: To investigate this hypothesis, a newly generated mouse line (Tecta-iCre) will be used in combination with electrophysiological recording and in-vivo calcium imaging to quantify the change in neural activity patterns in the cochlea, inferior colliculus, and the auditory cortex across developmental ages with an intact or disrupted tectorial membrane.

Results: Together, these studies will help define the interplay between spontaneous activity and early mechanotransduction in auditory circuit development, with relevance to restoration of hearing in individuals with congenital hearing loss.

Conclusions: Together, these studies will help define the interplay between spontaneous activity and early mechanotransduction in auditory circuit development, with relevance to restoration of hearing in individuals with congenital hearing loss.

MO121. Three-Dimensional Histopathologic Analysis of Human Inner Ears With Enlarged Vestibular Aqueduct: Implications for Radiologic Diagnosis and Cochlear Implantation

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Category: Inner Ear: Anatomy and Physiology

Background: Enlarged vestibular aqueduct (EVA) is the most common inner ear malformation in patients with sensorineural hearing loss (SNHL). Cochlear implantation outcomes in EVA patients are variable, and while some studies suggest this may be related to inner ear morphology, this has not yet been characterized by human histopathology. Here we present the first study that assesses vestibular aqueduct (VA) morphology, cochlear morphology, and spiral ganglion neuron (SGN) distribution in human temporal bone specimens with EVA.

Methods: The NIDCD National Temporal Bone Registry was searched for cases with descriptions of large VA, endolymphatic duct, endolymphatic sac, or sudden SNHL without documented EVA. All cases were histologically processed, and 20 um sections were digitized for three-dimensional (3D) analysis. Twelve cases met published diagnostic criteria for EVA based on midpoint or operculum measurements. 3D reconstructions were created and volumetric measurements of VA, whole cochlea and scala tympani (ST) were obtained. A machine learning algorithm was used to quantify SGNs. Midpoint width, operculum width, and VA volume were measured. VA, cochlear and ST morphology and SGN counts were compared between EVA and age and sex-matched control cases. Comparisons were substratified by isolated EVA (iEVA) versus EVA cases with incomplete partition type 1 or 2 (EVA+IP1/2).

Results: We evaluated 12 EVA cases, 6 iEVA and 6 EVA+IP1/2, and 12 controls. Mean VA volume was 32.9±32.7 uL (range 4.4-103.9 uL) in EVA cases and 5.3±4.7 uL (range 0.9-11.9 uL) in controls (p=0.020). Within the EVA cohort, EVA+IP2 cases had larger midpoint (p=0.008), operculum (p=0.026), and VA volumes than iEVA cases (p=0.002). Operculum width was strongly correlated with VA volume in control (r=0.75) and EVA cases (r=0.94). Midpoint width was poorly correlated with VA volume in control (r=0.27) and EVA cases (r=0.64). On histopathology, one iEVA case had a foreshortened cochlea with fused chambers, and another had a shortened cochlea with an apical scala communis. Neither case met criteria for CH-2 or IP1/2. There was no significant difference in cochlear or ST volume between iEVA and control cases. Mean SGN count in iEVA cases was 16,632±9,198 (range 1,548-25,992) and 49% (range 4%-77%) of normative age-matched controls.
Conclusions: Our results suggest there is a wide range of anatomical variations among patients with EVA. VA volumes were larger in EVA+IP1/2, and this measurement correlated strongly with opercular width. A subset of iEVA cases had cochlear malformations that did not meet criteria for IP1/2 or CH-2, suggesting that there may be a spectrum of EVA-associated cochlear anomalies not captured by existing classification systems. SGN counts ranged widely between EVA cases; one case demonstrated significant neuronal atrophy. Collectively, these results support the theory that individual differences in cochlear morphology and neuronal degeneration may contribute to variability in cochlear implant outcomes among patients with EVA.

MO122. Open Board

MO123. Expression of GJB2 and GJB6 Gene Transcripts in the Human Cochlea a Study Using RNAscope--- Confocal and Super-Resolution Structured Illumination Microscopy
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Category: Inner Ear: Anatomy and Physiology
Background: Connexin26 and 30 proteins are richly expressed in the human cochlea where they are involved in transcellular signaling, metabolic supply and fluid homeostasis. Disruption of their genes GJB2 and GJB6 cause profound autosomal recessive non-syndromic deafness. Here, we analyzed for the first time the various expression of GJB2 and GJB6 gene transcripts at different locations of the human cochlea using the RNAscope technique.
Methods: Archival paraformaldehyde-fixed sections of surgically obtained human cochleae were used to label single mRNA oligonucleotides using the sensitive multiplex RNAscope® technique with fluorescent-tagged probes. Positive and negative controls also included localization of ATP1A1, ATP1A2 and Kir4.1 gene transcripts to validate the specificity of cellular labeling.
Results: Confocal and super-resolution structured illumination microscopy (SR-SIM) detected single gene transcripts as brightly stained puncta. GJB2 and GJB6 genes transcripts were distributed in all three turns with some diminished expression against the base. The largest number of GJB2 and GJB6 transcripts was in the outer sulcus, spiral ligament and stria vascularis. Both oligonucleotides were demonstrated in supporting cells of the organ of Corti, spiral limbus fibrocytes and floor of the scala vestibuli. Multiplex gene data suggest that cells in the cochlear lateral wall contains either GJB2 or GJB6 gene transcripts or both. GJB6, but not GJB2 transcripts were found in the intermediate cells and none of them in the marginal cells. There were no GJB2 and GJB6 transcripts in type III fibrocytes or in spiral ganglion cells.
Conclusions: Localization of GJB2 and GJB6 mRNA gene transcripts could be assessed in the adult human cochlea using RNAscope® ISH and high resolution microscopy. Results suggest that cells in the human cochlea contains either GJB2 or GJB6 gene transcripts or both. This may be consistent with specialized GJ plaques having separate channel permeability and gating characteristics. Such information may be useful for future gene therapy.

MO124. Anatomical Variations of the Human Cochlea Using an Image Analysis Tool
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Category: Inner Ear: Anatomy and Physiology
Background: Preservation of neural structures and residual hearing is of high importance in cochlear implant (CI) patients. However, the delicate process of CI electrode insertion is prone to introduce damage

to cochlear structures. Cochlear damage may be mitigated by atraumatic surgical procedures and optimal electrode array designs. Cochlear size and morphology are known to have large interindividual variability as observed in recent studies. Manufacturers may offer electrode arrays that best match the needs of individuals by means of providing electrodes with different dimensions that are the most suitable for the candidates. Understanding the cochlear anatomy is crucial for developing atraumatic electrode arrays and insertion guidance systems for cochlear implantation.
Methods: This study analyses 1099 clinical temporal bone computed tomography (CT) images using a semi-automatic web-based image analysis tool (Nautilus, Oticon Medical). Global and local cochlear size and shape parameters such as A, B, and height dimensions, volume, area, duct lengths, wrapping factor, wrapping ratio, rollercoaster height etc. were obtained to determine statistics and perform regression and correlation analysis. Scala tympani duct diameter and area were also analyzed to determine the optimal characteristics for electrode array design. Additionally, the similarity between the contralateral ears was also analyzed for implantation scenarios in which a preoperative CT-scan is not available.
Results: The analysis revealed cochlear morphology follows Gaussian distribution while cochlear dimensions A and B are not correlated well to each other. Contrary to popular opinion, B dimension is more correlated to cochlear duct lengths, wrapping factor and volume than A dimension. The scala tympani size varies considerably among population with the size generally decreasing along insertion depth with dimensional jumps through the trajectory. The mean scala tympani radius was 0.32 mm near the 720° insertion angle. Inter-individual variability was four times compared to intra-individual variation. The left and right ear CT images are well correlated to each other. Moreover, on average both the ears are of similar dimensions. However, statistically significant differences were observed in clinically relevant dimensions between ears of the same patient suggesting that size and shape are not the same.
Conclusions: All previous studies either focused on uCT measurements which require a significant amount of effort limiting the population size or were restricted to only global dimensions such as A, B and lateral wall duct length. However, to guide electrode development, detailed information is required about the variability of parameters that describe the cochlear size and shape. In this study, variability, and correlation of cochlear parameters, extracted via artificial intelligence tools are investigated in a large set of 1099 cochleae. Harnessing deep learning based automated image analysis tools, our results yield important insights into cochlear morphology and implant development helping in reducing insertion trauma and preserving residual hearing.

MO125. Whole Organ Imaging of the Mammalian Vestibular System

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Category: Inner Ear: Anatomy and Physiology
Background: Aging is associated with an increased risk of falling, which is contributed to by diminished balance. However, the precise anatomical changes occurring within the vestibular system as it ages are debated (1, 2).
Methods: In this study, we used immunofluorescence, tissue clearing, and 2-photon microscopy to visualize the anatomical changes that occur as the vestibular system ages. The entire temporal bone was dissected and decalcified, instead of dissecting individual vestibular organs, to minimize tissue damage and distortions during dissection. We compared two clearing methods, an aqueous-based modified ScaleS method and a solvent-based ethyl cinnamate method (which have both been successful in clearing cochleae in previous studies) (3, 4), to find which works best for the vestibular organs. These methods allow imaging of the entire vestibular system sensory cells in their native orientations.
Results: Our goal is to quantify hair cells and synapses within the entire vestibular epithelia in mature (1-2 months) and aged (36-40 months) gerbils using automated analyses involving machine-learning algorithms. We provide some preliminary progress on imaging and analysis.
Conclusions: Our study will aid in a deeper understanding of the anatomical changes in the mammalian vestibular system with age.
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MO126. Preservation of Spontaneous Activity in the Auditory System of Connexin 26 Deficient Deaf Mice

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Category: Inner Ear: Anatomy and Physiology

Background: Supporting cells in the cochlea comprise a molecularly and physiologically diverse group of non-sensory cells that are required for hearing. Despite their distinct features, they are extensively coupled to each other through gap junctions, forming a functional syncytium that coordinates cellular maturation and ionic homeostasis. This intercellular coupling is enabled by the expression of connexins, including Connexin 26 (Cx26) encoded by Gjb2, mutations of which are the most prevalent cause of congenital hearing loss. In the absence of Cx26, fluid filled spaces in the sensory epithelium fail to form, airborne sound detection is profoundly impaired, and hair cells progressively degenerate. Cx26-mediated intercellular coupling is prevalent within inner supporting cells (ISCs) prior to hearing onset, a time when these cells initiate spontaneous bursts of activity to promote maturation of sound processing circuits. However, the consequences of Cx26 deficiency on pre-hearing spontaneous neural activity patterns and the development of central auditory pathways are unknown.

Methods: To understand how Cx26 deficiency influences spontaneous activity in the developing auditory system, we used a knock-in mouse line (Tecta-Cre) to restrict deletion of Cx26 to cochlear supporting cells (Cx26 cKO).

Results: Whole cell recordings in excised P7 cochleae from Cx26 cKO mice revealed that ISCs unexpectedly retained low membrane resistance and intercellular dye transfer indicative of preserved gap junctional coupling. Moreover, ISCs, hair cells and spiral ganglion neurons still exhibited robust bursts of spontaneous activity. Consistent with these observations, in vivo calcium imaging revealed that neurons in the inferior colliculus (IC) of P7 Cx26 cKO mice continued to exhibit temporally and spatially correlated bands of activity aligned to future isofrequency domains. Despite the persistence of pre-hearing neural activity in Cx26 cKO mice, at hearing onset (~P12-P15) they exhibited significantly higher auditory thresholds than controls (>50dB SPL), as assessed through auditory brainstem measurements and in vivo calcium imaging, indicating that acoustic sensitivity is impaired despite preservation of hair cells and neurons. However, neurons in IC and auditory cortex (AC) of awake, unanesthetized Cx26 cKO mice at this age were activated by suprathreshold tonal stimuli with spatial and temporal neuronal activity patterns remarkably similar to controls, demonstrating that tonotopic organization of the auditory system remains largely intact in these hearing-impaired mice. With increasing age, neural responses to suprathreshold tonal stimuli became larger and prolonged, with more tone-responsive cells observed across broader AC territories in Cx26 cKO mice, an adaptive response to the lack of peripheral input after cessation of spontaneous activity.

Conclusions: Together, these results indicate that cochlear spontaneous activity is preserved in the absence of Cx26, enabling activity dependent maturation of auditory circuits and homeostatic control of acoustic sensitivity. Preservation of this early patterned activity may help establish neural networks that can be engaged by cochlear prostheses to enable hearing.

MO127. Loss of PEX1 Affects Inner Hair Cell's Ribbon Synapse Maturation and Auditory Function

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Category: Inner Ear: Anatomy and Physiology

Background: Peroxisome Biogenesis Disorders (PBD) or Zellweger syndrome spectrum disorders (ZSD) are a group of rare genetic multisystem disorders characterized by partial or total defect in peroxisome synthesis, assembly, and/or function. PBD-ZSD is associated with neurosensory hearing loss, retinopathy, multiple organ dysfunction and psychomotor impairment. Mutations in 14 peroxin (PEX) genes have been found to cause PBD-ZSD. Mutations in PEX1 are the most common, representing 70 percent of the cases (Reuber et al. 1997). Based on genotype-phenotype correlations, PBD-ZSD has been classified into class I (less severe, survival of 2 years to above 45 years) or class II (more severe, survival of less than 12 months). Limited research has focused on the impact of peroxisomal disorders on auditory function, hampering the development of treatments for PBD-ZSD patients.
Hypothesis: As hair cells are particularly sensitive to metabolic changes, we hypothesize that mutations in PEX1 leads to an increase of oxidative stress leading to hearing loss by affecting hair cell functions and survival along the cochlea.
Methods: Global deletion of the Pex1 is neonatal lethal in mice impairing any postnatal studies. To overcome this limitation, we created two conditional knockout (cKO) mouse by breeding Pex1fl/fl mouse with either Gfi1-CRE (Gfi1cre/+Pex1fl/fl) or VGlut3-CRE (VGlut3cre/crePex1fl/fl) mice to allow for selective deletion of Pex1 in the inner ear. We measured auditory brainstem responses (ABR) to click and pure tone stimuli from 5.6 to 32.0 KHz at 1 and 4 months of age. Whole mount cochleae were stained with Myo7a, CtBP2 and GluR2 antibodies to assess inner hair cell's (IHC) synapse. Samples were imaged using the Zeiss LSM800 confocal microscope and analyses were done using Imaris Cell Imaging software (Oxford Instruments). The level of peroxisomes was determined by western blot with PMP70 antibody (labeling peroxisomal protein). We also evaluated electrophysiological changes in IHCs using whole cell patch clamp recordings.
Results: Pex1 excision in IHCs leads to progressive hearing loss associated with significant decrease in ABR wave I amplitudes ($P<0.0001$). To determine if this change was caused by alterations in IHC-SGN synapses, cochleas were stained with CtBP2 (pre-synaptic) and GluR2 (post-synaptic) markers. We observed a decrease in ribbon synapse number and volume especially in VGlut3cre/crePex1fl/fl mice (strong deletion of Pex1 in IHCs). Associate to that, we observed a decrease in peroxisomal number that we hypothesize is the result of oxidative stress imbalance.
Conclusions: Taken together, these results suggest a critical function of Pex1 in development and maturation of IHC-SGN synapses as well as hearing function.

MO128. Adaptive Stimulus Selection to Efficiently Estimate Auditory Brainstem Response Hearing Threshold Across Frequencies

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Category: Inner Ear: Anatomy and Physiology
Background: Evoked potentials from the Auditory Brainstem Response (ABR) can be used to estimate the hearing threshold of animals and humans who are unable to complete a behavioral audiogram. In a typical experiment, measurement of ABR waveform is repeated for a pre-determined list of test frequencies and levels, while not all test conditions are informative about hearing thresholds. Further, the gold standard in determining ABR threshold is visual inspection, which may introduce bias related to human subjectivity. Here, we present an adaptive procedure designed to (1) improve the efficiency of ABR threshold detection by iteratively leveraging a subset of stimuli and (2) reducing the subjectivity of traditional ABR by defining a statistical criterion for threshold.
Methods: The threshold is determined using a Bayesian adaptive procedure that samples the stimulus space (frequency and level) in an optimized sequence. First, the response is collected from a predetermined small set of (~8) initial stimuli. A Gaussian process (GP) model is fitted to the collected data in both the frequency and level dimensions simultaneously. The GP model returns the predicted response across the entire stimulus space. The threshold is defined according to a statistical criterion at each frequency. From this point, each subsequent stimulus condition (frequency and level) is iteratively optimized so that the expected uncertainty of the model-predicted threshold is minimized. The algorithm terminates after 20 iterations, including possible repeated presentations of the same stimulus.
The adaptive procedure is validated using previously collected data from two species: mice and budgerigars, comprising 19 ears with and without hearing loss, with approximately 70-100 stimuli per dataset. It is necessary to identify appropriate parameters (e.g., covariance assumptions in the GP model) to account for

the different frequency range and stimulus levels available in each dataset. The algorithm is validated through numerically simulated experiments, in which simulated ABR waveforms are generated by adding characteristic noise to the existing measurements. The algorithm-estimated threshold is compared with human-rater estimated thresholds. Test/retest reliability is evaluated.
Results: The algorithm performs satisfactorily with respect to the human-estimated threshold for the normal hearing mice and budgerigars. The algorithm performs less well on the hearing-impaired mice and budgerigars. With only minor modifications to the initialization parameters, the same algorithm can estimate the thresholds for two different species, despite considerable differences in the range of testing frequencies (mice: 4 – 32 kHz; budgerigars: 0.5 – 6 kHz). Threshold estimates from repeated simulation runs are reasonably consistent, comparable to the variability among human raters.
Conclusions: The results indicate that the adaptive algorithm may be able to improve the efficiency of ABR-based threshold estimation in multiple species of lab animals with minimal modifications, saving both time and resources.

MO129. Alpha9alpha10 Knockout Mice Show Altered Physiological and Behavioral Responses to Signals in Background Noise

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Category: Inner Ear: Anatomy and Physiology
Background: The medial olivocochlear (MOC) efferent system modulates peripheral responses to incoming sounds to support encoding of signals in noise and protect from acoustic injury. MOC neurons reduce outer hair cell motility through specialized nicotinic acetylcholine receptors. Transgenic mouse models that lack subunits of these receptors, such as alpha9 receptor subunit knockouts (α9KOs), lack classic cochlear suppression effects. These mice have normal hearing sensitivity in quiet and noise, but show abnormal temporal, spectral, and spatial hearing. A transgenic model that is deficient for both the alpha9 and alpha10 nicotinic acetylcholine receptor subunits (α9α10KO; Morley et al. 2017) may represent a more complete genetic lesion of the MOC pathway. Like α9KOs, α9α10KOs have normal auditory brainstem response (ABR) thresholds and weak MOC reflexes. Here, we provide further characterization of auditory function in adult α9α10KO mice by assessing: 1) ABRs in quiet and noise, 2) acoustic startle responses (ASRs) in quiet and noise, and 3) frequency and intensity difference limens using prepulse inhibition (PPI) of the ASR.
Methods: Subjects were 3- and 5-month-old (m.o.) α9α10KO (n = 9, 9) and C57BL/6J mice (wildtype controls; n = 10, 7). ABRs were recorded to clicks and tonebursts (4, 8, 12, 16, 24, 32 kHz; 90-10 dB SPL, 10 dB steps) in quiet and in 50 dB SPL broadband noise. ABR thresholds and Wave I-IV amplitudes and latencies were measured. ASRs were recorded to short pulses (70, 80, 90, 100, 105 dB SPL; 10 times per level) in quiet and in 60 dB SPL broadband noise. PPI was recorded to pulses of 105 dB SPL using a continuous tone (10 kHz, 65 dB SPL) that changed in frequency (+/- 0.5-3 kHz) or intensity (+ 5-15 dB) prior to the pulse. Statistical analyses using linear mixed effects models were conducted to evaluate effects of strain, age, and sex.
Results: 3 m.o. wildtypes and α9α10KO mice had similar ABR thresholds, amplitudes, and latencies in quiet and noise. In contrast, the 5 m.o. α9α10KO mice showed significantly higher 8 kHz masked ABR thresholds and significantly shorter masked ABR latencies than 3 m.o. α9α10KOs and wildtypes. ASR in quiet and noise, FDL, and IDL functions were similar across all groups. However, startle amplitudes were 25-50% smaller in 5 m.o. α9α10KOs in all conditions.
Conclusions: Masking noise had a greater impact on sensitivity at 8 kHz in 5 m.o. α9α10KOs, consistent with MOC dysfunction. Shorter masked ABR latencies in 5 m.o. α9α10KOs may reflect differences in synaptic transmission or neural conduction. Reduced acoustic startle responses in 5 m.o. α9α10KOs in the absence of elevated hearing thresholds may reflect motor deficits rather than reduced reactivity to loud sounds. These findings should be taken into consideration when using α9α10KOs to study hearing and MOC dysfunction.

MO130. Development of a Chronic in Vivo Cochlear Window to Image Afferents and Efferent Fibers

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Category: Inner Ear: Anatomy and Physiology
Background: Chronic two photon in vivo imaging of subcellular neuronal structures—such as axons and dendrites—has become a ubiquitous practice in the central nervous system (CNS) following the development of glass window implantations. Imaging preparations over superficial CNS structures—such as the cortex, inferior colliculus, cerebellum, and spinal cord—have used these glass windows, whereas imaging of deep brain structures has been achieved by incorporating prisms, gradient index (GRIN) lenses, and three photon imaging.
Two sets of neurons, the afferent neurons of the spiral ganglion and the efferent neurons of the olivocochlear (OC) system, connect the central auditory nervous system to the peripheral auditory nervous system. The dendrites and axons of these neurons, respectively, that project to the organ of Corti are encased deep within the otic capsule of the cochlea. The cochlea's position within the base of the skull—and in rodents and several other mammals, additional enclosure by the auditory bulla—make this a uniquely difficult area to image.
As a result, structural and functional studies of these afferent and efferent neurons within the inner ear have been mostly limited to in vitro, ex vivo, and fixed preparations. Typical ex vivo cochlear preparations such as those used for patch clamp electrophysiology have short (hours-long) lifespans and lack the cell bodies of efferent neurons as well as critical feedback from the brain. Additionally, these preparations are generally limited to younger ages due to the fragility of the tissue at older ages.
Methods: We test various considerations including surgical approach, objective lens selection (refractive indices, immersion medium, working distance, magnification, and numerical aperture), structure and activity indicators (fluorophore choices, appropriate multiphoton excitation and emission spectrums, virus and transgenic mouse line availability), patch clamping compatibility, anesthesia choices and tradeoffs, head fixation, sealant preparations, stimulation paradigms, and long-term tolerability for the mice.
Results: We present the beginning stages for developing an in vivo cochlear window for mice through three stages of increasing intactness: 1) an extracted cochlea with the temporal bone intact, 2) an acute, unsealed in situ prep within an anesthetized mouse, and ultimately, 3) a chronic sealed preparation in an awake mouse.
Conclusions: The successful development of an in vivo imaging window into the cochlea could provide a wealth of new research opportunities to study these afferent and efferent projections. While each stage of preparation will allow study under scenarios far closer to a natural state than typically achieved, the ultimate goal of a chronic, sealed preparation would provide the opportunity to measure long term effects of auditory stimulation on these neurons.

MO131. Macro- and Micro-Mechanical Vibration Patterns in the Apical Turns of the Guinea-Pig Cochlea

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Category: Inner Ear: Cochlear Mechanics
Background: There exist many claims, old and recent, that the mechanics in the apex of the cochlea is fundamentally different from that in the base. On the other hand, recent observations in the base have revealed that several phenomena previously thought to be unique to the apex (strong rectification; compressive I/O functions over a very wide frequency range), are found just as well in the base. They had been missed because the old measurement techniques in the base were confined to the basilar membrane (BM).
Methods: Sound-evoked vibration patterns were mapped out in the apical turns of the cochlea in deeply anaesthetized guinea-pigs using optical coherence tomography (OCT). We obtained high-resolution vibration maps of the whole cross section of the organ of Corti in which the basilar membrane and Reisner's membrane can be clearly distinguished.
Results: Each location exhibited broad, asymmetric frequency tuning, with a much steeper roll-off at high frequencies than at low. From mechanical responses to tone complexes we determined the best frequency (BF) and the frequency-dependent group delay (GD) relative to stapes motion. We found that BF and GD at each site varied systematically with longitudinal location, with BF GDs ranging from ~2 ms at 800 Hz nearer to the base of the cochlea (in turn 3) to ~4 ms at 140 Hz nearer to the apex (in turn 4).

Conclusions: These findings contrast quite markedly with those reported by other investigators using similarly 'modern' observation techniques in the apex of the guinea pig, but are more consistent with other, older ideas and data, including auditory nerve measurements in the cat (Kim et al, 1980; Liberman 1982) and the guinea pig (Palmer and Shackleton, 2008). Just like these older data, and recent OCT data from the 2.5-kHz region of the gerbil cochlea (Meenderink et al, 2022), our findings corroborate the tonotopical organization of the apex, with BFs systematically decreasing towards the apical end. Our observations also provide further evidence that micro-mechanical movements, i.e. motions that occur within and across the volume of the organ of Corti, include longitudinally-directed movements, as well as anti-phasic motion at the bases and apices of the outer hair cell soma.

MO132. Motion of the Cochlear Partition of Intact Human Cadaver Temporal Bones Measured With Optical Coherence Tomography and Vibrometry

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Category: Inner Ear: Cochlear Mechanics
Background: Recent work had found substantive anatomical and motion differences between the human cochlear partition (CP) and that of most laboratory animals at the cochlear base. For example, motion of the scala tympani (ST) surface of the basal human CP—which includes the surface of the osseous spiral lamina (OSL), bridge, and basilar membrane (BM) —were measured with laser Doppler vibrometry (LDV) after removal of the round window membrane. It was found that the OSL has a hinged plate-like motion and the bridge moves with the BM. In the present study, our aim is to image through the intact round window with optical coherence tomography (OCT) to visualize and measure the motion of structures beyond the ST surface of the CP.
Methods: CP motion in response to sound was measured while visualizing specific structures with OCT vibrometry in three very fresh (<29 hrs postmortem) cadaveric human temporal bones. We made measurements of 20-26 radial locations within the CP in each specimen. Cross-sectional imaging and vibrometry measurements were made using a Spectral-Domain OCT system with a 900-nm center wavelength and a line-scan camera frame rate of 46-kHz (GAN620C1, Thorlabs, Germany). The axial resolution is 2.23 µm (in water) and the lateral resolution is ~8 µm, using a 36 mm, 0.055NA, 2x objective lens. Custom-built LabVIEW-based software (VibOCT v2.1.5) recorded images and made vibration measurements. SyncAv (v0.47) generated pure-tone sequences from 0.1-21 kHz equalized for constant SPLs of ~80-110 dB in the ear canal. Stapes velocity was also measured with LDV as a reference for cochlear input. All measurements were measured synchronous to input sound.
Results: This study presents detailed OCT images and vibrometry of very fresh intact human cochleae. CP structures and details can be appreciated, such as the vestibular and tympanic plates of the OSL, the bridge, the structures of the organ of Corti (OoC), the limbus, and the tectorial membrane (TM). The motion of the CP surface facing ST was generally consistent with previous findings measured with LDV. For example, the BM and bridge surface facing ST vibrated like a beam with respect to radial position at most frequencies. However, structures such as the reticular lamina surface and the TM had a more complex motion that varied with frequency.
Conclusions: The ST surface of the bridge and BM (which has running continuous collagen fibers) has a simple beam motion independent of frequency. The scala-media surface of the OoC—the RL surface—was not always the same as the BM-bridge, and these differences were frequency dependent. This may reflect the compliant properties of the OoC structures as compared to the BM-bridge.
Supported by grants R01 DC013303 and T32 DC000038 (Training for Speech and Hearing Sciences) from the NIDCD/NIH

MO133. MET Channel Blockade Causes Endolymphatic Hydrops in Mice

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Category: Inner Ear: Cochlear Mechanics
Background: Noise or blast trauma is associated with stereociliary damage, cochlear synaptopathy, and hair cell death. Endolymphatic hydrops (ELH), defined as an increase of endolymph volume in the membranous labyrinth of the inner ear, also occurs after noise or blast and is predictive of cochlear synaptopathy. ELH may occur after trauma because impaired K+ recycling results in K+ accumulation and an increased osmotic load within scala media after mechanoelectrical transduction (MET) is impaired. Glutamate excitotoxicity may also be involved in the development of ELH. Here we test the working hypothesis that MET channel blockade is sufficient to cause ELH. To do so, we perfused streptomycin, an aminoglycoside known to partially block MET channels, throughout the perilymphatic fluids via a posterior semicircular canal (PSCC) injection and studied the cochlea in vivo using volumetric optical coherence tomography and vibrometry (VOCTV).
Methods: We injected adult wild type (CBA/CaJ), Vglut3KO, salsa, and TectaC1509G/C1509G mice of either sex with streptomycin (50 mM) or artificial perilymph via a PSCC canulation. We used our previously described VOCTV system to collect cross-sectional images of the apical cochlear turn and to measure sound-evoked basilar membrane vibrations. This was done both before and up to three hours after injection. The ratio between the cross-sectional areas of scala vestibuli and scala media were used to quantify changes in endolymph volume.
Results: Wild-type mice had active cochlear mechanics and no ELH. After streptomycin injection, cochlear mechanics became passive and ELH developed, consistent with the concept that MET channel blockade causes ELH. No changes occurred after perfusion of artificial perilymph, confirming that the PSCC injection procedure itself does not alter cochlear physiology. In transduction-impaired mutants (salsa and TectaC1509G/C1509G), cochlear mechanics were passive. After perfusing streptomycin, mechanics remained passive and no individual changes in endolymph volume occurred. This argues that functional MET channels are required for streptomycin to induce ELH in wild-type mice. We then studied Vglut3KO mice, which do not release glutamate, to determine whether glutamate was involved in the development of ELH. Before streptomycin injection, these mice had active cochlear mechanics and no ELH. After streptomycin injection, cochlear mechanics became passive and ELH developed, indicating that glutamate excitotoxicity is not required for the development of ELH.
Conclusions: Our results indicate that MET channel block is sufficient for producing ELH and that glutamate excitoxicity is not required. MET blockade may therefore be involved in the development of ELH in Meniere's disease, for which there is no definitive treatment. Untangling the mechanisms underlying the development of ELH may help to identify new therapeutic targets.
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MO134. Optical Coherence Tomography Vibrometry Tuning Curve Morphology Maintained With Smaller Gold Nanoparticle Size After Posterior Semicircular Canal Injection in Mice

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Category: Inner Ear: Cochlear Mechanics
Background: Nanoparticles can carry and deliver large payloads of macromolecular therapeutics, however the safety and effect on hearing after nanoparticles reach the inner ear has yet to be determined. Posterior semicircular canal injection is a hearing preservation technique which can be used to deliver a large concentration of nanoparticles directly into the inner ear space. We sought to use optical coherence tomography to both visualize nanoparticles within the cochlea and measure cochlear mechanics after posterior semicircular canal injection of gold nanoparticles.
Methods: Wild type normal hearing CBA/CaJ mice 6-10 weeks old were anesthetized and surgery was performed to expose the cochlea and posterior semicircular canal. Optical coherence tomography images of the cochlea and organ of Corti vibrometry measurements were recorded to assess cochlear physiology at baseline, before and after a canalostomy, and during and after posterior semicircular canal injection at 0.5 uL/min of 1 uL of gold nanoparticles suspended in artificial perilymph. Gold nanoparticles were synthesized starting with either a 20nm, 50nm, or 80nm spherical gold core and functionalized using AlexaFluor488-

polyethylene glycol-OPSS and 5kDa methoxy-polyethylene glycol-SH. All animal experiments were approved by the USC Institutional Animal Care and Use Committee.
Results: Gold nanoparticles with a size of 52.3 +/- 0.7 nm, 83.1 +/- 2.0 nm, and 101.8 +/- 1.0 nm, synthesized from 20nm, 50nm, and 80nm spherical gold cores respectively, were found to be stable and do not aggregate in physiologic salt condition. However, the two largest sized nanoparticles settled over time, whereas the smallest nanoparticles remained suspended in solution. After posterior semicircular canal injection of nanoparticles that were suspended in solution, we found that they travelled throughout the cochlea to the apical turn, around the helicotrema, and back down to the basal turn. A low frequency notch developed in tuning curve morphology after perilymphatic perfusion of the larger size particles but not for the smallest size nanoparticle. Despite this, characteristic frequency and cochlear gain at the characteristic frequency were preserved.
Conclusions: Gold nanoparticles can be reliably delivered throughout the cochlear perilymph using posterior semicircular canal perfusion. Larger nanoparticles modulate cochlear mechanics presumably by settling and altering cochlear impedance. Thus, nanoparticle size may be helpful in directing therapeutic agents to specific cochlear targets.
This work was supported by NIDCD grants DC017741, DC014450, DC003896, and the American Otological Society.

MO135. A Biomechanical Model of the Stapes to Simulate Preloads on the Stapedial Annular Ligament
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Background: The stapedial annular ligament (AL), which constitutes a compliant connection of the stapes at the oval window, acts important roles in ossiculoplasty surgeries with preloads. Though the human AL comprises two fibrous face layers and a core layer between the fibrous layers, the AL was treated as a homogeneous structure in most of previous mechanical models. Further, varying inclination of the fibrous face layers along the stapes footplate boundary has not been implemented in any previous mechanical model. On the other hand, preloads on the AL have been implemented by increasing stiffness values in previous models.
Methods: The inertial properties of the stapes were calculated using mass distribution data obtained from micro-CT imaging. The model geometry of the AL was obtained using morphometric data from multiphoton imaging, which include varying inclination and dimension of the face layers. Against acoustic stimuli, only the axial stiffness along the longitudinal direction of the fibers was considered and bending stiffness of the fibers was ignored unless preloads are applied on the fibers. Under preloaded condition, new dimension and position of the fibers were calculated, and stiffness component along a direction perpendicular to the axial direction was added as an effect of the preload. The axial stiffness of the fibers was maintained the same under the preloaded condition. The mass and stiffness matrices under natural and preloaded conditions were calculated from the corresponding kinetic and potential energies, respectively. The Young's modulus of the fibers was estimated by comparing simulation of the model with the experimental stiffness data of the isolated stapes with the AL.
Results: Vibration of the stapes could be constrained without bending stiffness. The model simulation under preload conditions on the stapes showed similar trends as the experimental data in ossiculoplasty surgery using partial ossicular replacement prostheses (PORPs), with and without concurrent tension of the stapedial muscle. The attenuation of the middle-ear transfer function due to the preload was most pronounced in the low- and mid-frequency range below 4 kHz, with the maximum attenuation around 1 kHz. Constraining stapes motion by tension of the stapedial muscle became less effective under larger preloads.
Conclusions: A comprehensive biomechanical model of the stapes and the AL was established with the following key features: 1) implementation of the real and detailed geometry of the sub-layers of the AL, 2) only axial stiffness of the fibers in AL without preload, and 3) additional stiffness of the fibers along a direction perpendicular to the axial direction under preloaded condition. The novel implementation of the AL geometry and preload description in the analytical model was confirmed by constrained motion of the stapes without bending stiffness of the fiber layers and similar trends to the experimental data of the middle-ear reconstruction using PORPs.

MO136. Designing a Coupled Common-Mode OCT Probe With a Voltage Electrode for Simultaneous Intracochlear Motion and Voltage Measurements in Guinea Pig

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Category: Inner Ear: Cochlear Mechanics

Background: Two of the most valuable data types for understanding cochlear mechanics in response to sound stimuli are motions of structures in the cochlea and outer hair cell (OHC) transduction currents and voltages. Optical coherence tomography (OCT) provides the ability to image and measure motion of structures within the organ of Corti complex (OCC) in vivo. There are physical, optical, and practical limitations currently preventing simultaneous motion and voltage data from being taken at the same location. We propose a coupled fiber optic/electrode probe that simultaneously records voltage and OCT-measured motion.

Methods: The proposed device consists of an insulated tungsten electrode glued to an OCT probe previously developed by our lab that has been adapted to use a common-path setup. This dual probe utilizes a fiber optic cable with a Graded Index (GRIN) lens (focal length of 325um in water) which is attached to a scanning piezoelectric bimorph. The distance between the fiber tip and the electrode tip is approximately 300um.To record data, the dual probe is situated in the scala tympani perilymph, scans above the basilar membrane, and records images and motion data of the basal Guinea Pig OCC in vivo. Motion and voltage data are taken with the scanner off.

Results: A prototype probe without an electrode imaged the base of the Guinea Pig OCC. Prototypes have also successfully combined the fiber and electrode; ex vivo experiments have shown that the presence of the electrode does not interfere with the imaging or scanning functionality of the piezoelectric bimorph.

Conclusions: The coupling of voltage and motion measurements this probe provides can offer insight into the intricacies of cochlear mechanics. By using the imaging capabilities, the electrode can be positioned at a precise and effective recording location close to the Basilar Membrane (BM) in the OHC region to measure local cochlear microphonic. Simultaneous motion and voltage data from the same location can offer insight into the feedback relationship between mechanical and electrical changes in the OCC.

MO137. Superior Frequency Selectivity F Echolocation Produced by Bat's Uniquely-Shaped Basilar Membrane

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Category: Inner Ear: Cochlear Mechanics

Background: Bats perceive the distance, direction, and size of objects by emitting ultrasonic waves and listening to their echoes. And, they fly around in the dark without colliding with walls or other mates for predatory activity. “Echolocation” makes this possible. For example, CF-FM bats use ultrasonic sounds to emit a constant-frequency sounds, followed by a rapid drop in frequency. However, how bats perceive these echoes remains to be elucidated. The configuration of the bat's auditory is quite similar to that of the humans’. However, only the shape of the basilar membrane is significantly different in terms of its width and thickness. The purpose of this study is to elucidate the mechanism of echolocation in bats by estimating the acoustic properties of the bat’s basilar membrane.

Methods: As an example of a bat basilar membrane, consider a structure in which three types of fan-shaped basilar membranes are connected in series. The first stage basilar membrane, which is closest to the base of the cochlea, becomes wider and thinner towards the cochlear apex. The second basilar membrane becomes narrower and thinner toward the apex. And, finally, the third basilar membrane becomes wider and thinner toward the apex. Then, a simulation was performed by modeling the bat basilar membrane in the authors’ cochlear fluid dynamics model that assumes compressible perilymph.

Results: From the simulation results, we found that the higher frequency sounds generated a traveling wave in the first and the second basilar membranes and did not generate it in the third one. While, the lower frequency sounds generated the traveling wave in only the third basilar membrane. This result indicated that bats’ auditory can distinguish the lower sounds from the higher ones clearly in the level of displacement of the basilar membrane.

Conclusions: Ecologically, bats are thought to use the lower frequency sounds to explore their surroundings and the high ones to focus on specific objects. It can be said that the simulation results are consistent with

their activities. In other words, the unique shapes of the bats' basilar membrane provide a strict frequency-band-based selectivity in hearing, and enable their echolocation.

MO138. Virtual Rhesus Inner Ear Model Predicts Effect of Electrode Placement on Cochlear Mechanics

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Category: Inner Ear: Cochlear Mechanics

Background: The implantation of cochlear electrodes (CI's) affects the fluid motion of the inner ear, which in turn affects residual hair cell function. Minimizing the obstruction of inner ear fluid movement by CI's will result in preserving maximum residual cochlear function, allowing for better post-operative hearing in patients. Therefore, understanding the effect of cochlear implantation on inner ear mechanics is a priority in electrode design. A computational model of the rhesus macaque inner ear is critical for electrode and stimulus protocol design, allowing for quick, repeatable experiments to be performed at low cost. The objective of this study is to create a virtual model of the rhesus inner ear in order to accurately simulate the effects of electrode implantation on cochlear fluid mechanics.

Methods: Geometry of the inner ear was isolated from µMRI scans of the rhesus macaque. A simulated cochlear electrode array was designed alongside and placed within the scala tympani. The dimensions of this geometry were consistently cross referenced with existing anatomical literature to ensure accuracy.
This geometry was then used as the basis for designing a finite element model in Altair HyperMesh. From there, simulations will be run in Ansys Workbench to model basilar membrane displacement dependent on cochlear location and frequency. The output of these simulations will be compared against existing data. The model's mechanical properties will then be repeatedly tuned till the simulation's output accurately matches the real-life data.

Results: The model is expected to accurately mirror real-life acoustic behavior of the inner ear once tuning is completed. From there, various scenarios involving the presence of cochlear electrode arrays may be tested. Different angles and depths of insertion are likely to create variations in the displacement of the basilar membrane, and thus cause differences in sound perception. By iterative testing of different array locations, it should be possible to determine optimal placement strategies for real-world patients.

Conclusions: A virtual computational model of a rhesus money was created for a variety of virtual experiments. The rhesus monkey model may present an inexpensive alternative for preliminary testing of inner ear implants and reduce costly animal use for initial implant design procedure.

MO139. Auditory Lipidomics, an Approach to Identify Unique Molecular Effects of Noise Trauma

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Category: Inner Ear: Damage and Protection

Background: Hearing loss is a health problem of epidemic proportions. It is well established that even moderate noise levels, such as those experienced in daily life, e.g., concerts, can produce long-lasting hearing deficits. However, the mechanisms by which different levels of noise damage the ear are not well known. To interrogate this issue, our group previously compared the inner ear metabolome of noise exposed and control mice by measuring aqueous metabolites, and identified novel noise-modulated metabolites and pathways, as well as some already linked to noise exposure or cochlear function such as neurotransmission and oxidative stress. To expand upon the metabolic changes induced by noise, we are now exploring the lipidome.

Methods: Awake mice were exposed bilaterally for 2 hours to varying intensities of noise (98, 100, 110 dB @ 8-16 kHz). Immediately following the exposure, otic capsules were dissected and flash frozen. For controls, animals were placed in the exposure chamber for 2 hours without presenting noise and tissue collected in the same way. Otic capsules were mechanically lysed and the lipid fraction was extracted by the University of Michigan metabolomics core, who then performed untargeted lipidomics and lipid identification. To narrow down which lipids are influenced by hair cell activity we used both hearing and deaf mice (salsa mutants and Pou4f3-DTR mice in which hair cells were ablated by diphtheria toxin injection).

Results: Lipidomics results identified approximately 650 lipid species from ~40 classes. Analysis was initiated by grouping lipids into biologically relevant groups based on their lipid class, saturation status, and length. Then, principal component analysis was performed to calculate the 'Lipid score' for each group.

Within each principal component, each lipid received a factor loading, i.e., the correlation coefficient between the lipid and the component. Lipid scores were created by multiplying the lipid factor loading by the lipid standardized peak intensity and then adding together these values for all lipids within a group. Lipid scores were compared between the exposures and hearing/deaf mice to identify lipid families of interest.
Conclusions: Our initial analysis has identified significant differences in the lipidome of deaf and hearing animals, as well as differences in the levels of several lipid groups following noise. We are in the process of validating these lipid species and groups of interest. Also, to further investigate the effect of altering of lipid metabolism on hearing and the effects of noise exposure, we are also testing the impact of pharmacologic agents that prevent normal fatty acid metabolism. Though preliminary, to our knowledge this is the first lipidomic study following noise exposure.
This work was supported in part by R21DC017916 (GC).

MO140. Distribution of Cisplatin in the Cochlea after Intraperitoneal or Intravenous Injection
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Category: Inner Ear: Damage and Protection
Background: Cisplatin chemotherapy is used in 10-20% of all cancer treatment regimens, particularly in ovarian, lung, bladder, and head and neck cancers. One severe sequela of the treatment is the ototoxic side effect, in which cochlear function is compromised and hearing threshold elevated. During cancer therapy, cisplatin is intravenously (i.v.) injected. In contrast to the clinical practice, cisplatin is injected intraperitoneally (i.p.) in animal studies. This raises the questions whether ototoxic effects and target structures are the same for the two different delivery methods. In this study, we would like to investigate whether cisplatin deposition patterns in the cochlea differ after i.v. or i.p. injection using X-ray fluorescence microscopy (XFM).
Methods: The ongoing study uses both male and female mice between 2-3 months (weight 20-30 grams). The mice are randomly assigned to different experimental groups: Cis_IP and Cis_IV groups receive different doses of cisplatin (10 or 16 mg/kg) through i.p. or i.v. (tail vein) injection, respective. A control group is included which receives no cisplatin injection. Thresholds of auditory brainstem responses (ABRs) serve as the outcome measure for hearing and are measured before (baseline) and after (1, 7, and 14 days) the treatment. Animals are euthanized and cardiac perfused at different time points after the treatment (1 hour, 1 day, 7 days, and 14 days). Tissues including liver, kidney, brain and intestine are harvested for histology and XFM imaging and analysis. XFM is performed at Beamline 2-ID-E and 2-ID-D at Argonne National Laboratories on cross-sections of cochlear and other tissues. The number of mice in the study is justified by statistical analysis with G*Power using an alpha of 0.05 and a power of 0.08.
Results: The results so far have shown that the animal survival rate in the Cis_IP group is greater than in the Cis_IV cisplatin group with the same dose. This may indicate that the IV effects of cisplatin are more systemic and seem to have greater cytotoxic effects. ABR threshold also showed a 15-20 dB elevation at frequencies over 16 kHz at day 14 in the Cis_IV group but not in the Cis_IP group. XFM scans show preliminary that platinum signal is detected in the inner hair cells and outer hair cells, and the concentration and distribution of platinum in the cochlea is different between the Cis_IV and Cis_IP groups.
Conclusions: The study is still ongoing. Preliminary data of ABR thresholds show that the effect of cisplatin differs between the Cis_IV and Cis_IP groups. Likewise, XFM images show differences in cisplatin distribution patterns in the cochlea between the two groups. We suggest that i.v. injections in animal models may be more predictive of cisplatin ototoxicity in the cochlea.

MO141. Immediate Effects of Noise Damage on the Actin Structures Supporting Stereocilia in Mammalian Auditory Hair Cells
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Category: Inner Ear: Damage and Protection

Background: During sound stimulation, each stereocilium of the auditory hair cell pivots around its base, where its actin core becomes denser and forms a rootlet protruding into the cuticular plate. It is believed that actin-based cuticular plate provides a stable mechanical support for stereocilia, while rootles are responsible for their pivotal flexibility and life-long resilience to mechanical stimuli. Not surprisingly, classical studies in the lizard hair cells identified breaks in the actin filaments at the base of the stereocilia (Tilney et. al, 1982). Similar studies in mammalian hair cells reported damage to the stereocilia bundles and rootlet displacements as hallmarks of the permanent noise-induced hearing loss (NIHL) (Liberman, 1987). Yet, despite decades of NIHL studies, it is still unknown whether these ultrastructural changes in the stereocilia and their rootlets occur immediately after acoustic trauma. In this study, we compare the changes in the hair cell actin within minutes after exposure to damaging noise that typically causes temporary or permanent NIHL.
Methods: First, we recorded auditory brainstem responses (ABR) to identify noise intensities that reliably generate temporary (TTS) or permanent (PTS) shifts of hearing thresholds in the adult (P25-P26) C57Bl/6 mice. We have found that a broadband noise of 100dB SPL for 30 minutes and 110dB SPL for 1h would, respectively, produce TTS and PTS at the frequency region of 16-20kHz. We determined the hair cell location corresponding to these characteristic frequencies based on the place-frequency map of the organ of Corti (Müller et al., 2005). Next, other cohorts of male/female mice, which were exposed to noise causing either TTS or PTS, were euthanized immediately after damage. Organ of Corti samples from unexposed control and noise-exposed animals were stained with ALEXA fluor 488 for high-resolution confocal microscopy or high pressure frozen and freeze-substituted for serial sectioning with focused ion-beam (FIB) and backscatter scanning electron microscopy (FIB-SEM).
Results: Although Alexa Fluor 488 phalloidin did not label rootlets in most of the samples, rootlet insertions were clearly seen as prominent label-free holes in the cuticular plates. We systematically quantified the distances between these insertions within (intra) and between (inter) first and second row stereocilia in control, TTS, and PTS inner hair cells. We did not find significant changes in the intra-row distances after either noise exposure. However, the inter-row distances were significantly decreased in TTS but not PTS. Additionally, preliminary FIB-SEM images revealed noise-induced changes in the actin density at the bottom of cuticular plates after PTS noise. Moreover, PTS damaged hair cells exhibited cytocauds – abnormal F-actin bundles extending from the cuticular plate into the cell body.
Conclusions: We conclude that noise-induced damage may initiate complex multi-step re-arrangements of actin in the stereocilia and cuticular plate.
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MO142. Potential Use for Immunomodulatory Drugs to Prevent Endotoxemia Potentiated Aminoglycoside-Induced Hearing Loss in Mice

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Category: Inner Ear: Damage and Protection
Background: The role of the immune system in relation to the propagation or protection of the inner ear from ototoxic insults has come into focus in the last several years. Central to immune system development, polarization, and proliferation is the important JAK – STAT signaling pathways. Furthermore, interest in moderating the significant inflammatory reactions in patients with sepsis or significant infections in order to improve clinical outcomes is increasing. With the development of highly specific drugs has come the opportunity to study this potential in a meaningful way. We hypothesize that the improvement of JAK inhibitors, which have garnered significant excitement for autoimmune conditions such as rheumatoid arthritis, might also be useful in controlling excessive inflammation in sepsis or infections that can potentiate the negative effects of aminoglycoside use, thus protecting patients from these effects. Here we have utilized specific JAK inhibitors to assess the roles of these upstream receptor tyrosine kinases with respect to lipopolysaccharide (LPS) and kanamycin (KM) mediated cochleotoxicity.
Methods: To investigate the role of JAK signaling in ototoxicity we utilized a clinically relevant aminoglycoside model in B6N(Cg)-Cdh23tm2.1Kjn mice. In this model, animals were treated with Lipopolysaccharide (LPS) at 1 mg/kg I.P. 3 times over a 14-day period in combination with subcutaneous injection of Kanamycin (KM) at 500 mg/kg two times a day, 6 hours apart for 14 days. Experimental animals were co-treated with KM and Fedratinib - 50 mg/kg via oral gavage (OG). (JAK2 specific

inhibitor). Functional hearing assessments were collected via auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE). ABRs were tested at 4,8,16,22,32,45, and 64 kHz from 100 dB SPL to 20 dB SPL in 5 dB increments. DPOAEs were tested at 5.6, 8, 11.3, 16, 22, and 32 kHz from 75 dB SPL to 10 dB SPL in 5 dB increments. We have also assessed the histopathology of the kidneys, spleen, and liver to assess changes imparted by the LPS and KM treatment regimens.
Results: Fedratinib treatment rescued hearing in animals exposed to the LPS+KM treatment regimen across the middle and basal turn frequencies including 22kHz, 32kHz, 45kHz and 64kHz. Interestingly, 22 kHz and 32 kHz thresholds were no different than the age matched control group. Similarly, Fedratinib co-treatment resulted in protection of DPOAE thresholds at 16, 22, and 32 kHz.
Conclusions: This study has uncovered the potential use of JAK2 inhibitors like Fedratinib in the prevention of aminoglycoside related hearing loss. In addition, this work suggests that intervention in the inflammatory processes that are associated with infections treated with aminoglycosides might be able to control or prevent subsequent hearing loss. This finding is consistent with previous results that have suggested that endotoxemia and inflammation potentiate the negative side effects of aminoglycosides in the cochlea.

MO143. Effect of Viral-Like Inflammation on Hearing and Cochlear Uptake of Aminoglycosides

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Category: Inner Ear: Damage and Protection
Background: The effect of viral-like inflammation on drug-induced hearing loss is poorly understood. We tested whether an agonist for TLR7 that detects single-stranded viral RNA induced viral-like inflammation and modulated cochlear or serum levels of gentamicin, an ototoxic aminoglycoside. We also tested a dose-range of this same agonist, gardiquimod, to determine their efficacy in eliciting inflammatory responses and/or hearing loss. Finally, we tested if a specific dose of gardiquimod increased cochlear uptake of gentamicin without elevating serum gentamicin levels.
Methods: C57BL/6 mice received DPBS (control) or gardiquimod (1-20 mg/kg; s.c.; N≥4 per group). Three and 24 hours later, blood and cochlear tissues were collected to determine cytokine expression levels via qRT-PCR or Luminex ELISA assays, including: IFNα, IFNβ, IFNγ, MCP1, MIP1α, NFκB, TNFα, IP10, IL1α, IL1β, IL2, IL6, IL10, IL12α, IL12β. To determine the effect of gardiquimod on cochlear and serum concentrations of gentamicin, C57BL/6 mice received DPBS or gardiquimod (up to 20 mg/kg; N=6 per group), and 24 hours later, mice received gentamicin (20 mg/kg) for 1 hour (i.p.). Blood and cochlear samples were then collected to evaluate gentamicin levels using an ELISA kit. To evaluate the effect of gardiquimod on mouse hearing, 5 groups of mice received DPBS or increasing doses of gardiquimod (1, 5, 10, 20 mg/kg; s.c.; N≥6 per group). ABRs and DPOAEs were obtained before, and 1, 7 and 14 days after, treatment.
Results: Gardiquimod induced dose-dependent inflammatory responses, with increased serum and cochlear levels of cytokines that are associated with viral infections, including IP10, MCP1, MIP1α, IL6, TNFα, compared to DPBS-treated mice. Inflammatory responses were greater at 3 hours compared to 24 hours after treatment. Gardiquimod increased cochlear levels of gentamicin without modulating serum levels of gentamicin. Selected doses of gardiquimod significantly elevated ABR thresholds at 32 kHz 14 days post-treatment.
Conclusions: Our data show that gardiquimod induced robust inflammatory responses that mimic virally-induced inflammation in humans. Furthermore, gardiquimod increased cochlear, but not serum, levels of gentamicin. Last, elevated ABR thresholds at 32 kHz 14 days after a single injection of gardiquimod provides evidence that viral-like inflammation can alter hearing performance, as observed in some patients after viral infection. Thus, activated TLR7 signaling could increase the risk of hearing loss, and potentially exacerbate ototoxin-induced hearing loss.
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MO144. Noise-Trauma Induced Dysregulation of Vesicular Zinc Promotes Cochlear Damage and Hearing Loss

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Category: Inner Ear: Damage and Protection
Background: Vesicular zinc is a critical signaling molecule in the nervous system for fine-tuning synaptic transmission and sound processing, and vesicular zinc signaling is modulated by sensory experience. Following injury, however, the dysregulation of vesicular zinc can exacerbate neurodegenerative processes and hinder regeneration. The vesicular zinc transporter, ZnT3, loads zinc into vesicles, and mRNA for ZnT3 is expressed in the cochlea, although its cellular location and cochlear function remain unknown. Therefore, we sought to determine the expression pattern of ZnT3 and vesicular zinc in the cochlea, as well as the role of vesicular zinc dysregulation in noise-induced hearing loss.
Methods: To determine the cellular expression pattern of ZnT3 in adult mouse cochleas, we used a novel ZnT3-CreERT2/Rox mouse line bred to a fluorescent reporter line (Ai14) to label ZnT3-expressing cells in cochlear whole mounts and cryosections. To determine the expression pattern of vesicular zinc in the cochlea and how it is modulated by noise exposure, we performed zinc autometallography (Timm) stain on sham- and noise-exposed mouse cochlear whole mounts and cryosections.
To determine the impact of ZnT3 deletion on recovery from NIHL, we performed auditory brainstem response (ABRs) and distortion product otoacoustic emissions (DPOAEs) experiments in noise-exposed ZnT3-knockout (KO) and wild-type mice, 1 and 14 days after noise exposure. To further evaluate if zinc, specifically, is involved in cochlear degeneration, we treated wild-type mice with zinc chelator, TPEN, either systemically or locally via surgical application of a TPEN-infused polymer gel, and measured ABRs and DPOAEs 1 and 14 days after noise exposure. Cochlear whole mounts from these mice were additionally evaluated for hair cell loss and cochlear synaptopathy.
Results: We observed fluorescence in ZnT3-CreERT2/Rox:Ai14 mice in the inner hair cells, outer hair cells, and spiral limbus, suggesting that ZnT3 is expressed in these cells. Furthermore, we found prominent zinc staining in these same cell types, further suggesting that hair cells and cells of the spiral limbus contain vesicular zinc. After noise exposure, we observed a profound dysregulation of vesicular zinc, as zinc staining was strongly increased and disorganized in hair cells and the spiral limbus.
After noise exposure, ZnT3-KO mice showed enhanced recovery of ABR and DPOAE threshold shifts relative to wild-type mice, and ZnT3-KO mice did not show evidence of cochlear synaptopathy. Additionally, mice treated with TPEN (systemically or locally) also showed enhanced recovery of ABR and DPOAE threshold shifts, suggesting that removal of dysregulated vesicular zinc enhances cochlear recovery from NIHL.
Conclusions: Together, these data suggest that noise-induced dysregulation of vesicular zinc in the cochlea contributes to cochlear degeneration and NIHL. Moreover, by targeting noise-induced zinc dysregulation, recovery from NIHL is enhanced, thereby suggesting zinc dysregulation as a potential therapeutic target for the treatment of NIHL.

MO145. TLR4 Expression Shows a Time-Dependent Increase in Sensory Cells of the Inner Ear after Noise Damage

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Category: Inner Ear: Damage and Protection
Background: Noise damage to the cochlea elicits an inflammatory response marked by immune cell infiltration and pro-inflammatory cytokine upregulation. However, the mechanisms behind this response have not been delineated. TLR4 is a receptor that recognizes microbial pathogen-associated and damage-associated molecular patterns. It was recently detected in inner hair cells (IHCs) and supporting cells of the organ of Corti. We show that TLR4 is expressed at basal levels in both sensory hair cells and spiral ganglion neurons of mice. We investigated whether TLR4 expression increases in all frequency regions after noise exposure.
Methods: Adult mice were exposed to 106dB SPL broadband noise for 2 hours. Distortion product otoacoustic emissions (DPOAEs) thresholds were measured before noise exposure, 2 days post-noise, and 7 days post-noise. Cochleae were harvested on either day 2 or 7. Immunofluorescence staining was performed using TLR4 and CD45 to determine the expression of TLR4 and total immune cell numbers.

Results: Noise exposure led to significant threshold shifts at 2 days (21dB SPL ± 5) and 7 days (23dB SPL ±7), respectively, post-noise. Immunofluorescence data revealed a time-dependent increase in the number of CD45+ immune cells in the spiral ganglion, stria vascularis and basilar membrane, but these results showed no statistical significance. TLR4 immunoreactivity was observed in IHCs and OHCs of the organ of Corti as well as spiral ganglion neurons of both noise-exposed and control mice. The apical region of the cochlea showed stronger TLR4 immunoreactivity compared to the basal region. In the apical region, TLR4 fluorescence intensity also increased in a time dependent manner with control cochleae displaying the least intensity and day 7 cochleae displaying the strongest (8.9 ± 1.2, 15.31 ± 0.5, 55.92 ± 2.03). However, TLR4 expression remained the same in the basal region, regardless of noise exposure, and there was no significant difference among control and noise-exposed cochleae at different timepoints. Unexposed mice with a targeted deletion of oncomodulin (OCM KO) also have increased basal TLR4 expression.
Conclusions: Our preliminary findings show that TLR4 is constitutively expressed in the sensory cells of the organ of Corti and spiral ganglion neurons and that its expression varies by frequency region. Immune cell numbers and TLR4 expression increase in a time-dependent manner after noise damage. Higher levels of TLR4 in OCM KO mice suggests a role for Ca2+ homeostasis in TLR4 signaling. Future directions in our lab will involve studying the developmental pattern of TLR4 expression and modulating the TLR4 pathway using a small molecule inhibitor to combat noise damage.

MO146. Genesis and Cellular Composition of the Flat Epithelium in Adult Mice

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Category: Inner Ear: Damage and Protection
Background: The organ of Corti shows varying degrees of damage depending on the severity of the injury. In severe cases, hair cells and supporting cells are lost, resulting in the replacement of the sensory epithelium by a layer of flat cells, which is defined as the flat epithelium. The flat epithelium has been observed in human temporal bone specimens and is thought to represent a shared final state of degeneration after noise or ototoxic exposure, and in the most frequent profound genetic deafness. A consequence of this observation is that the basis for regenerative therapy differs between patients with surviving organ of Corti supporting cells versus patients with flat epithelium cells. Thus, understanding the genesis and the cellular and molecular composition of the flat epithelium is essential to develop therapies for patients with a flat epithelium. An adult mouse model with a complete replacement of the organ of Corti by a flat epithelium is missing.
Methods: We infused a single high dose of the ototoxic aminoglycoside sisomicin into the posterior semicircular canal of one-month-old FVB mice to elicit flat epithelium formation. Immunohistochemistry was conducted at different time points post-infusion to characterize the molecular characteristics of the emerging flat epithelium cells. We used antibodies to known hair cell and supporting cell markers as well as tight junction-associated proteins and cell adhesion molecules. To reveal functional deficits associated with sisomicin-induced flat epithelium, we recorded Auditory Brainstem Responses (ABRs) and Distortion Products of Otoacoustic Emissions (DPOAEs).
Results: We instigated the replacement of the sensory epithelium by flat cells from base to apex with a single high dose of sisomicin. Hair cells and supporting cells died 24 hours post-sisomicin infusion, except for the inner pillar cells. Preliminary results revealed that 48h after infusion, inner pillar cells were still present, but four days after infusion, all supporting cells, including the inner pillar cells died. At these early stages we observed a rearranging epithelium consisting of hair cell and supporting cell corpses. This immunohistopathology changed when we analyzed specimens two weeks post-sisomicin infusion, showing a complete replacement of the sensory epithelium by flat cells. Immunohistochemistry revealed that flat epithelium cells were negative for known hair cell and supporting cell markers but displayed epithelial characteristics. Consistently, no ABRs and DPOAEs were detectable in mice with flat epithelium.
Conclusions: We established a novel, fast, and reproducible adult mouse model to study the flat epithelium. We aim to understand its origin and molecular composition using immunohistochemistry and transcriptomic techniques, which will allow us to target flat epithelium cells in future gene therapy approaches.

MO147. Characterizing the Surviving Cochlear Hair Cells After Noise-Induced Damage in Mice With Pou4f3 Overexpression

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Category: Inner Ear: Damage and Protection
Background: The irreversible loss of sensory hair cells in the mammalian cochlea is caused by multiple insults including exposure to excessive noise, ototoxic drugs, and ageing, which results in permanent hearing loss. Germline knockout and mutation studies demonstrated the critical role of the transcription factor Pou4f3 in regulating hair cell survival during development. Our recent findings demonstrate that overexpression of Pou4f3 protects outer hair cells (OHCs) from death in a subset of mice at 1 or 2 weeks after noise exposure. However, OHC quantification only used myosin VIIa which is not sufficient to indicate signs of possible damage or degeneration. Here, we are characterizing the health of these surviving OHCs based on prestin expression (a key protein expressed in OHCs) and assessment of stereocilia morphology. Additionally, to determine whether Pou4f3 overexpression causes delayed OHC death post-noise or is actually preventing the death of the OHCs, we are investigating a later timepoint (i.e. 4 weeks post-noise) for OHC loss quantification and analysis.
Methods: We used tamoxifen inducible PrestinCreERT2:CAG-loxP-stop-loxP-Pou4f3-IRES-mCherry mice to overexpress Pou4f3 in OHCs at 4 weeks of age. Baseline auditory brainstem responses (ABRs) were performed one day prior to noise exposure (8-16 kHz octave band noise at 105 dB SPL for 1 hour), which was given at 5 weeks of age. Hearing function was then evaluated by ABR at either 1, 2, or 4 weeks after noise exposure, followed by temporal bone collection. OHCs were manually quantified from confocal images of whole-mount cochlear turns immuno-stained with an anti-myosin VIIa antibody. Cre-negative littermates were used as controls. Samples showing increased numbers of OHCs compared to control were further examined by immunostaining of the second cochlea for the OHC marker prestin and the stereocilia marker espin.
Results: Quantification of OHCs from samples analyzed at 1 or 2 weeks after noise exposure showed massive loss of OHCs in control samples, whereas the samples with Pou4f3 overexpression had variable levels of OHC death and were assigned to a protection or no protection (showing > 80% OHC loss in at least 2 cochlear turns) group. We observed protection in ~ 40% of the samples at 1 week and ~ 20% of the samples at 2 weeks after noise exposure, but there were no significant changes in ABR threshold shifts among control and Pou4f3 overexpression mice in the protection group. Analysis of samples at 4 weeks post-noise and assessment of OHC health using prestin and espin are in progress.
Conclusions: While these preliminary data suggest that overexpression of Pou4f3 might have a limited otoprotective effect, the detailed assessment of the stereocilia and morphology in surviving OHCs will help determine whether Pou4f3 is a viable target for future clinical use.

MO148. Training and Testing a Machine Learning Model to Predict Drug Ototoxicity

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Category: Inner Ear: Damage and Protection
Background: While over 600 drugs, and many more in clinical trials, are potentially ototoxic, testing for ototoxicity is not a clinical drug trial standard. This oversight could be mitigated with computational modelling, which has grown into a powerful tool for predicting toxicity in other tissue types. We could apply such a tool to pre-emptively screen drugs for ototoxic effects.
Methods: We used PubMed to compile a database of over 200 validated ototoxins and non-ototoxins, then created a simple ototoxicity prediction model with our database using a modified Tanimoto Similarity Index. To increase predictive accuracy, we then combined our training dataset with an additional published dataset of over 700 compounds. With this combined dataset, we developed a machine-learning model that uses PyBioMed to compute molecular features of each potential ototoxin and principal component analysis to determine features most likely to predict ototoxicity. Selected features are used to determine potential ototoxicity of new drugs relative to our training dataset of known ototoxins and non-ototoxins. We then validated our computational models by testing the predicted ototoxins in the zebrafish lateral line.
We also sought to use our model to correlate chemical features with mechanisms of ototoxicity. Given that many ototoxins enter hair cells through the mechanotransduction (MET) channel, we sorted ototoxins with known MET channel interactions into a smaller training data set. To increase the size of our MET-channel interactors dataset, we also tested for MET-channel effects of several other drugs from our original training dataset. These drugs included the cardiovascular medications nimodipine and salicylic acid, COVID-19 therapies ritonavir and lopinavir, and spermine, important for eukaryotic cell metabolism. We treated

zebrafish larvae with the corresponding ototoxin, then quantified intensity of FM1-43X dye, which enters hair cells through the MET channel and serves as a proxy for channel function.
Results: By combining our initial dataset with a larger published dataset and applying principal component analysis, we increased the prediction accuracy from 75% to 85% and reduced bias in our model, further reducing the risk of overfitting. For the MET-interaction experiments, we found that salicylic acid significantly reduced FM1-43X fluorescence, suggesting an interaction with the MET channel. Future research will include additional MET-channel interactor drugs to add to our model and will validate newly identified ototoxins using the zebrafish system.
Conclusions: We have shown that machine learning models can successfully predict ototoxicity based on chemical features of each drug. Our research paves the way for an efficient method to flag drugs for patient audiometric testing during clinical trials. By preemptively screening drugs for ototoxicity, we can mitigate hearing loss as an unforeseen side effect in patients seeking treatment from many FDA approved drugs.

MO149. The Role of Resident and Migrating Macrophages Towards Sensory Hearing Loss in Chronic Suppurative Otitis Media

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Category: Inner Ear: Damage and Protection
Background: CSOM is a worldwide disease that afflicts 330 million people worldwide and is the most common cause of hearing loss in children in the developing world. We have previously found that macrophages are the main immune cells in the cochlea mirroring the timing of hair cell loss. In this report we investigated the function of resident and migrating cochlear macrophages towards hair cell loss in CSOM.
Methods: We investigated in our novel pseudomonas aeruginosa PA CSOM animal model, previously validated to mimic the human disease. We depleted cochlear resident macrophages by using the CSF-1 receptor inhibitor PLX5622 before inoculating them with PA. We determined macrophage numbers in the cochlea and hair cell loss at different timepoints (1, 3, 7, and 14 days) during the infection course using immunohistochemistry and confocal microscopy.
Results: We found that depletion of cochlear resident macrophages did not affect hearing or cause hair cell damage in wild type mice. This shows that resident cochlear macrophages are not required to maintain hearing. Total macrophages were significantly reduced in the cochlea after depletion of resident macrophages at all assessed timepoints during the infection, compared to the control group without depletion ($p < 0.05$). In CSOM, we did not find any hair cell loss after 1 and 3 days in both groups. However, we found hair cell loss at 7 and 14 days in both groups. We found significantly less hair cell loss at 14 days when resident cochlear macrophages were previously depleted ($p = 0.04$). The number of hair cells in the basal turn of the cochlea remained as 29/100 µm of the basilar membrane after depleting macrophages and 19/100 µm of the basilar membrane in the control group.
Conclusions: These data suggested that both the resident and migrating macrophages play a role in CSOM associated hair cell loss. Our further research plan will focus on the underlying molecular mechanism between macrophages and hair cell loss.

MO150. Hdac Inhibitor, Trichostatin a Inhibits Hearing Loss in a Model of Alport Syndrome

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Category: Inner Ear: Damage and Protection
Background: Genetic hearing loss (GHL) is one of the most prevalent sensory disorders. Alport syndrome (AS) is caused by a mutation in type IV collagen, one of the major proteins in the basement membrane. Kidney and cochlea are frequently involved causing glomerulonephritis and hearing loss. Hearing in AS is usually normal at birth, progressively deteriorating thereafter. To evaluate the impact of acquired genetic changes on disease progression, we tested the effects of treatment with Trichostatin A (TSA), an HDAC inhibitor, on hearing in an AS model.
Methods: Knockout (KO) mice of type IV collagen alpha 3 chains (Col4α3) were used in the AS model (Jackson lab, 129x1/SVJ). The treatment group was intraperitoneally injected with TSA (CAYMAN CHEMICAL COMPANY, 89730) from 3 weeks of age. Hearing levels were measured by auditory

brainstem response audiometry (ABR) from 4 weeks of age to 9 weeks when homozygous KO began to die of end-stage renal failure. Cochlear were harvested at 9 weeks, decalcified in EDTA cut, and stained Immunofluorescence for ICAM2 and 4-Hydroxynonenal (4-HNE) staining. Basement membrane thickness was checked using TEM (Transmission Electron Microscope). TSA(1uM) was treated to HEI-OC1 cell and qRT-PCR was done to see the molecular changes.
Results: From week 4, the hearing threshold gradually increased in AS mice, reaching a plateau of approximately 50±5 dB at 5 weeks of age. The KO group treated with TSA had a better ABR threshold of 40±3 dB at 7 weeks of age in AS mice, while the untreated group was 50±5 dB. Col4α3 KO showed dispersed and enlarged lateral wall, especially in the stria vascularis. Spiral ganglion neurons and hair cells in the organ of Corti appeared to be normal. Basement membrane thickness of the TSA-treated group was comparable to the control in Col4α3 KO mice. Instead, 4-HNE was significantly elevated in KO mice and TSA reduced it. In HEI-OC1 cells, inflammatory molecules TGFβ1, TNFα, IL-6 and IL-1β were down-regulated by TSA treatment.
Conclusions: Hearing thresholds were gradually increased in Col4α3 KO mice and HDAC inhibitor TSA inhibited this hearing impairment in AS mice. Oxidative stress marker 4-HNE was increased in AS mice in which TSA reduced it. TSA reduced inflammatory cytokines in HEI-OC1 cells. TSA is a candidate molecule for inhibiting hearing deterioration in AS model.

MO151. The Outcome of the Combination of Mouse Embryonic Stem Cell (mESC) and Photobiomodulation (PBM) Delivery in Auditory Neuropathic Animal Models

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Category: Inner Ear: Damage and Protection
Background: A cochlear implant is known as the most effective clinical approach for hearing rehabilitation for sensorineural hearing loss. The prognosis of cochlear implants is good when sufficient nerve cells are secured, but the prognosis is poor in patients with secondary auditory neuropathy. Studies on the differentiation of hair cell-like cells using stem cell (SC)s have been reported, and the application of SC transplantation to damaged neurons in the cochlea is emerging as a promising strategy. However, intra-cochlear stem cell transplantation has limitations in overcoming the specific environment of the inner ear structure and limited survival time. It has been reported that PBM using light energy intensity (< 500 mW) activates cells without generating heat inside the cells. PBM also promotes the differentiation of stem cells into neurons and allows external light energy to be delivered to the inner ear.
This study is to investigate the effects on combination treatment of mESC and PBM on auditory function and auditory neurons in chronic auditory neuropathic model.
Methods: 5-week-old, C57BL/c6 mice and a green fluorescent protein (GFP)-tagged mESCs were used. The Kanamycin (KM, 150 mg/kg) was placed into the round window to deliver the drug through the scala tympani (ST). A total volume of 3 μL of GFP-mESCs (2×104 cells/μL) was injected into the ST of the neurodegenerative cochlea. PBM using an 808nm diode laser (at 40 mW/cm2, 1 h/day for 5 days) was performed after SC transplantation. ABRs were measured before and after KM surgery and 1 and 2 weeks after combination therapy. eABRs were assessed 2 weeks after combination therapy. Oct4 and Nanog expression was investigated.
Results: At 2 weeks after KM surgery, the ABR thresholds had increased at 8, 16, and 32 kHz frequencies; no further change was observed through 8 weeks. At 2 weeks after combination therapy, ABR thresholds had increased at all tested frequencies. The waveform of eABR after 2 weeks of combination therapy showed no peak relative to baseline in all treated groups. GFP-expressing cells were observed only in KM+SC+PBM, confirming that the combination could increase the viability of mESC-like cells in OC. However, aberrant GFP expression was also observed in the cochlear. The Oct4 expression was not detected in aberrant GFP-positive cochlear cells in the KM+SC+PBM. Nanog expression was also not detected in GFP-positive cells transplanted into the cochlea after combination treatment.
Conclusions: Transplanted GFP-positive cells were connected or contacted. The cochlear cell viability can be improved by the combination treatment of mESCs and PBM in transplanted cochlea, but it did not affect the hearing recover. PBM induced explosive proliferation of cochlear implanted SCs, which differentiated into non-carcinogenic cells other than auditory cells.

MO152. Evaluation of Auditory Damage After Blast Exposure in CBA/J Mice

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Category: Inner Ear: Damage and Protection

Background: Substantial evidence in animal models and human temporal bones suggests that in the early stages of sensorineural hearing loss (SNHL), auditory nerve fibers are damaged and synapses between the auditory nerve and hair cells are reduced before hair cells loss occurs. This phenomenon has been named cochlear synaptopathy (CS) and is considered to be the hallmark pathology behind "hidden hearing loss". Symptoms of hidden hearing loss such as tinnitus and difficulty hearing in noise have been observed in individuals exposed to blast injury. Experiments in chinchillas have shown that CS can occur following blast exposure (Hickman TT et al. Sci Rep. 2018). In this study, we explore the auditory effects of serial blasts of varied peak pressures on CBA/J mice. Our goal is to define blast parameters for the creation of a blast-induced CS model in mice.

Methods: Male CBA/J mice aged 6 weeks or older were assigned to one of the following groups: (A) no blast control, (B) 1 blast at 165dBP SPL, (C) 10 blasts at 165dBP, (D) 1 blast at 170dBP, (E) 10 blasts at 170dBP, (F) 1 blast at 175dBP, (G) 2 blasts at 175dBP. We used a shock tube described by Hickman et al. that can reliably generate blasts with peak pressures between160-180dBP SPL by varying compression chamber static pressure, membrane material, and membrane thickness. Blast pressure was measured with two 1/8" condenser microphones placed at mouse ear level. Cochlear function was assessed by ABR and DPOAE pre-exposure, 24 hours and 1 week after exposure. All procedures were approved by the IACUC of the Massachusetts Eye and Ear.

Results: In control group (A), ABR and DPOE thresholds were present and within the normal range. Mice exposed to (B) 1 blast at 165dBP and (D) 1 blast at 170dBP had either no or only small changes in ABR/DPOAE thresholds after 24 hours or one week. Groups C (10 blasts at 165dBP) and E (10 blasts at 170dBP) showed no auditory responses at 24 hours and 1 week, consistent with profound hearing loss, which also occurred in group G (2 blasts 175dBP); however in group F (1 blast 175 dBP) threshold changes were less severe. In several ears exposed to either 1 blast at 170dBP (D) or 1 blast at 175dBP (F), we observed temporary threshold shifts and reduced ABR wave I amplitude. No perforation of the tympanic membrane was observed by endoscope in any ear.

Conclusions: A single exposure blast between 170-175dBP SPL can produce a temporary threshold shift and reduced ABR wave I amplitude in the CBS/J mouse. Histologic evaluation of blast exposed inner ears is necessary to identify CS. Described methods may aid in the development of useful models of blast induced CS in mice.

MO153. Investigating Mechanisms of Cochlear Synaptopathy Using Vglut3KO and TectaC1509G/C1509G Mice

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Category: Inner Ear: Damage and Protection

Background: Cochlear synaptopathy is a type of hearing loss defined by synaptic loss between hair cells and afferent auditory nerve fibers. Synaptopathy alters neural encoding at higher sound intensities, not auditory thresholds; therefore, it is referred to as "hidden" hearing loss. Hair cell bundle stimulation triggers

glutamate release at the base of the hair cells. Glutamate is the primary afferent neurotransmitter that excites auditory nerve fibers. We used Vglut3KO and TectaC1509G/C1509G mutant mice to examine the role of glutamate excitotoxicity in producing synaptopathy.
Methods: CBA/CaJ (JAX#: 000654), Vglut3KO (JAX#: 016931), and TectaC1509G/C1509G (Xia et al., 2010) mice of either sex aged 6-8 weeks were exposed to a blast wave with peak pressure ~196 dB SPL. Eight days later, cochleae were dissected, the organ of Corti divided into apical, middle, and basal sections, immunohistochemistry performed, and imaged using a Leica SP8 confocal microscope (63x/1.4 objective) with analysis via Imaris software. Another set of mice received a ~200 dB SPL peak pressure blast wave for synaptic analysis via transmission electron microscopy.
Results: Presynaptic ribbons per inner hair cell (IHC) were counted for apical, middle, and basal sections of the cochlea. Synaptopathy was confirmed by a loss in ribbons/IHC between CBA/CaJ unexposed mice and CBA/CaJ mice exposed to a blast wave. Preliminary results show no difference in ribbons/IHC counts for unexposed vs. blast wave exposed cochleae for either Vglut3KO or TectaC1509G/C1509G mutant mice. Across all mouse strains there was no difference in % orphan ribbons between unexposed mice and mice exposed to a blast wave.
A comparison of electron micrographs from CBA/CaJ unexposed mice and mice exposed to a blast wave revealed ultrastructural changes to the IHCs and surrounding structures. These changes included dendrite swelling, cell/neural membrane retraction in synaptic regions, increased extracellular space, mitochondrial damage, and the presence of lipid droplets.
Conclusions: The absence of cochlear synaptopathy in both Vglut3KO and TectaC1509G/C1509G mutants after blast trauma indicates that hair cell overstimulation mediates synaptopathy through glutamate excitotoxicity. This pathway appears to be associated with dendritic swelling. Next steps include measuring dendritic swelling in vivo after blast exposure as we anticipate dendritic swelling to correlate with cochlear synaptopathy.

MO154. Efficacy of Non-Invasive Mild Therapeutic Hypothermia Delivery to the Inner Ear

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Category: Inner Ear: Damage and Protection
Background: The direct otoprotective effect of mild therapeutic hypothermia (MTH) on the auditory system following trauma such as noise exposure, cochlear implantation and cisplatin ototoxicity has been extensively reported in several studies. Acute MTH modulates the release of proinflammatory cytokines, mitochondrial dysfunction and oxidative stress that can lead to initiation of programmed cell death within the sensorineural cochlear structures and consequent hearing impairment. To improve the translational potential for applications of MTH minimizing acoustic, mechanical, vibrational, and ototoxic trauma, we developed and tested novel non-invasive approaches to improve target temperature management. Efficacy of these devices and approach were assessed experimentally and numerically in preclinical and human cadaver models.
Methods: Custom cooling devices for localized cooling of the inner ear were designed and tested. MTH was induced unilaterally in both preclinical and human cadaver models using a copper tip probe placed in the ear canal to reach desired temperature (32 °C) or external cooling packs. The preclinical model utilized female and male Brown Norway (160-320g) rats. In acute experiments cochlear temperature changes were recorded in anesthetized rats using micro thermistors implanted through the round window. Body temperature was measured rectally and maintained at 37°C with electric isothermal pads. In another set of experiments, temperature recordings were collected from human cadaver heads. Specimens were pre-warmed and maintained at a temperature range of 35.5-38°C using a metal bead bath to simulate average human conditions. Thermistors were placed at the middle ear, round window, scalp, bead bath and probe tip. Finally numerical analysis was performed on accurate 3D anatomical models of the temporal bone derived from imaging of the cadaver samples.
Results: The data collected from our preclinical model showed that mild hypothermia effectively reduced the cochlear temperature, averaging a 4-6°C from the baseline while the rectal temperature was within the normothermic range (36-38°C). Both ear canal and externally placed cooling devices were equally effective

in the cadaver samples (drop of 3-4ºC) as well. Numerical simulations corroborated the experimental results, including the temporal profiles of MTH.
Conclusions: The numerical analysis and experimental temperature measures from preclinical and cadaver models highlight clinical utility of our custom approach to deliver MTH to the inner ear.

MO155. Dynamic Changes in Auditory Sensitivity After Impact Acceleration Traumatic Brain Injury
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Category: Inner Ear: Damage and Protection
Background: Acquired brain injuries from car accidents, falls, and explosions can lead to auditory deficits including hearing loss, hyperacusis, and tinnitus. Models of different traumatic brain injury (TBI) mechanisms have been developed to replicate the physics of a TBI, but few models have characterized factors contributing to functional variability observed across time and individuals. The impact acceleration model of TBI (IA-TBI) is used to simulate the most common neuropathology in most TBIs, diffuse axonal injury, following which many humans experience hearing loss. Simulating this injury in mice and tracking hearing status and underlying structural damage is an effective way to begin understanding the heterogeneity of injury and functional outcomes. Here, we aimed to longitudinally characterize auditory function in 2-month-old (m.o.) CBA/CaJ mice by assessing ABRs and the status of cochlear hair cells and afferent terminals before and after injury for up to 90 days.
Methods: Subjects were 2 m.o. CBA/CaJ mice (n = 10 sham, n = 16 IA-TBI). ABRs were recorded to clicks and tones spanning the normal mouse hearing range (8, 12, 16, 24, and 32 kHz; 90-10 dB SPL) at various time points (baseline, 3, 7, 14, 30, 60, and 90 days). ABR thresholds and Wave I-V amplitudes and latencies were measured. The number of inner and outer hair cells (IHC; OHC) per 100 microns and the number of CTBP2-positive puncta per IHC and OHC were quantified in both experimental groups after injury. The status of tunnel-crossing fibers was also evaluated. Statistical analyses using linear mixed-effects models were conducted to evaluate ABR thresholds, amplitudes, and latencies across experimental groups, time points, and sexes. Hair cells and synaptic ribbons were evaluated across groups and frequencies (4, 5, 8, 11, 16, 22, 32, 45, 64 kHz).
Results: Compared to shams, IA-TBI subjects had higher thresholds, smaller amplitudes, and delayed latencies of ABR responses after injury. Preliminary cochlear assessments show no loss of IHCs or OHCs, but a significant loss of IHC synaptic ribbons at 16 and 32 kHz in injured subjects. Tunnel crossing fibers also variably showed pathological features.
Conclusions: The increase in auditory thresholds after IA-TBI suggests that even a mechanical injury that doesn't involve acoustic overpressure (as in blast exposures) causes hearing loss. The reduction in amplitudes and delayed latencies replicate findings in other mechanisms of TBI further suggesting examination of the morphology of the ABR response may be useful in identifying auditory system pathologies in individuals with TBI. The corresponding loss of synaptic ribbons in mid and high-frequency regions of the cochlea corresponds to physiological response deficits and provides evidence of TBI-induced cochlear synaptopathy. This high-throughput model linking auditory dysfunction with peripheral neural damage after TBI will be useful for evaluating therapeutics and prevention strategies.

MO156. TREM-1 Gets Upregulated on Cochlear Macrophages and Its Inhibition Promotes Recovery of Hearing Thresholds Following Acoustic Trauma
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Category: Inner Ear: Damage and Protection
Background: Triggering receptor expressed on myeloid cells-1 (TREM-1) is a receptor for the immunoglobulin superfamily, that activates neutrophils and monocytes/macrophages by signaling through the adapter protein DAP12. TREM-1 amplify inflammatory response and play a critical role in sepsis, cancer, and neurodegenerative diseases. Blockade of TREM-1 by LR12 peptide has neuroprotective effects. Sensorineural hearing loss is an inflammatory and neurodegenerative disease but the role of TREM-1 in the pathophysiology of hearing loss and cochlear neurodegeneration is unknown. Due to the short-half-life of

LR12 peptide, we have developed small chemical inhibitors of TREM-1, GJ073 and GJ004. In this study, we seek to examine TREM-1 expression and its role in the cochlea via its inhibition after acoustic trauma.
Methods: B6N(Cg)-Cdh23tm2.1Kjn/Kjn mice (both sexes, 6-8 week of age) were exposed for 2 hours to a noise level of 106 dB SPL at 8-16 kHz octave band. Auditory brainstem responses (ABRs) and distortion product of otoacoustic emission (DPOAEs) were measured prior to, at 1 day and 15 days after acoustic trauma. Following functional measurement at 1 day after acoustic trauma, mice were unilaterally trans-tympanically injected with vehicle (1.6% DMSO in sterile Poloxamer 407 hydrogel) or single dose of one of the TREM-1 inhibitors; peptide (LR12) peptide, small molecules (GJ073 or GJ004). Mice were euthanized at 2 months after acoustic trauma and excised cochleae were processed for cryosectioning and multilabel fluorescent immunohistochemistry to examine hair cells, macrophages, and spiral ganglion neurons by confocal microscopy.
Results: GJ073 and GJ004 were identified as potential TREM-1 antagonists by in-silico molecular docking into the TREM-1 receptor. Surface plasma resonance analysis exhibited greater TREM-1 binding affinity of GJ004 (Kd = 5.1 uM) than LR12 (Kd = 9.2 uM) and GJ073 (Kd = 14.3 uM). TREM-1 is found to be upregulated on circulating macrophages and neutrophils that are recruited into the noise-damaged cochlea. Acoustic trauma led to a permanent shifts in hearing thresholds of ~ 20-70 dB at all stimulus frequencies in the mice that were treated with vehicle. Transtympanic treatment with LR12 peptide (20 µg/5 µl) at 1 day after acoustic trauma showed ~ 10-25 dB recovery in hearing thresholds across tested frequencies. Similarly, treatment with the small molecules, GJ073 (426 ng/5 µl) or GJ004 (389 ng/5 µl) also showed recovery in hearing thresholds and between the two small molecules, GJ004 showed most robust recovery of ~ 30-50 dB across the tested frequencies. DPOAE levels were reduced after acoustic trauma but did not show recovery with any of the TREM-1 inhibitors.
Conclusions: TREM-1 is upregulated in the cochlea after acoustic trauma and its inhibition partially protects against noise-induced hearing loss. The difference in recovery of hearing thresholds could indicate differences in drug efficacy or potency in different parts of the cochlea.

MO157. mGlu7 Receptor Negative Allosteric Modulator (NAM) Prevents Chronic Inflammation and Cell Death Induced by Noise Trauma in the CBA/CaJ Mouse Cochlea

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Category: Inner Ear: Damage and Protection
Background: Noise-induced Hearing Loss (NIHL) is the major cause of acquired hearing loss in both civilian and military populations. Chronic inflammation is one of the important mechanisms that promotes cochlear damage resulting from NIHL, involving the transcription factor NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and AKT, which are key mediators of inflammatory responses. In this study, we tested the hypothesis that young adult CBA mice, following noise trauma (NT), would have a dramatic reduction of cochlear damage with treatment of a newly developed mGlu7 receptor negative allosteric modulator (PRAGMA Therapeutics) including reduction of cochlear sensory cell death.
Methods: CBA/CaJ mice with normal hearing at age 3-6 months were randomly placed in control and treated groups. Our novel NAM compound was administered orally to the treatment group, and placebo (saline) to the control group at 1 hour pre-noise exposure, and immediately following the exposure. Unanesthetized mice were exposed to an octave-band of noise at 110 dB SPL for 45 minutes. Five months post-exposure mice were sacrificed and the cochleae were processed and dissected. Immunohistochemistry staining and imaging with confocal laser scanning microscopy were used, along with qRT-PCR and ligation mediated -PCR (LM-PCR), to detect gene expression and DNA fragmentations (apoptosis), respectively. Auditory function was evaluated by measuring auditory brainstem responses (ABRs) and distortion product acoustic emissions (DPOAEs) thresholds and amplitudes.
Results: We measured gene expression changes of NF-κB and AKT in both treated and non-treated cochleae, and both genes showed decreased expressions in mice treated with NAM vs placebo controls. The protein expressions of NF-κB, AKT and phosphor-AKT (ser473) were detected using Immunofluorescence and quantified by image densitometry analysis. Protein expressions of NF-κB and AKT were decreased in

the NAM treatment mice vs. placebo group, indicating that anti-inflammatory effects of our new mGlu7 NAM are key mechanisms for NIHL prevention. In addition, apoptosis inducing factor (AIF) cell component relocation was found in the nucleus of the placebo group cochlea but not in the mGlu7 NAM treatment group. Also, we observed DNA fragmentations in the genomic DNA isolation extract of the noise-exposed placebo group but not in the mGlu7 treated mice.
Conclusions: Our findings support previous reports that NIHL promotes inflammation and cell death in the cochlea, and the novel results here indicate that modulation of mGlu7 receptor activity can inhibit noise-induced cochlear cell death and inflammation.

MO158. Noise-Induced Cochlear Synaptopathy and Time Course in Young Adult Rats
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Category: Inner Ear: Damage and Protection
Background: Noise-induced cochlear synaptopathy (NICS) has been widely studied but mainly using CBA/CaJ mice. While it appears that spontaneous synaptic regeneration does not occur after NICS in CBA/CaJ mice, in other species, e.g., guinea pigs, regeneration of lost synapses has been observed. This raises the question of which species represents the exception and which the rule. Here we performed a pilot study of NICS in rats to ask whether NICS occurs in young adult rats and, if so, whether synapses can spontaneously regenerate up to 6 weeks after noise exposure. For further development of means to prevent synaptopathy or promote regeneration, inclusion of a rat model will be advantageous.
Methods: 8 week-old Sprague-Dawley rats were exposed to noise calibrated to generate NICS comparable to that used for CBA/CaJ mice. For rats, this was 2 hr of 104 dBSPL, 6-12 kHz octave band noise. ABRs were measured at 6, 12 and 24 kHz prior to noise exposure to obtain a baseline for each rat, again on postnoise day 1 (PND1) to assess the temporary threshold shift, and again on PND14, PND28 and PND42. Wave-I amplitude was normalized to the pre-noise measure for each rat to determine % decline. Rats were euthanized after the PND42 ABR measure and the cochleae processed for immunofluorescent visualization of the synapses. Presynaptic ribbons and postsynaptic densities were labeled with, respectively, anti-CtBP2 and anti-PSD95 antibodies. Synapses were counted in organ of Corti wholemounts at 6, 12 and 24 kHz locations.
Results: Consistent with our previous studies in CBA/CaJ mice, ABR thresholds in the noise-exposed rats were elevated and wave-I amplitudes depressed on PND1. By PND14, ABR thresholds recovered to prenoise levels but wave-I amplitudes were depressed relative to prenoise and did not recover significantly subsequently. It should be noted that in non-noise-exposed control rats, ABR thresholds remained stable from 8 to 14 weeks of age but wave-I amplitude declined significantly between 8 and 10 weeks. Nevertheless, the within-subject measure of wave-I amplitude decline confirms that NICS causes a significant amplitude decline. Synapse numbers in rats are slightly higher than in mice: 6, 12 and 24 kHz locations in rats having, respectively, ~6, ~2, and ~4 more synapses/inner hair cell (IHC) than equivalent 8, 16, and 32 kHz locations in mice. Synapse/IHC counts were significantly decreased in noise-exposed relative to age-matched non-noise-exposed control rats.
Conclusions: In summary, we find that NICS occurs without spontaneous recovery in rats. ABR wave-I amplitudes appear to decline with age, especially from 8 to 10 weeks, suggesting that NICS experiments should avoid exposing rats <10 weeks old to noise. Use of within-subject ABR amplitude measures can overcome individual variation in absolute magnitudes and provide a more consistent measure in animal models. (Support from R01 DC015790)

MO159. RIPOR2-Mediated Autophagy Dysfunction is Critical for Aminoglycoside-Induced Hearing Loss
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Category: Inner Ear: Damage and Protection
Background: Aminoglycosides (AGs) are potent antibiotics capable of treating a wide variety of life-threatening infections, however, they are ototoxic and cause irreversible damage to cochlear hair cells. Despite substantial progress, little is known about the molecular pathways critical for hair cell function and survival that are affected by AG exposure.
Methods: Using biochemical screening, immunostaining, live cell imaging and mouse genetics methods

Results: We demonstrate here that gentamicin, a representative AG antibiotic, binds to and triggers within minutes translocation of RIPOR2 in murine hair cells from stereocilia to the pericuticular area. Then, by interacting with a central autophagy component GABARAP, RIPOR2 affects autophagy activation. Reducing the expression of RIPOR2 or GABARAP completely prevents AG-induced hair cell death and subsequent hearing loss in mice. Additionally, abolishing the expression of PINK1 or Parkin, two key mitochondrial autophagy proteins, prevents hair cell death and subsequent hearing loss caused by AG.
Conclusions: In summary, our study demonstrates that RIPOR2-mediated autophagic dysfunction is essential for AG-induced hearing loss.

MO160. Sparsentan Protects From Hearing Loss, Improves Kidney Function and Prolongs Lifespan in Alport Mice With Developed Auditory and Renal Pathology

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Category: Inner Ear: Drug Delivery
Background: There are no approved therapies for Alport syndrome and none in development target hearing loss. In Alport Syndrome, endothelin type A receptor (ETAR) activation is an important trigger for renal and cochlear pathologies. Our previous work showed Sparsentan ,a single-molecule dual endothelin type-A and angiotensin II type 1 receptor antagonist (DEARA) administered to young COL4A3-/- (Alport KO) mice prevented increases in proteinuria, hearing loss, stria vascularis as well as renal structural dysmorphology. Whether these effects translate into preservation of kidney function, increased lifespan, and protection from hearing loss in older mice with developed cochlear and renal pathology is unknown.
Methods: The ABR was utilized at 8-8.75 weeks (W) of age to assess hearing and cochlear ability to tolerate a metabolically stressful noise (10 kHz OBN, 106 dB SPL, 10 Hr) in wild-type and Alport KO mice treated via oral gavage with vehicle or Sparsentan 120 mg/kg from 5W. Sparsentan levels in cochlear lateral wall (stria and spiral ligament) tissue and plasma were determined at 9W of age after 6W dosing. In renal studies, Alport KO mice were gavaged daily with Sparsentan starting at 4, 5, 6, or 7W of age. Glomerular filtration rate was measured at 9W in mice dosed from 4W and glomerulosclerosis was evaluated at 10W in kidney sections stained for fibronectin.
Results: Sparsentan initiated at 5W improved post noise thresholds with significant prevention of hearing loss at all frequencies tested from 8 to 40 kHz. Sparsentan crossed the blood-labyrinth barrier into LW tissue at levels expected to antagonize both receptor targets. Sparsentan initiated at 4W abrogated the decline in glomerular filtration rate at 9W compared to vehicle-treated Alport KO mice and provided significant protection from glomerulosclerosis at 10W. Sparsentan extended the median lifespan even when dosing began in mice with detectable glomerulosclerosis.
Conclusions: Sparsentan prevented hearing loss in Alport KO mice likely in-part through direct effects on strial ETAR and AT1R. Maintenance of glomerular filtration rate and lifespan extension occurs in mice with developed strial and renal structural changes. If these results are translated successfully into the clinic, sparsentan might be unique in its potential to preserve both hearing and renal function even when therapy is started after significant proteinuria is present.

MO161. Application of Acoustic Multi-Frequency Stimulation to Increase Apical Drug Concentrations After Intratympanic Drug Administration in Guinea Pigs – a Pilot Study

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Category: Inner Ear: Drug Delivery
Background: Systemic drug delivery to the inner ear requires high drug dosages to bypass the blood-labyrinth-barrier and is therefore often associated with systemic side effects. On the contrary, the local application of drug compounds into the middle ear presents an alternative way to achieve high intracochlear

drug concentrations while bypassing the risk of systemic adverse effects. Nevertheless, drug delivery to the apical cochlear regions remains especially precarious yet critically important in order to treat the sensory cells responsible for detection of low frequency sounds like the human voice. Using an in silico approach, the effect of multi-frequency stimulation on intracochlear drug distribution was recently investigated. By triggering a travelling wave along the basilar membrane eliciting continuous fluid motions within the perilymph called “steady streaming”, drug particles could be actively transferred into the apical regions of the cochlea. The goal of this study was to investigate this effect of multi-frequency stimulation on the intracochlear drug distribution in a small animal model.
Methods: A corticosteroid, triamcinolone-acetonide (TAAC), formulated in a thermoreversible hydrogel was injected into the middle ear of 25 guinea pigs. Subsequently, the animals were either exposed to a multi-frequency stimulation for 2.5 or 24 hours (100 or 85dB), to the high frequencies of the stimulation sound for 24 hours (85dB) or used as negative controls without noise exposure for 2.5 or 24 hours (n=5 per group). After the respective time, perilymph was sequentially sampled from the cochlear apex and drug concentration was subsequently measured using high performance liquid chromatography-mass spectrometry. Furthermore, acoustic brainstem response audiometry (ABR) measurements were performed before application and sampling.
Results: Several animals exposed to the multi-frequency stimulation for 24 hours showed higher overall and apical TAAC concentrations compared to animals without acoustic stimulation for 24 hours. However, this difference proved not to be statistically significant. Moreover, no statistically significant differences in total, apical, or basal TAAC concentrations between the two groups with 2.5 hours stimulation/observation or between all three groups with 24 hours follow-up were observed. Click-ABR thresholds were slightly increased in animals after acoustic stimulation (mean±SD; before: 30.4±6.0dB, after: 36.8±4.2dB; p=0.002).
Conclusions: The results of this pilot study suggest a possible influence of multi-frequency stimulation on the intracochlear fluid dynamics and the distribution of drug compounds within the cochlea. To further investigate this effect and to find a possible significant increase in apical drug levels, more animals are planned to be included in this study and exposed to multi-frequency stimulation for 24 hours. Nonetheless, before clinical implementation of this technique, the stimulation protocols must be adapted to ensure a safe and reliable clinical application of this method.

MO162. Modulating Inner Ear Exposition of Hydrophobic Active Pharmaceutical Ingredients Using Lipid-Based and Gel Formulations

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Category: Inner Ear: Drug Delivery
Background: BCS class II and IV drugs include active pharmaceutical ingredients (API) with low solubility and either low or high permeability. They generally require formulation with amphiphilic excipients to increase their bioavailability. When it comes to otic administration by transtympanic (TT) injection, similar considerations still apply, as the drug aims to cross the round window membrane and diffuse in the cochlear perilymph. In addition, the formulation must be sufficiently retained in the middle ear to allow long-lasting delivery, enabling ample drug diffusion from the base to the apex of the cochlea.
In these experiments, four different hydrophobic small molecules, with high potential for treating hearing disorders, were specifically prepared in different formulation systems for TT delivery to study their pharmacokinetic (PK) profiles in the inner ear, and to evaluate formulation behavior in the middle ear and possible effects on hearing, resulting from ototoxicity or conduction impairments.
Methods: Four hydrophobic drug candidates were formulated as either suspensions in thermoreversible gel or lipidic mucoadhesive solutions. Each molecule formulation was transtympanically injected in Male Wistar rats (30 µL/ear; both ears). Using Auditory Brainstem Responses (ABR), hearing was measured prior and 6 days after this single TT injection. At different timepoints post-injections (1h, 24h and up to 7 days, n=5 per time point), otoscopic evaluations and gross middle ear assessments were performed, and the inner ear fluids were sampled ex vivo to determine molecule concentrations using LC-MS/MS.).
Results: The different formulations of the 4 molecules did not induce any modification of the eardrum appearance, as revealed by otoscopic evaluation. In addition, no significant increase of ABR thresholds was observed 6 days after the single TT injection, demonstrating that any formulation induced neither ototoxic effects, nor conductive hearing loss, as confirmed by gross middle ear assessments. Regarding the PK

results, whatever the formulation, each molecule reached the inner ear fluids rapidly after the TT injection. However, only the thermoreversible gel and Phosal 53 MCT formulation enabled drug presence up to 7 days post-injection.
Lipidic excipients were able to achieve one-week sustained-delivery of hydrophobic compounds to the inner ear, as demonstrated by pharmacokinetic profiles. Their use as stand-alone lipidic mucoadhesive solutions, or in combination with poloxamer-based thermoreversible gel, significantly influences the duration of delivery and the overall resulting exposure. CremophorEL and Phosal 53 MCT, as well as mixtures with poloxamer-based hydrogels, appear well tolerated in the middle ear and do not cause significant auditory threshold increases.
Conclusions: CILcare and Drugabilis report here their expertise in assessing novel excipients to formulate hydrophobic drugs for transtympanic injection, enlarging the possibilities of drug repurposing in various otic disorders.

MO163. Interpreting Drug Concentrations of Perilymph Samples – An Analysis by Simulations

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Category: Inner Ear: Drug Delivery
Background: When small molecule drugs are applied to the inner ear they do not distribute homogenously. Rather, gradients develop along the scalae from the site(s) of drug entry to more distant regions. As a result, perilymph samples collected for analysis can be extremely difficult to interpret accurately. Drug concentration depends on where the sample was collected, how much volume was taken, and how fast the sample was collected.
Methods: The FluidSim Cochlear Fluids Simulation Program (v 4.05, available for download from https://www.turnerscientific.com/) was used to simulate fluid sampling from 3 sites of the guinea pig inner ear after an intratympanic application of dexamethasone. The sampling sites were: 1) the lateral semi-circular canal; 2) the cochlear apex; and 3) the round window. For each location we compared sample concentrations for sequential sampling (in which repeated small samples of 1 to 2 µL in volume are collected as the fluid is expelled from the ear) and for single large samples of different volumes, ranging from 1 to 20 µL.
Results: Taking a single large sample of 1-20 µL from the lateral canal provides a good representation of drug levels in the vestibule but was almost unaffected by drug levels in scala tympani. Taking a single large sample of 1-8 µL from the cochlear apex is likely to grossly underestimate the drug level at the cochlear base. Taking a small sample of 1-2 µL from the round window can provide a meaningful representation of basal turn perilymph composition but will be highly influenced by the prevailing middle ear composition during sampling, whether the round window niche is rinsed, and whether any perilymph is lost to the middle ear prior to sampling. For all 3 sites, the sequential sampling method provides a more detailed representation of drug distribution in perilymph than is possible with single samples.
Conclusions: Simulations of sampling techniques provide valuable insights into the difficulties encountered interpretating perilymph sample data, and allow for more efficient and cost-effective in vivo studies. Simulations show that in some situations, substantial errors will occur when the sample concentration is assumed to represent the perilymph concentration prior to sampling.

MO164. Chronic In-Vivo Test of Anti-Inflammatory Coated Cochlear Implants

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Category: Inner Ear: Drug Delivery
Background: Hearing loss affects more than 460 million people worldwide and is a major socio-economic burden. If the auditory nerve is intact, the cochlear implant (CI) is a possible treatment. After CI implantation, connective tissue grows around the electrode array. This leads to poorer signal transduction and increased impedances at the electrode contacts. In this study, anti-inflammatory substances like diclofenac and immunophilin inhibitor MM284 released from polymeric CI coatings and surface patterning were investigated for their efficacy to reduce connective tissue growth in vivo.

Methods: Cochlear implant electrodes (MED-EL, Innsbruck, Austria) with 4 contacts and dexamethasone-loaded silicone body were implanted in guinea pigs for 28 days. Six different types of electrodes were used: (control (no coating), Poly-L-lactid acid (PLLA) coating, PLLA with diclofenac, PLLA with MM284, surface patterning without coating, surface patterning with diclofenac in PLLA). Each electrode type was implanted in a group of 6 animals. Hearing thresholds were determined on day 0 after implantation. Cochleostomy and multiple insertions during implantation were used to provoke connective tissue growth. Impedances were measured daily for 14 days and on days 21 and 28. On day 28, another hearing threshold determination was performed, and the animals were sacrificed. The cochleae were removed, fixed in paraformaldehyde and decalcified in ethylenediaminetetraacetic acid. After optical clarification of the samples and staining for vimentin, cochleae were scanned for auto-fluorescence and the vimentin signal.
Results: On day 0, impedances were initially 7 to 10 kOhms, and then decreased until day 1 to about 4 to 6 kOhms. Subsequently, a continuous increase was observed at all contacts in all groups. The groups treated with diclofenac and immunophilin inhibitor MM284 showed a slower impedance increase in the first 10 days at contacts 1 and 2 compared to the control group. At day 28, impedances were comparable in all groups (10 to 14 kOhms). On the implanted side, the animals showed significant hearing loss with a threshold shift of more than 50 dB whereas the contralateral side remained unaffected. Histological analysis revealed great variability in tissue growth, confined to a small layer around the array or massively extending onto the apex, regardless of the anti-inflammatory coating. PLLA-coated electrodes caused extended damage to the cochlea due to increased stiffness, which resulted in an increased amount of connective tissue especially in middle and apical turns. There were no significant differences in tissue growth between the PLLA group and the PLLA with diclofenac and PLLA with MM284 groups.
Conclusions: The findings indicate that diclofenac and immunophilin inhibitor MM284 delay the connective tissue growth after CI implantation. However, a long-term effect was not observed, and more flexible polymeric coatings should be used in future.

MO165. Surgical Considerations in Inner Ear Gene Therapy From Human Temporal Bone Anatomy
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Category: Inner Ear: Drug Delivery
Background: Sensorineural hearing loss (SNHL) has remained the most common form of hearing loss due to the deterioration of hair cells (mechanosensory receptors) of the inner ear whether the underlying etiology be genetic, age-related, ototoxicity, or noise-induced. The widespread prevalence of SNHL which is increasing with aging populations highlights the need for the development of restorative and preventative treatments. Safe and effective delivery of therapies are essential in developing SNHL treatments. The primary aim of this study was to describe anatomical measurements relevant for inner ear drug delivery which may be complicated by the delicate anatomy of the cochlea.
Methods: Human temporal bone specimens were evaluated (1) to assess critical distances from the round and oval windows to the membranous labyrinth in human temporal bones, (2) to compare parameters between age-grouped populations, and (3) to compare the utility of different imaging modalities (histology slides, micro-CT, and synchrotron radiation phase contrast imaging).
Results: Mean width and depth of the anterior portion (AL) of the annular stapedial ligament for the overall sample population were 0.107 mm and 0.406 mm. The means of the width and depth of the posterior portion (PL) of the annular stapedial ligament were found to be 0.070 mm and 0.414 mm, respectively. Remaining means for the distance to saccule from the AL, distance to the saccule from the center of the footplate (FP), FP width, round window membrane (RWM) width, and distance from the round window to the osseous spiral lamina were 1.67 mm, 2.07 mm, 0.0614 mm, 0.112 mm, and 1.75 mm respectively. We observed a significant difference between age groups for the depth of the anterior portion of the annular stapedial ligament, the distance from the center of the footplate to the saccule, and the distance from the anterior portion of the annular stapedial ligament to the saccule. No significant differences for remaining measurements were reported. Differences between imaging modalities was seen between micro-CT and histology slides for the depth of the anterior portion of the annular stapedial ligament only.
Conclusions: The combined analyses between age-controlled groups and imaging modalities demonstrate critical dimensions to consider when inserting delivery vehicles into the cochlea and designing the instruments to do so.

MO166. A Computational Model for Sensitivity to Fast Frequency Chirps in the Inferior Colliculus

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Category: Midbrain: Structure and Function

Background: Recent physiological recordings in inferior colliculus (IC) demonstrate a sensitivity to fast frequency chirps contained in Schroeder-phase (SCHR) stimuli. Neurons respond to the periodicity of SCHR stimuli in a manner consistent with their modulation transfer function (MTF, rate versus modulation frequency). Similarly, responses to chirp velocity can be described using a rate-velocity function (RVF). RVFs are generated using responses to aperiodic chirp stimuli, consisting of isolated chirps with different velocities and frequency-sweep directions, presen¬¬ted in random order. Currently, state-of-the-art computational models of the IC do not simulate sensitivity to chirp direction or velocity. Broad, asymmetric inhibition has been proposed as a mechanism for several IC feature sensitivities, including sensitivity for frequency-modulation direction. Here, we show that physiologically realistic MTFs and RVFs can be simulated by a single model that receives excitation flanked by two inhibitory inputs, with higher and lower characteristic frequencies (CFs), approximating broad inhibition and narrow excitation.

Methods: Inputs to the IC model were derived from an auditory-nerve (AN) model. IC model inputs were treated as non-homogeneous Poisson processes and combined over a coincidence window using a strategy described by Krips and Furst (2009; Neural Computation, 21:2524-2553). Using a database of single-unit IC recordings, model parameters were fit to maximize the correlation coefficient between data and model feature vectors, consisting of the example neuron's MTF and RVF. Physiological MTFs were constructed using responses to wideband sinusoidally amplitude-modulated noise (100 Hz – 10 kHz, 30 dB spectrum level), and RVFs were constructed using responses to aperiodic chirps (1/25 – 1/600 s durations, 65 dB SPL). Aperiodic-chirp velocities were selected to match SCHR-chirp velocities. The free parameters in the model were the numbers of identical inhibitory inputs at each CF (analogous to strength) (Mlo , Mhi), delays of inhibitory inputs relative to excitatory (dlo, dhi), and coincidence window duration (ΔCD). Inhibitory delays were permitted to be negative, indicating that the inhibition arrived before the excitation. For preliminary simulations, the CFs of inhibitory inputs to the IC model were held constant, and centered around the excitatory CF.

Results: Using optimized parameters, the model successfully simulated the MTF and RVF shapes of IC neurons, including a wide variety of RVF shapes, demonstrating the model's robustness over a range of chirp-response profiles. Preliminary simulations successfully portrayed band-enhanced MTFs, which have increased rates in response to modulated stimuli relative to unmodulated stimuli.

Conclusions: Here, we simulate IC chirp sensitivity in a computational model for the first time, based on a mechanism of asymmetric, broad inhibition and narrow excitation. This work towards a physiologically relevant chirp model provides a new tool to investigate the relevance of chirp sensitivity to neural coding of complex sounds, such as speech, in the IC.

MO167. Distinct Neuronal Populations Process Auditory and Non-Auditory Activity in Shell Inferior Colliculus

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Category: Midbrain: Structure and Function

Background: Active listening requires not only correctly identifying primary sound features, but also learning their behavioral relevance. Behaviorally relevant representations are abundant in auditory cortex and thalamus, but whether similar activity is present earlier in the auditory pathway is unclear. The non-lemniscal nuclei of the inferior colliculus (shell IC) receive a variety of acoustic, multi-sensory and neuromodulatory signals, suggesting in integratory role in perceptual learning. We used multiphoton Ca2+-imaging, machine learning, and a reward-based discrimination task in mice to test if behaviorally relevant signals are present in shell IC neurons, and whether shell IC neurons change their responses to task relevant features across learning.

Methods: We trained 11 CBA/C57 Bl-6J mice to detect the presence of amplitude modulation in a bandpass noise stimulus using a GO/NOGO paradigm. Using 2-photon microscopy, we tracked GCaMP6f fluorescence signals in the same dorsal shell IC neurons over several weeks, first in a passive condition, and subsequently during task learning. Once mice had become experts, modulation depth was varied to obtain

psychometric functions. We analyzed the population- and individual activity during the process of learning as a whole and at various epochs during psychometric trials, and used a support vector machine (SVM) classifier to predict several behavioral and acoustic variables from neural population activity.
Results: Our data show that neurons change their responses over several days during task acquisition. We further found that the average differences in neural population trajectories and principal components during correct and incorrect responses stabilized over time, suggesting that representations of task-related variables are robust in the shell IC. We predicted the outcome of each trial from the neural data using an SVM classifier. Significant classification was achieved even if activity was integrated over only the first or second half of the sound stimulus, and even when the SVM was exclusively trained on neural activity occurring prior to mice's behavioral response.
Conclusions: Collectively, our data argue that neural population activity in the auditory midbrain encodes behavioral relevance of sounds, and reflects a mixed selectivity of task-relevant pre- and post-behavior information in response to behaviorally relevant sound features.

MO168. Neurochemical Characterization of the Porcine Inferior Colliculus

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Category: Midbrain: Structure and Function
Background: The mammalian inferior colliculus has classically been parcellated into three main subdivisions: a central nucleus considered to be the main lemniscal nucleus, and the dorsal and lateral cortices, considered to be non-lemniscal. Chernock et al. in 2004 demonstrated that the rat lateral cortex contains periodic modules that stain intensely for several markers, including GABA, parvalbumin, cytochrome oxidase, nicotinamide adenine dinucleotide phosphate-diaphorase, and acetylcholinesterase. Later studies showed that these modules are also present in mice and determine the input/output relationship of the lateral cortex. What is not clear is whether these modules represent a specialization only seen in rodents, or if they are present more generally across mammals.
Methods: We performed a series of neurochemical or immunohistochemical stains on sections through the porcine inferior colliculus using standard techniques to detect glutamic acid decarboxylase, parvalbumin, cytochrome oxidase, acetylcholinesterase and nicotinamide adenine dinucleotide phosphate-diaphorase.
Results: We observed striking modularity in the distribution of a number of markers in the inferior colliculus, most prominently for acetylcholinesterase and cytochrome oxidase. GAD and parvalbumin immunostaining revealed positive islands of staining in the caudal-most region of the inferior colliculus, but modularity was less evident in the rostral regions, where the central nucleus stained very intensely for both markers.
Conclusions: These data suggest that the presence of neurochemical modularity of the lateral cortex of the inferior colliculus is not isolated to the rodent, but also suggests that some differences exist between the rodent and porcine model. Future studies are planned to examine if the neurochemical modularity present in the porcine inferior colliculus also predicts its connectivity, as it does in the mouse.

MO169. Neuropeptide Y Y1R Neurons Provide Extensive Local Excitatory Inputs in the Inferior Colliculus

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Category: Midbrain: Structure and Function
Background: The inferior colliculus (IC) integrates information from numerous auditory nuclei, making the IC an important hub for sound processing. The IC contains neurons that project intrinsically, as well as principal neurons that send long-range projections to other brain regions. Most studies have focused on understanding the role of principal neurons in the IC. However, local circuits almost certainly shape the output of IC principal neurons, and the functional roles of local circuits in the IC remain largely unknown. We recently identified a distinct class of IC GABAergic neurons that express Neuropeptide Y (NPY). NPY neurons are principal neurons and also project locally in the IC. We also recently showed that a population of glutamatergic neurons in the IC expresses the NPY Y1 receptor (Y1R neurons) and that application of the Y1R agonist [Leu31, Pro34]-NPY decreases the excitability of Y1R neurons. Here, we find that Y1R neurons provide extensive excitatory input to other local IC neurons.

Methods: To selectively target Y1R neurons, we crossed Y1R-Cre mice with Ai14 reporter mice. First, to validate Y1R-Cre x Ai14 mice, we used in situ hybridization with probes targeted to tdTomato, Y1R and vGlut2. Next, to investigate local Y1R neuron projections, Y1R-Cre x Ai14 mice were injected with a Cre-dependent adeno-associated virus to selectively express the excitatory opsin Chronos in Y1R neurons. Y1R and non-Y1R neurons were recorded in current clamp in acutely prepared brain slices, and flashes of blue light were presented to activate Chronos-expressing terminals.
Results: In Y1R-Cre x Ai14 mice, we found that 78% of tdTomato+ neurons expressed Y1R and 91% of tdTomato+ neurons expressed vGlut2. By quantifying the remaining glutamatergic cells, we found that tdTomato neurons represent 92.5% of IC glutamatergic neurons. To test if activation of Y1R neuron terminals evoked EPSPs in neurons in the local IC, we recorded from Y1R and non-Y1R neurons while activating Chronos-expressing Y1R terminals. Our data show that optogenetic activation of Y1R terminals elicited EPSPs in both populations of neurons.
Conclusions: Our data show that most IC glutamatergic neurons express Y1R and that Y1R neurons provide excitatory inputs to Y1R and non-Y1R neurons in the local IC. Since our previous data shows data NPY hyperpolarizes Y1R neurons in the IC, it is likely that NPY signaling can modulate the strength of Y1R neuron synapses onto local postsynaptic targets. Future experiments will focus on understanding how NPY signaling shapes local IC computations in vivo.

MO170. Gerbil Middle Ear Development: Soft Tissue and Bones

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Category: Middle and External Ear
Background: The present study aims to better understand the middle ear's sound transmission changes during early development. Using synchrotron X-ray micro-tomography, we quantified dimensions and ossification of the middle ear ossicles in gerbils aged 0-21 days. Measures include the stapes and oval window footplate areas, the distance between ossified sections of the ossicles at joint faces, and volume fractions of the bones and soft tissue structures. In contrast to previous studies of middle ear development, this study considers soft tissue and monitors the progression of ossification.
Methods: Gerbils of either sex, aged 0 to 21 days, were included in this study. After euthanizing the animals with a lethal dose of ≥100 mg/kg Euthasol (390 mg/ml sodium pentobarbital and 50 mg/ml sodium phenytoin), the temporal bones were harvested and placed immediately in a -40 degrees freezer and kept frozen until imaging at the I13L beamline at Diamond Light Source, Oxfordshire, UK. The central energy of the pink beam was 30 keV. The imaging setup consisted of a rotating sample stage and a PCO Edge 5.5 CCD camera with an objective lens providing a total magnification of either 2.5x or 4x. For the tomographic scans, the samples were secured in an upside-down Eppendorf tube. A saline solution was placed in the cap of the Eppendorf tube to reduce sample dehydration. Data was recorded in fly scan mode over 180 degrees and with 2160 projections. The exposure time was 0.1 s. Twenty dark field and bright field projections were collected. The sample-detector distance was 323 mm for the ossified and 600 mm for the non-ossified or decalcified middle ears. Reconstruction procedures included flat-field correction, image distortion correction, phase retrieval, image reconstruction, and ring artifact reduction. The resulting slices were rendered to a three-dimensional volume with customized Python code.
Results: Due to the use of different animals for each of the 17-days, there is greater variability and random scatter in the results. Image rendering provides three-dimensional models of the ossified sections of the developing ossicles. Measurements from images of ossified middle ear ossicles showed that the footplate and oval window growth were similar to previously reported measures in gerbils (which is?). Measurements from non-ossified or decalcified ossicles did not show size changes between 0 and 21 days.
Conclusions: Volume fraction has not previously been measured in gerbils, but the growth pattern mirrors the pattern reported in humans, supporting gerbils as an animal model for middle ear development. The decalcified tissue does not change over time; these findings may challenge results from previous studies using microtomography and middle ear development. Understanding the development process of decalcified tissue may provide a novel understanding of the manifestation of diseases and the function of hearing.

MO171. Implementing Global Sensitivity Analysis to Investigate Impact of Variations of Middle-Ear Parameters and Interactions Among Them

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Category: Middle and External Ear

Background: The middle ear is made up of several structures and even if we assume that all these structures have linear elastic behaviour, we will end up having tens of material and geometrical parameters. Ranking the importance of each of these parameters and their possible interactions with each other facilitates the creation of new patient-specific models of the middle ear. It also can provide a deeper insight into the biomechanics of the middle ear. One strategy to systematically analyze the importance of each of the parameters is to use sensitivity analysis methods. Most sensitivity analysis studies in the literature are focused on a few structures and a limited range of excitation frequencies. Furthermore, many of these studies used local sensitivity analysis techniques that do not consider the interactions among parameters. In this work, we employed a global sensitivity analysis method to analyze the global impact of variations of middle-ear parameters and their interactions for a wide range of model parameters and excitation frequencies.

Methods: We used a finite-element model, which was validated with experimental data, to find the outputs of the middle ear for a range of middle-ear parameter values. To reduce the computational cost of performing the global sensitivity analysis, we exploited these outputs to create surrogate models that can be used in lieu of costly finite-element models. The surrogate models were created using the polynomial chaos expansion method. These surrogate models were used in combination with Sobol' global sensitivity analysis method to investigate the importance of each parameter and the interactions among parameters. We studied the effects of variations of mechanical properties (i.e., Young's modulus, damping, and Poisson's ratio) of all structures in our middle-ear model as well as the thickness of the tympanic membrane.

Results: The global sensitivity analysis results show that the parameters with the greatest global impact on the motions of the umbo and stapes footplate are the Young's modulus and thickness of the tympanic membrane, Young's modulus and damping of the stapedial annular ligament, and the Young's modulus of the ossicles. Also, our results reveal that although the most considerable interactions happen between the Young's modulus and the thickness of the tympanic membrane, there are some weaker interactions among other parameters at some specific frequencies.

Conclusions: The results of the global sensitivity analysis method can significantly reduce the computational cost of creating new patient-specific models of the middle ear. Moreover, these findings can facilitate the interpretation of diagnostic measurements of the middle ear.

MO172. An in Vitro Model to Evaluate Tympanic Membrane Drug Permeability

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Category: Middle and External Ear

Background: Oto-therapeutics utilizing non-invasive localized drug delivery have seen limited innovation in part to drug development and screening costs. Current screening methods relying heavily on expensive animal models with limited relevance to human tissue. A cost-effective, high-throughput, in vitro tympanic membrane (TM) model to rapidly characterize drug permeation could allow for increased discovery of novel drug candidates and delivery systems. New topical oto-therapeutics may reduce the need for problematic systemic treatments, improve patient outcomes, and reduce overall healthcare costs.

Methods: A 3D TM tissue model consisting of primary human keratinocytes cultured at an air liquid interface was developed and evaluated histologically at various growth intervals. Barrier function was evaluated by trans-epithelial electrical resistance and tight-junction protein immuno-staining. Drug flux across the model was evaluated in several template drugs with a range of log P values (dexamethasone sodium phosphate, ciprofloxacin HCl, gentamicin sulfate, and fluorescein). Drug solutions were placed on the apical surface of the tissues and the underlying basal receiver solution was collected at various timepoints over a 24-hour period. Receiver solutions were analyzed by UV-HPLC or fluorescence to quantify drug permeation.

Results: Using a physiologically based approach to model the layers of the TM, we show that the outer epidermal layer of the TM is expected to be the only significant contributor to overall TM drug permeability, while the middle lamina propria and the inner mucosal epithelial layers display comparatively low resistance

to drug permeation. Therefore, our TM model was simplified by modeling only this outer epidermal layer, which we show is fully developed after 11 days at air-liquid interface. Drug permeation studies show fluorescein displayed the highest permeability coefficient, followed by ciprofloxacin, then gentamicin sulfate, a trend which displays decreasing permeability correlating with decreasing logP. Dexamethasone sodium phosphate, which displayed very low permeability despite a high logP, deviated from this trend, although we speculate this likely due to phosphatase-mediated metabolism to its active form rather than a lower overall permeability. We also find overlap between the drug permeability in this in vitro model of the TM and drug permeability evaluated in in situ cadaveric TM.
Conclusions: This simple in vitro model can be used estimate drug permeability across the TM and allow for low-cost, high-throughput screening of novel drug candidates and delivery systems of oto-therapeutic targeting middle or inner ear tissues. Further validation and development of this model may allow for additional uses and even disease state modelling.

MO173. Experimental Study of Fracture-Related Properties of Gerbil Tympanic Membrane Using Hypodermic Needle Insertion Measurements

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Category: Middle and External Ear
Background: The current treatment of multiple ear diseases (e.g., acute otitis media and otitis media with effusion) is dependent on surgical procedures on the tympanic membrane (TM) such as myringotomy and tympanostomy tube insertion. Therefore, a better understanding of the mechanical properties of the TM can pave the way for developing novel surgical technologies. Although some of these properties including the viscoelastic characteristics of the TM have been of interest to researchers for many years, its fracture-related properties have remained unexplored. In this study, we implemented an experimental approach to investigate these properties for the gerbil TM.
Methods: We used hypodermic needles with different gauge sizes to perform needle insertion experiments into gerbil TMs. All measurements were carried out using a displacement-controlled loading technique; while the needle was inserted into the TM with a constant vertical speed, the reaction force applied to the needle was continually measured to obtain the force-displacement plot. Moreover, we conducted a double-insertion approach (i.e., two consecutive insertions at the same point) to extract the fracture toughness of the TM based on the difference between the first and second insertion plots. Using this approach, the effect of frictional and deformational components on force-displacement plots was eliminated to extract solely the fracture-related component.
Results: In this study, we investigated the effect of multiple parameters including needle gauge sizes, insertion speed, lubrication, and insertion location on force-displacement plots. In all the cases, we measured the maximum insertion force in the first insertion (TM puncture force), needle displacement in puncture instance (TM puncture displacement), the maximum insertion force in the second insertion (needle jump-in force) for an existing crack, and the frictional force between the needle and the TM. We also estimated the fracture toughness of the TM based on the difference between the force-displacement plots obtained from the first and second insertions and the measured crack area under various conditions.
Conclusions: In general, the cutting characteristics of the TM depend on the needle insertion procedure in terms of needle geometry (e.g., gauge size) and loading approach (e.g., insertion speed and with or without lubrication). The results obtained from experimental measurements of needle insertion into the TM can be used to develop novel surgical tools and advance haptic surgical technologies.

MO174. Visualization of Human Middle Ear Abnormality Using Optical Coherence Tomography

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Category: Middle and External Ear
Background: Optical coherence tomography (OCT) is a technology for performing real-time high-speed and high-resolution cross-sectional imaging on the micro-scale in situ, which has recently been introduced in auditory research to visualize structures of the ear requiring minimally invasive operation. OCT can also be adapted for vibrometry to measure the sound-induced, sub-nanometer vibrations of the middle and inner ear. The depth-resolved anatomical (structure) and functional (vibrations) information obtained with OCT

overcomes several limitations of classical imaging and vibrometry techniques, and together becomes a powerful tool to pinpoint the origins of conductive or sensory hearing loss.
Methods: Spectral domain OCT (SD-OCT, Thorlabs Telesto III TEL321C1) was used for non-invasive 3D structural and functional evaluation of the human middle ear through the ear canal. Fresh human temporal bones were used. The ear canal was shortened to compensate for the working distance of our OCT system, facilitating middle ear imaging and vibrometry through the tympanic membrane (TM). TM thickness was calculated from 3D images of the TM using custom developed software in Matlab. Middle ear sound transmission was assessed by measuring vibratory responses of the TM, and along the ossicular chain at the umbo and stapes, respectively.
Results: The TM thickness was reconstructed from 3D OCT images. The thickness of normal TM was between 100-150 um and distributed almost evenly. It thickened at the edges and along a line overlying the manubrium. Vibratory response patterns across the TM changed with stimulus frequency, but the amplitude of the umbo response was relatively smooth across frequencies. Compared to the umbo, stapes motion was attenuated. In one ear there were two TM retractions/atelectasis of Sade Grade 1 and 2, resulting in local thinning and thinning plus perforation of the TM, respectively. These common abnormalities changed the vibration patterns across the TM; it vibrated less and "non-symmetrically" compared to normal ears, and led to smaller responses of the umbo, and stapes. These findings explain the conductive hearing loss observed in humans with early grade TM retractions.
Conclusions: Our findings confirm that OCT can be a powerful clinical tool to examine middle ear injuries non-invasively. The combination of imaging and vibrometry can pinpoint the cause-and-effect of middle ear injuries and related conductive hearing loss.
This study was supported by VA Rehabilitation Research and Development Service Merit Award 101RX002296 (W. D), and the Department of Otolaryngology Head and Neck Surgery, at Loma Linda University Health. These contents do not represent the views of the U.S. Department of Veterans Affairs or the United States Government. There is no conflict of interest for all authors.

MO175. Effects of Early Acoustic Deprivation in Sensory Gating and Acoustic Startle Reflex

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Category: Multisensory Processing/Interactions
Background: The rodent's first days of life represent a critical period during development of the auditory system. Changes in auditory input during this period can impact auditory processing later in life, as is seen in humans Here we investigate the impact of early acoustic deprivation impairs the sensory gating and acoustic startle reflex, using prepulse inhibition (PPI) and gap-prepulse inhibition of acoustic startle reflex (GPIAS).
Methods: Swiss mice from P14 to P45 (CEUA 133/2018) were acoustically deprived with silicon ear plugs applied at t hearing-onset (P14) and removed 30 days later. Ear plugs raised auditory thresholds in XX% and they remained elevated even 5 days after plug removal. PPI test consists of five minutes of a background acclimatation (65 dB SPL of WN sound) followed by a six startle-reflex (SR) stimulus (115 dB SPL, WN with 40ms) and then 8 PPI stimulus (80 dB SPL, WN with 20ms of duration given50ms before the startle stimulus) and 8 trials with no pre-pulse, in a pseudorandom order. The GPIAS was accessed in five different frequencies: WN and 4, 10, 16 and 20 kHz preceded or not by a silence gap of 50 ms duration, 50 ms before the startle stimulus, in a pseudorandom order
Results: We found that the startle reflex of the animals was decreased in ear-plugged animals about 50% ($p<0.05$) and the proportion of inhibition of prepulse (%PPI) was smaller in ear-plugged animals (55%) group in comparison with the sham. The proportion of inhibition of gap-prepulse (%GPIAS) was significantly different ear plugged animals (25%) in comparison with Sham (47%) and Control (45%) animals in 4kHz. Another difference was found in 16kHz frequency. The inhibition in ear-plugged animals was 31% counter 51% in sham and 57% in control animals.
Conclusions: These results suggest that the sensory gating and the acoustic startle reflex was decreased in the sound deprived animals. Moreover, a deficit in the GPIAS also suggests changes in cortical processing. We conclude that early acoustic deprivation impacts auditory processing substantially.

MO176. Understanding Head Orienting During Live Conversation Through Objective Measures and Manual Annotation

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Category: Multisensory Processing/Interactions
Background: Group conversation in a noisy environment is one of the most common communication challenges for hearing impaired listeners. During speech communication and conversational turn-taking, listeners direct their head and eyes to receive meaningful auditory and visual cues, and to relay nonverbal cues back to talkers. There is scant understanding of how listeners interact with multiple talkers, how this interaction may convey listener intent, and how hearing aids might take such movement or intention into account. This study investigated head movement behaviors during self-driven conversations among multiple partners.
Methods: Participants were tested in cohorts of three individuals seated around a circular table. Each participant wore a headset snugly fit to the head upon which were small sensors. Movement of the sensors was tracked in real time by an infrared camera system (OptiTrack V120-Trio). Participants watched an audio-video clip (~3 minutes) presented from a monitor that is visible and audible to each participant for the purpose of generating a common basis for subsequent conversation, followed by a 5-to-10-minute period of undirected discussion. A moderator was available to provide conversational prompts when necessary. Two noise conditions were tested: 1) ambient room noise and the sounds created by the participants, or 2) those sounds plus 60-dB babble presented from four loudspeakers. The entire session was audio and video recorded for annotation purposes by matching types of behaviors to head movement data. Communicational behavior annotation was based on three primary categories: head movement (e.g., face down, face forward, nodding, shaking, turning to right, turning to left), aural-oral communication (e.g., talking, listening to talker, listening to video), and others (e.g., writing, looking at cell phone, fidgeting). Two annotators independently coded the video files using the EUDICO Linguistic Annotator (ELAN) software application. Annotations were then co-registered with the head tracking data in post processing.
Results: Despite some variability in the cumulative events tagged by the annotators, there was good agreement (80% or greater) between annotators for certain behaviors such as head shaking, general talking, and general listening, quantified across all three participants. Movement trajectories showed that listeners move much bigger in both x and y translation within a noisy environment compared to a no-background noise environment.
Conclusions: The combination of objective measures of head movement and manual annotation of conversation behaviors and events provides a rich data set for characterizing natural conversations in ecologically valid settings. The resulting data have internal validity, face validity, and are essential for assessing the relationship between head movement and listener intent. While such experiments sacrifice some control relative to traditional “listening” experiments, the measurement procedures and coding system developed here is a first step towards characterizing head movements during conversations needed to predict listening intent and to create actions based on those predictions.

MO177. A Model of the Reference Frame of the Ventriloquism Aftereffect Considering Saccade Biases

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Category: Multisensory Processing/Interactions
Background: The ventriloquism aftereffect (VAE), observed as a shift in the perceived locations of sounds after audio-visual stimulation, requires reference frame (RF) alignment since hearing and vision encode space in different frames (head-centered vs. eye-centered). Previous experimental studies observed inconsistent results: a mixture of head-centered and eye-centered frames for the VAE induced in the central region vs. a predominantly head-centered frame for the VAE induced in the periphery. A previous model proposed to describe these data required different parameter fits to predict the central vs. peripheral data. Here, a new version of the model is introduced to provide a unified prediction of both data sets considering that saccade responses used to measure VAE might also introduce biases.
Methods: The model has two additively combined components: a saccade-related component and an auditory space representation component. The former component characterizes biases in auditory saccade responses in eye-centered RF. The latter is adapted by ventriloquism signals in a combination of head-centered and eye-centered frames.
Results: The updated version of the model provides a unified prediction for both central and peripheral aftereffect data, even if only head-centered RF is considered in the auditory space representation.

Conclusions: The results suggest that purely head-centered RF is used for adaptation of auditory spatial representation in the ventriloquism aftereffect, and that the apparently mixed eye-and-head centered RF observed experimentally is most probably due to saccade-related biases that are eye-centered. However, additional simulations need to be performed to determine whether eye-centered ventriloquism signals further improve the model predictions.
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MO178. Transfer Effects of Discrete Tactile Mapping of Musical Pitch on Discrimination of Vocoded Stimuli

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Category: Multisensory Processing/Interactions
Background: Many studies have found benefits of using somatosensory modality to augment sound information for individuals with hearing loss. Amongst, a widely popular approach is to provide frequency information of sound input through tactile vibrations via vibro-actuators after low-pass filtering such as Luo and Hayes (2019) Front. Neurosci. 13:1145. However, few studies have explored the use of tactile patterns associated with discrete pitch classes that can be learned through exposure. This study builds on the advantage of single tactile stimulus cue synchronized with each musical note onset for melody recognition performance, found by Huang, et al, (2019), Ear and Hearing. We further employed tactile stimulus mapped spatially on multiple fingertips that expresses an ascending order of the musical scale pitch classes. We hypothesized that this form of tactile stimulation may potentially allow participants to encode additional pitch information through association learning with the goal of improving music perception.
Methods: Each of eight musical diatonic scale notes were associated with one of unique digit 2-5 patterns in the dominant hand, where a pneumatic tactile stimulation apparatus was attached to each of the four fingertips. We tested 10 normal-hearing participants in one session consisting of pre and post-test with the learning phase in-between. During the learning phase, participants had to identify common American nursery song melodies similar to Huang et al. (2019) presented with simultaneous auditory-tactile stimulus for about 10 minutes, using non-vocoded (original) audio. Pre- and post-tests examined stimulus discrimination for 4 conditions: original audio+tactile, tactile only, vocoded audio only, and vocoded audio+tactile. The audio vocoder used cochlear implant 4 channel simulation.
Results: For the three conditions during the 2FAC higher pitch discrimination task, vocoded audio (Av), vocoded audio with tactile (AvT), and tactile only (T), the participants' accuracy improved at the post-test compared to the pre-test. A paired t-test showed that participants performed significantly better ($p = 0.029$) during the tactile only (T) post-test ($M = 0.76$, $SD = 0.14$) than the pre-test ($M = 0.66$, $SD = 0.15$). Also, participants performed significantly better ($p = 0.043$) during the vocoded audio without tactile (Av) condition during the post-test ($M = 0.88$, $SD = 0.11$) than the pre-test ($M = 0.82$, $SD = 0.11$). Comparison between pre-test versus posttest for AvT was approaching to significance ($p = 0.070$).
Conclusions: Our preliminary results demonstrated that the audio-tactile learning indeed improved participant's performance on the vocoded audio+tactile tasks. The learning phase of 10 minutes appear to be sufficient in allowing participants to use the tactile cues conveyed through the spatial pattern mapping on the fingers, which itself successfully represented the ascending order of pitch classes, evident in the tactile only performance. We are currently adding more participants in both normal hearing and cochlear implant listeners.

MO179. Concussion Disrupts Auditory Processing: Evidence From Interactive Metronome and Frequency Following Response

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Category: Multisensory Processing/Interactions
Background: Concussions can result in auditory processing impairments. However, this symptom domain is often underrecognized compared to other cognitive, sensory, neurologic, and socio-emotional consequences.
This study is aimed at shedding light on the impact of concussion on auditory processing through the lens of Interactive Metronome (IM)—a validated rhythm-based assessment tool that requires accurately following

rhythmic auditory cues in a select goal-directed manner —and the Frequency-Following Response (FFR)— an auditory evoked potential that reflects neural processing of stimulus features.
Methods: Seventy-eight children and adolescents (age range 8 – 17) were assessed on both IM and FFR during a clinic visit acutely after their concussion injury and diagnosis
Part 1 of this study tested whether IM performance relates to the Post Concussion Symptoms Scale (PCSS), a self-report measure that records the severity of post-concussion symptoms; and whether IM performance of concussed children is different from a control group (N = 85).
Part 2 tested whether concussed children differ from a control group (N = 78) with respect to fundamental frequency (F0) encoding captured in the FFR, a key acoustic cue previously shown to be weakened by a concussion.
Results: Part 1 results reveal a positive relationship between IM performance and PCSS and poorer IM performance in concussed participants compared to the control group.
Part 2 results reveal that concussed children had lower responses to F0 compared to the control group. Smaller encoding of harmonic frequencies and lower overall response magnitude further differentiate them from age-matched controls.
Conclusions: Overall, these results show that concussions impact auditory processing. Part 1 supports the ability of IM to capture concussion symptoms and motivates its use for systematic post-concussion intervention. Part 2 strengthens previous evidence reporting a poor F0 encoding as a distinctive trait of the concussion signature and motivates its use as an adjunct diagnostic tool and a monitor for recovery.
Supported by NAMM, NOCSAE, Knowles Hearing Center

MO180. Audio-Visual Integration During Physical Inference
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Category: Other, Audio-Visual Integration
Background: Understanding the physical world involves inferring object interactions and their hidden physical causes. Humans are known to make physical inferences from vision, but less is known about the role of hearing, and about how information from different senses might be integrated in real-world settings. One setting in which audio-visual integration could be important is motion perception, in which audio provides cues to object contact (e.g. impact or scraping events) that could help disambiguate visual cues to the three-dimensional trajectories traversed by objects through space. We investigated whether motion perception is influenced by a combination of visual and auditory cues to physical events.
Methods: We used a virtual environment with simulated physics (ThreeDWorld) to render audio and video of objects sliding across a table, and measured the effect of inconsistencies between the auditory and visual stimuli on motion perception. Specifically, we rendered the audio corresponding to an object briefly coming to a halt in the middle of its trajectory, and paired this with a video showing continuous motion. To test the influence of physics knowledge on perceived motion, we varied the physical appropriateness of the sounds.
Results: We found that a short gap in the scraping audio that occurred in the middle of the object's motion caused observers to see the object briefly stopping despite the continuous visual stimulus. The effect was reduced when the audio signal was less physically compatible with the object.
Conclusions: We introduce a new audio-visual integration phenomenon in which visual motion perception is influenced by auditory cues to object interactions. The results suggest that physical inferences about the world involve multisensory integration and that this integration depends on implicit knowledge of physics.

MO181. Noise Inside and Outside the Brain
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Category: Other, Auditory Plasticity
Background: Noise exists around us and within us, impinging on communication. The brain's ability to deal with noise is determined by a combination of biology and experience.
Methods: These effects are revealed through the frequency-following response (FFR), a neurophysiological response to speech that reflects microsecond-fast precision in neural processing.
Results: A chief mechanism for overcoming the noise around us is to boost the strength of the fundamental frequency (F0) in the neural response to sound. This mechanism is strengthened in bilinguals, who must

compete with environmental noise as well as juggle two languages in their brain. In contrast, musicians utilize a distinct method: they fine tune harmonic encoding, providing an additional cue for understanding speech in noise. While these experiences can alter how we deal with the noise around us, neither influences how we minimize the noise within us. Rather, socioeconomic status (SES) and athletic experience exert their influence on the levels of noise in the brain, with low-SES leading to a noisier brain and athletic expertise leading to a quieter one. Biologically, aging also leads to an increase in this noise.
Conclusions: Initially, our understanding of these experiential and biological effects came from comparing groups who differ on a single factor; yet, we now know that the imprint of each factor layers upon one another within an individual's FFR. Thus, the FFR is a neural fingerprint, telling the story of an individual, their life in sound, and their ability to cope with noise that is around and within them. Supported by NIH NIDCD R21DC019448 and R01-NS102500 and the Knowles Hearing Center, Northwestern University

MO182. Transgenic C57BL/6J (Foxp2Cre and GP4.3) x CBA/CaJ Hybrid Mice Show Normal Hearing in Old Age and Parental Transgenic Traits

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Category: Other, Auditory Research Mouse
Background: The C57BL/6J (C57) strain of mice is a common background strain for creating transgenic mice, many of which are valuable in neuroscience research, e.g. the B6.Cg-Foxp2tm1.1(cre)Rpa/J (Foxp2Cre) mice which express cre recombinase in layer 6 of cortex and the C57BL/6J-Tg(Thy1-GCaMP6s)GP4.3Dkim/J (GP4.3) mice which express the green fluorescent calcium indicator, GCaMP6s, in neurons. However, the C57 strain shows hearing loss at young age. This generates obstacles in using these transgenic mice in long-term experiments, e.g. sophisticated behavioral training. CBA/CaJ (CBA) is a strain having normal hearing at young and middle age. Previous research showed that the C57 x CBA hybrid had normal hearing at 3-month, 1-year, and 2-year age, while the C57 completely lost hearing since 1-year age. We crossbred the Foxp2Cre or GP4.3 mice with CBA and compared the hearing ability of the Foxp2Cre x CBA (Foxp2/CBA) and the GP4.3 x CBA (GP/CBA) mice with wildtype CBA and C57 mice at 3-month, 6-month, 9-month, 12-month, and 19-month age. We also confirmed the cre expression of the Foxp2/CBA mice and the GCaMP6s expression of GP/CBA mice.
Methods: Auditory Brainstem Response (ABR) was used to measure the hearing threshold to flat noise and the pure tones of 4, 8, 16, and 32 kHz. At each age, six mice in each mouse type were measured. Two Foxp2/CBA mice were injected with cre-dependent-GFP-expressing virus in AC. Two GP/CBA mice were done craniotomy over AC and measured the sound-induced green fluorescence activity.
Results: The ABR results showed that at 6 months, the C57 mice showed a significant loss in high-frequency (32 kHz) hearing and at 12 months, the C57 mice started to drop significantly in the mid-high-frequency (16 kHz) hearing, while the other 3 mouse types had normal hearing until then. At 19 months, the C57 mice had a significant hearing loss across the whole spectrum, while the hearing of two hybrid mice was normal. However, the C57 hearing ability to 8 and 16 kHz and noise was not completely lost. The Foxp2/CBA mice kept the expression of cre recombinase in layer 6 as the cre-dependent-GFP-expressing virus marked the neurons in layer 6 of AC with GFP. The GP/CBA mice kept the expression of GCaMP6s as tones induced a green fluorescence increase in AC and the response location to different tones formed a tonotopic map.
Conclusions: The Foxp2/CBA and GP/CBA hybrids had better hearing ability than C57 mice since 6-month age and the hearing of the hybrids was normal even at 19 months. The transgenic traits of the parental strain were kept in the hybrid mice. This indicates that by crossbreeding transgenic C57 mice with CBA mice, we can perform long-term experiments requiring normal hearing on the useful transgenic mice.

MO183. Experimental Investigation of the Influence of the Skull Vault Contents on the Skull Bone Motion

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Category: Other, Bone Conduction
Background: The mechanism of the bone conduction pathways through non-osseous skull contents, such as brain and cerebro-spinal fluid (CSF), is still not well understood. This study investigates the influence of the skull vault contents on the skull bone surface wave propagation.

Methods: Experiments were conducted on three Thiel embalmed whole head cadaver specimens. The electromagnetic actuators from commercial bone conduction hearing aids were used to provide stepped sine stimulus in the range of 0.1-10 kHz. Osseous pathways (direct bone stimulation or transcutaneous stimulation) were sequentially activated by mastoid stimulation via a percutaneously implanted screw, Baha® Attract transcutaneous magnet, and a 5-Newton steel headband. Non-osseous pathways were activated by stimulation on the eye, neck, and dura via a 5-Newton steel headband. All stimulation conditions were done on each sample with intact and drained skull contents, sequentially. The response of the skulls was monitored as motions of the ipsi-, top, and contra-lateral skull surface at ~200 points (~ 15-20mm pitch) via a three-dimensional laser Doppler vibrometer system.
Results: Limited differences were observed in the average response of the skull surface between fluid-filled and drained heads, with the largest changes under dura stimulation. In addition, the resonance pattern on the skull surface showed overall longer wavelengths, corresponding to an upshift (~1/2 octave) in natural frequencies for drained heads. Such change is consistent with the reduced mass loading and reduced damping in the absence of CSF.
Conclusions: Overall, the intracranial fluid affects to a limited extent the average response and spatial composition of the skull motion.

MO184. Stimulus Feature Decoding Using Frequency Following Responses

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Category: Other, Brainstem and Cortex: Human and Animal studies
Background: Frequency following responses (FFRs) are scalp-recorded electrophysiological signals that reflect ensemble neural activity that faithfully represent the spectrotemporal characteristics of the stimulus, and contain rich information about the processing of multidimensional speech features. However, FFRs are a measure of stimulus representation across distributed networks. Recent efforts using FFRs to multiple stimuli have aided in understanding the FFR representation across the different auditory centers in the brain in human and animal models using a representational similarity analysis framework (RSA). The RSA framework utilizes the confusability and distances across stimulus features/categories to establish to infer similarities in representation. This framework works best with multiple exemplars per stimulus feature. One previous study has used RSA for cross-species and cross-modal comparisons of FFRs. However, the previous work was limited to single stimulus feature and a single exemplar per stimulus feature and only used electrophysiological approaches. In this study we leverage both electrophysiology and high resolution 7TfMRI neuroimaging with multiple features (speaker, lexical tone category, sex of the speaker) and multiple exemplars per feature to thoroughly characterize FFR representation with both high spatial (fMRI) and temporal precision (FFR). Further we assessed FFR decoding in Guinea pigs which has been used recently as a successful animal model to assay auditory processing.
Methods: Twenty-one human participants and 2 Guinea pigs were recruited for the study. Sixteen stimuli were used to elicit the FFRs. These stimuli were all syllable /di/ spoken by 4 speakers (2 male and 2 female), in four lexical pitch trajectories (high-flat, low-rising, low-dipping, high-falling). The FFRs were obtained for 700 trials of each stimulus. A linear SVM classifier using k-fold cross-validation was used to decode the FFRs. The stimulus features decoded were, stimulus identity (16 categories), lexical pitch trajectory (4 categories), speaker (4 categories), and speaker sex (2 categories). RSA of the data across the FFR vs fMRI and human vs guinea pig model is underway using the off-diagonal elements of the confusion matrices resulting from stimulus decoding. Gaussian-copula mutual information, spearman rho, and multidimensional scaling were for RSA.
Results: Strong (>60%) and above-chance decoding performances were obtained for testing average size of as few as 20 trials for all stimulus categories in FFRs and fMRI. The guinea pig model showed FFR decoding accuracies than owing to higher signal-noise-ratio. RSA analysis is underway to understand the features that drive the decoding performances and to cross-modal and cross-species dis/similarities.
Conclusions: FFRs to multiple features can be reliably used to decode the different multidimensional features of speech in FFRs. The RSA framework can be employed to understand cross-species and cross-modal comparison of stimulus representation with high spatiotemporal precision.

MO185. Characterization of a Newly Established Humanized Mouse Model for DFNA9

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Category: Other, Development: humanized mouse models
Background: DFNA9 is an autosomal dominant inherited disorder characterized by vestibular dysfunction and adult-onset progressive hearing loss caused by different heterozygous mutations in the COCH gene. In Belgium and the Netherlands, DFNA9 is perhaps the most frequent type of AD inherited post-lingual SNHL, with about 1.000 patients confirmed by routine genetic testing. The majority of patients in these regions are carriers of the c.151C>T mutation, resulting in a replacement of amino acid Proline by Serine at position 51. The COCH gene encodes for cochlin, which is a secreted protein expressed at high levels in the inner ear, more specifically in the fibrocytes of the spiral ligament and spiral limbus. Although the exact molecular mechanism underlying DFNA9 remains unknown, it is believed that mutations in cochlin lead to the formation of high-molecular-weight aggregates that sequester wildtype cochlin proteins. In order to study the pathophysiology of DFNA9 more thoroughly and investigate new therapeutic strategies to tackle hearing and vestibular loss in carriers of the p.P51S founder mutation, we developed a clinically relevant mouse model for DFNA9 patients within Belgium and the Netherlands.
Methods: We generated DFNA9mWT/hWT and DFNA9mWT/hP51S mice bearing humanized mutant and humanized wildtype COCH alleles. More specifically, exon 3 to exon 6 of the murine Coch gene was codon-optimized in order to display human nucleotide sequence (for gRNA targeting), while retaining murine amino acid sequence (for cochlin functionality). As these mice were created using the C57BL/6N strain carrying the Cdh23ahl allele, we bred them with C57BL/6N mice where the Cdh23ahl locus was removed from the genetic background. At this moment, we already obtained Cdh23ahl corrected DFNA9hWT/hWT, DFNA9hWT/hP51S and DFNA9hP51S/hP51S mice who are now characterized in terms of hearing and vestibular function in addition to an excessive investigation of gene expression to assess wildtype and mutant cochlin expression.
Results: An extensive in vitro investigation of hybrid Coch pre-mRNA splicing did not reveal any alternatively spliced transcripts, which we confirmed in the inner ear of DFNA9hWT/hWT and DFNA9hWT/hP51S mice. In addition, direct sanger sequencing showed that the c.151C>T mutation was successfully introduced in DFNA9hWT/hP51S mice and DFNA9hP51S/hP51S mice. Phenotyping of DFNA9hWT/hWT demonstrates normal hearing function and vestibular function up to three months confirming that humanization did not lead to any adverse effects on otovestibular functioning. Currently, Cdh23 corrected DFNA9hWT/hWT, DFNA9hWT/hP51S, and DFNA9hP51S/hP51S mice are reaching the age of 6 months and are assessed for hearing and vestibular function.
Conclusions: Clinical characterization of the humanized DFNA9 mouse model carrying the p.P51S variant is ongoing. This will allow us to increase our understanding of the pathophysiology underlying DFNA9. In the next step, this mouse model will be used to investigate the specificity and efficacy of a CRISPR/Cas9-based gene therapy to target the mutant COCH allele.

MO186. Open Board

MO187. A New Methodology for Evaluation of Large Vestibular Aqueduct in CT and MRI Images: Finding the Clinical Correlation

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Category: Other, Inner Ear Pathology/ Imaging
Background: The gold standard to diagnose large vestibular aqueduct(LVA) are CT images, using Valvassori and Clemis or Cincinnati criteria. The previous studies did not agree on whether audiological/clinical data are correlated to radiological data. The goal of our research is the development of a new method to evaluate LVA/large endolymphatic sac anomaly (LESA) using CT/digital volume

tomography (DVT)/MRI images. The other objectives are to compare measurements of MRI with CT/DVT, to detect additional inner ear malformations, and to find relationships between the radiological measurements and clinical course of the disease.
Methods: Retrospective analysis of radiological images from 173 patients (315 ears), who were diagnosed with LVA/LESA based on CT/DVT and/or MRI images of the temporal bone, was performed in the tertiary center. CT/DVT images were available for all 173 patients and 93 (161 ears) of those patients had also MRI images. The following measurements were performed on both modalities: orifice, length, external aperture measurements of vestibular aqueduct/endolymphatic duct and sac. Additional measurements like volume of extraosseus endolymphatic sac, maximal contact diameter of the dura mater and extraosseus endolymphatic sac in the axial pictures were made on MRI. The clinical course of hearing loss was defined using the patient's history, audiometry and the patients were divided into two groups: 1. congenital deafness and 2. patients with progredient hearing loss with and without episodes of sudden hearing loss, progredient hearing loss, which starts in second-third decade, sudden deafness, normal hearing.
Results: LVA was reported to be bilateral in 82,08 % (142 patients) and unilateral 17,92% (31 patients) of cases. In 117 patients (214 ears), LVA was the only radiographically detectable inner ear anomaly, whereas in 56 patients (101 ears) it was accompanied by other inner ear abnormalities. Comparison of MRI and CT/DVT measurements showed a strong correlation (0.65) in external aperture, moderate (0.57) in orifice, and weak (0.33) in length measurements ($p<0.05$). Measurements in MRI (maximal contact diameter of the dura mater and extraosseus endolymphatic sac, volume of extraosseus endolymphatic sac) showed a strong correlation (0.84, $p<0.05$).
The logistic regression model showed that length of vestibular aqueduct and external aperture measurement in CT images are statistically significant to distinguish congenital deafness and the other group. From the MRI measurements only maximal contact diameter of the dura mater and extraosseus endolymphatic sac was statistically significant to distinguish the two previous explained groups.
Conclusions: The patients with the shorter vestibular aqueduct, brighter operculum, longer dura mater and extraosseus endolymphatic saccus contact are prone to have more profound hearing loss compared to the other group. The new method can become a valuable tool to predict the course of hearing loss in children and adult population as well as to help to consult the patients about future treatment.

MO188. Heterogeneity and Plasticity of Olfactory Bulb Dopaminergic Neurons

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Category: Other, Olfactory system
Background: Dopaminergic (DA) neurons in the olfactory bulb regulate the transmission of information at the earliest stages of sensory processing and are one of the few neuronal types in the mammalian brain continually generated throughout postnatal life. Here, we ask whether this continuous neuronal production results in a gradient of cell states within the resident population, and whether this affects neuronal plasticity.
Methods: Birthdating in 4-week old DAT-IRES-Cre/Flox-tdT mice revealed that resident DA neurons span an age range of at least 3 weeks. We next collected individual DA neurons by either manual sorting of tdT positive DA neurons, or aspiration after patch-clamp recordings in acute slices (Patch-seq), and performed deep single-cell RNA sequencing. Additionally, we performed single nuclei RNAseq of fluorescently sorted nuclei from dopaminergic neurons.
Results: Clustering analysis identified putative subpopulations of DA neurons, while cell trajectory analysis described a transcriptomic gradient that closely matched the clusters. Further analysis revealed differentially expressed genes, significantly enriched for GO terms related to neuronal and synaptic function, indicating that the identified gradient may reflect a transcriptional maturational gradient. Furthermore, the transcriptomic changes elicit by sensory deprivation differed across the putative subpopulations of dopaminergic neurons. Ongoing analysis of electrophysiological properties will reveal whether it describes a gradient of functional states.
Conclusions: In summary, we are exploring a hitherto unanticipated gradient of cell states within a specific neuronal subtype that could underpin differential plasticity properties, and the functional maturation of DA cells in the postnatal brain.

MO189. Does Contraction Bias Occur at Low or High Levels of Auditory Processing?

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Category: Other, Perceptual learning, Serial effects
Background: Serial dependence is a phenomenon where perceptual judgments are systematically biased (contracted) towards recent experiences. While serial dependence has been extensively documented in visual perception and was observed for a variety of stimuli and tasks, from simple to complex judgments, there is only little research on the auditory modality. A main debate is whether contraction occurs at a perceptual-decision level, or at a subsequent working-memory level. Both views assume Bayesian optimization at object levels, which promotes perceptual likelihood and stability with respect to external objects and events. The current study is, to our knowledge, the first that systematically analyze the mechanisms underlying the formation of perceptual priors by dissociating the contributions of high (abstract representation) and low (frequency-specific representation) auditory-processing levels. Furthermore, it evaluates the contribution of attention to contraction in the auditory modality, revealing whether these processes are bottom-up or top-down determined. It is also the first that investigates the source of pitch contraction when consecutive tone trials belong to different timbre categories.
Methods: We conducted a two-tone pitch-discrimination task with trials composed of pure or harmonic complex tones, to assess the influence of timbre categories on pitch contraction. Since the fundamental frequency of a complex tone determines its perceived pitch even when physically missing, we analyzed whether the contraction operates on high- or low-level representations of pitch by comparing contraction in presence or absence of the fundamental frequency. If at high level, no difference is expected, while if at low level, contraction should be observed only when the fundamental is physically present. An additional two-tone pitch discrimination task, involving only pure tones but including a simple arithmetic discrimination task on half the trials, was conducted to evaluate the influence of participant's attention on the bias magnitude.
Results: We found that pitch contraction is larger when consecutive trials have the same timbre, suggesting a high-level contribution of perceptual categories, in line with the high object-level account. Surprisingly, the cross-timbre bias reveals an early contribution within frequency-channels: when a complex tone lacks its fundamental frequency, there is no contraction to its pitch, although the latter is still perceived. Rather, contraction occurs to the physically-present frequency even when it differs from the perceived pitch, suggesting a bottom-up contribution. Additionally, attention is not necessary, since contraction occurs even when participants are actively attending to different sources of information in a bottom-up manner. Yet it enhances contraction, which also suggests a top-down contribution.
Conclusions: This combination of contributions reveals that integration of recent history operates at both low and high processing stages, through both bottom-up and top-down pathways. These results reveal the mechanism underlying the formation of perceptual priors and evidence the sources of contraction bias in the auditory modality.

MO190. Excitotoxicity Induced Synaptopathy: From Molecular Mechanisms to Targeted Therapies

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Category: Other, Synaptopathy
Background: The type I spiral ganglion neurons (SGN) contact the sensory inner hair cells (IHC) via their peripheral dendrites and relay auditory information to the brainstem via their central axon fibers. The disruption of the synaptic connection between IHCs and SGNs have been shown to occur early in many cochlear pathology conditions such as noise- or ototoxic drug-exposures or cochlear aging. It has been proposed that the excitotoxic process may be a primary initial event in the degenerative cascade observed after noise exposure or during cochlear aging.
Methods: To investigate the underlying molecular mechanisms involving in cochlear synaptopathy after noise exposure and to develop effective therapies, we examined the molecular basis responsible for kainate-induced loss of IHC–SGN synapses and degeneration of the distal type 1 SGN peripheral axons using a

cochlear explant culture from P3 mouse pups. In addition, we evaluated the efficiency of synapse regeneration using several novel BDNF mimetics.
Results: Our results revealed that disruption of the synaptic connection between IHCs and SGNs affected neurotrophin signaling pathways and induced increased levels of oxidative stress and upregulated some proinflammatory factors. Addition of BDNF mimetics increases axon growth and synaptogenesis.
Conclusions: These results suggest that understanding the pathways involved in excitotoxicity is of critical importance for the future clinical treatment of many auditory neurodegenerative diseases.

MO191. Ultrasound-Evoked Otoacoustic Emissions as a Novel Diagnostic Tool for Hearing and Intracranial Pressure Assessment

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Category: Otoacoustic Emissions
Background: Distortion Product Otoacoustic Emissions (DPOAEs) are sounds generated by the outer hair cells of the cochlea in response to pure tones. Facilitated by the non-linear mechanism of the cochlear amplifier, DPOAEs reflect the integrity of the outer hair cells and thus are a robust and non-invasive method for hearing assessment, especially as part of the newborn hearing exam. Previously, DPOAEs have been studied with air-conduction in humans. Recent studies have demonstrated that transcranial ultrasound (US), particularly amplitude-modulated (AM) US, can stimulate the cochlea through a fluid pathway. We aimed to determine if AM US can also elicit DPOAEs thereby providing a novel diagnostic method for hearing assessment and measurement of intracranial pressure (ICP) that has been described previously for acoustic pure-tones.
Methods: Five guinea pigs were utilized as an animal model for generating acoustic and AM US-based DPOAEs. With an IACUC-approved protocol, acoustic DPOAEs were recorded with primary (f1) frequency of 10 kHz at a level of 60 dB with two speakers each presenting a pure tone at a ratio of 1.2 to the secondary frequency (f2) for 1s duration. AM US-evoked DPOAEs were generated with two US transducers at carrier frequencies 100 kHz and 220 kHz coupled to the ipsilateral cranium approximately 1 cm rostral to the pinna with 70 kPa level. Modulated frequency of 10 kHz was applied to the carrier waveform for the primary frequency also at a ratio of 1.2. In both modalities with a single guinea pig, DPOAEs were recorded before and after euthanization. ICP was elevated hydrostatically with a column in communication with the subarachnoid space via a small craniotomy and DPOAEs were recorded at ICP steps of 4, 15, and 28 mmHg in a single guinea pig experiment.
Results: Acoustic, US, and bimodal stimulation generated DPOAEs at the 10 kHz primary frequency at intermodulation frequencies of 8, 6, 4, and 2 kHz . The cubic distortion product (8 kHz) was most prominent with levels between 10- and 20-dB SPL. The gradual cessation of DPOAEs after euthanization confirmed that the signals are otoacoustic emissions with reductions of 16- and 32-dB SPL for acoustic and AM US stimulation, respectively. When ICP was elevated from 4 to 15 mmHg, DPOAEs in all modalities remained stable but further increases to 28 mmHg caused diffuse reductions in DPOAE amplitude with mean decrease of 13.3 and 14.6 dB SPL for acoustic and AM US-evoked modalities, respectively.
Conclusions: We demonstrate that transcranial AM US generates DPOAEs that may be used as a novel hearing assessment method via a fluid pathway not utilized by conventional audiometry. With additional research to determine the optimum stimulation protocols, US-evoked DPOAEs may also be useful for non-invasive measurement of human ICP and diagnosis of intracranial hypertension.

MO192. Coupled Limit-Cycle Oscillators Vs Standing Wave Models of Spontaneous Otoacoustic Emission Generation: Comparing the Mechanisms and Characteristics of Cluster Formation

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Category: Otoacoustic Emissions
Background: The generation of sound by the ear in the absence of external stimuli, called spontaneous otoacoustic emissions (SOAEs), is a common feature of both non-mammalian and mammalian species. Two alternative theories have been proposed to describe SOAE generation: the local oscillator framework (LOF) and the global oscillator framework (GOF). In the LOF, the inner ear consists of an array of limit-cycle oscillators coupled to nearest neighbors with resistive (dissipative) and reactive (elastic) elements. In the

GOF, the individual elements of the cochlea are not oscillatory when considered in isolation; however, when coupled via to the cochlear traveling wave, cochlear roughness gives rise to reflections and the overall system exhibits spontaneous oscillations due to self-sustained standing waves. The goal of this study is to compare predictions from these two theories.
Methods: Both LOF (Vilfan and Duke, 2008), and GOF (Bowling et al, 2019) models were implemented in MATLAB using a state-space formulation. Cochlear roughness is introduced by adding some random perturbations in the values of model parameters. Linear stability analysis is first used to assess the presence of linearly unstable modes. The nonlinear dynamics of the models are simulated to predict the limit cycle oscillations of the system. The formation of "clusters" (groups of neighboring elements that oscillate at the same frequency) is evaluated from the steady-state dynamics of the systems, for both LOF and GOF models.
Results: In the LOF model, a large number of linearly unstable frequencies is observed in the linear stability diagram; however, the spectrum of the predicted SOAEs includes only a few discrete peaks. This occurs because of clustering due to self-synchronization of oscillators with neighboring oscillators when nonlinearity is considered. In the GOF model, only a few discrete unstable frequencies are observed, which can be linked to the spectral peaks observed in the SOAE spectrum. The spontaneous oscillations of the basilar membrane in the GOF model exhibits plateaus that are analogous to the oscillator clusters predicted by the LOF model. The influence of model parameters on the formation of clusters and plateaus in the LOF and GOF models is analyzed.
Conclusions: Although the LOF and GOF models start with seemingly disparate assumptions, the spectrum of both the SOAE and of the inner ear response exhibits striking similarities, including the formation of clusters. In the absence of intrinsic noise or external stimulus, both models appear equally capable of simulating SOAEs. This study lays the groundwork such that future comparisons relative to empirical data (e.g., effects of external stimuli) should reveal critical differences between these two theories and how to refine the models (e.g., proper inclusion of noise).

MO193. Components of Stimulus-Frequency Otoacoustic Emission in Mice
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Category: Otoacoustic Emissions
Background: Since their discovery, the paradigm surrounding otoacoustic emission (OAE) generation has stayed largely unchanged – outer hair cell (OHC)-driven amplification is most notable in the peak region, or where cochlear excitation is resonant along the basilar membrane (BM). However, recent advances in imaging techniques revealed that the motion of the organ of Corti doesn't have to follow the motion of the BM. Unlike the BM, the vibrations of the organ of Corti are actively amplified in both the peak and tail regions of the wave, prompting us to revisit the nature of OAE generation and cochlear amplification.
To provide further answers to this question, we use different stimulus parameters to manipulate cochlear amplification and its reflection in stimulus-frequency (SF)OAE responses. We predict that SFOAE components originating in the tail region of the traveling wave should demonstrate near-linear growth with stimulus intensity as these are characteristics of the amplified motions of the organ of Corti in the tail region of the wave. The SFOAE components originating in the peak region, on the other hand, are expected to display compressive growth, as demonstrated in both BM and organ of Corti vibrations. Ototoxic drugs may be used as additional manipulators of amplification, especially at the peak region.
Methods: We measured SFOAEs in deeply anesthetized mice (CBA/CaJ). As SFOAE overlaps in time and frequency with evoking stimulus, we use a suppression method to extract different emission components. We hypothesize that by varying the frequency ratio of the suppressor tone relative to the evoking probe tone (from a narrow to wide ratio), one may extract OAE components from cochlear locations corresponding to either peak or tail amplification regions. We measure narrow- and wide-ratio SFOAEs across stimulus intensities, as well as before and after application of ototoxic drugs.
Results: The SFOAE collected with wide suppressor to probe ratios tend to have short latencies and grow linearly with stimulus intensity. The narrow ratio SFOAEs, on the other hand, tend to have longer latencies and growth deviating from linearity.
Conclusions: This preliminary data confirms the hypothesis that SFOAE components extracted with different ratios reflect on cochlear mechanics in either tail of the peak region. Application of ototoxic furosemide may allow greater manipulation of the peak region separately from the tail region, and provide more robust confirmation of our hypothesis.

MO194. Measurement of Swept Level Distortion Product Otoacoustic Emission (DPOAE) Growth Functions Simultaneously Across Frequencies

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Category: Otoacoustic Emissions

Background: Otoacoustic emissions (OAEs) are low-level sounds generated by the inner ear that provide a non-invasive assessment of cochlear health and function. OAEs measured across a range of stimulus levels are termed growth functions. OAE growth functions have proven useful for a variety of applications, including for estimating behavioral threshold and detecting subclinical cochlear aging. Here, we demonstrate a novel method for measuring distortion product OAE (DPOAE) growth functions by sweeping the evoking stimuli in level while simultaneously measuring at multiple frequency pairs. We demonstrate that our novel recording method yields results (magnitude and phase estimates) that are equivalent to those measured using traditional (discrete) stimuli. Notably, this novel technique offers the distinct benefit of obtaining near-continuous growth functions – better capturing inflection points than discrete measures – across multiple frequencies in just 4-5 minutes.

Methods: DPOAE growth functions were obtained from participants by fixing L1 at 70 dB FPL and varying L2 from 0-70 FPL. L2 levels were either varied continuously at a rate of 10 dB/s (swept level experimental condition) or varied discretely in 10 dB steps (discrete level control condition). Growth functions were obtained at f2 = 1, 4, 8, and/or 10 kHz. Growth functions were recorded with either a single frequency pair (control condition) or with multiple frequency pairs presented simultaneously (experimental condition). DPOAE magnitudes and noise floors measured in all conditions were estimated using a least-squares fitting procedure.

Results: DPOAEs measured using swept level stimuli (novel experimental condition) were equivalent to those measured using discrete tones (control condition) at equivalent stimulus levels, both on average and for individual participants. Additionally, DPOAE growth functions measured using multiple frequency pairs presented simultaneously (novel experimental condition) were equivalent to those measured using a single frequency pair (control condition). Average absolute differences within and between stimulus conditions were typically within +/- 5 dB, and always within +/- 10 dB; these differences are within the accepted range of test-retest reliability for OAEs. Finally, DPOAE growth functions obtained using the swept level technique were highly repeatable for a given participant across probe insertions, test sessions, and various measurement protocols. Taken together, these results suggest that our novel swept level technique can be used to obtain DPOAE growth functions that are equivalent to those measured using discrete stimuli.

Conclusions: Using a novel measurement technique, we demonstrate that DPOAE growth functions can be obtained using swept level stimuli with multiple frequency pairs presented simultaneously. This novel recording technique offers several benefits and may open the door for more advanced OAE applications to be adopted in both clinical and research settings.

MO195. Cortical Oscillations Modulated by Vagus Nerve Stimulation in Auditory Pathways

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Category: Primary Auditory Cortex

Background: Vagus nerve stimulation (VNS) activates cholinergic, and noradrenergic systems and modulates perception and cognition. We previously found that VNS strengthened stimulus-evoked activities in the superficial layer of the sensory cortex, but not in the deep layer, suggesting that VNS altered the balance between feedforward (FF) and feedback (FB) pathways. Because the cholinergic and noradrenergic system modulates a stimulus gain in the cortex, we hypothesized that VNS modulates the FF-FB balance through the cholinergic and noradrenergic system.

Methods: We investigated how VNS modulates auditory-evoked activities in the auditory cortex of rats using a surface microelectrode array, and how the modulation changed with cholinergic and noradrenergic antagonists. Thirty-eight male Wistar rats with postnatal weeks of 9-12 were used in the experiments. The electrophysiological recordings were performed at least five days post-implantation of VNS system. A click was provided as a test stimulus every second. The current from pulse generator was set to 500μA with the width of 130μs at a rate of 30Hz. VNS of 900 pulses was applied at 5-min intervals. Antagonists were

locally applied on the auditory cortex. The putative modulation of FF and FB pathways were estimated by high- and low-frequency power, respectively, in time-frequency analyses.
Results: Consistent with our previous work, VNS increased the auditory cortical evoked potentials and shortened the latency. Furthermore, we found that VNS increased the auditory-evoked gamma and beta power, and in turn decreased the theta power. Gamma and beta power were not modulated by VNS with a cholinergic antagonist. Theta power was not modulated by VNS with a noradrenergic antagonist.
Conclusions: Our results suggest that VNS modulates the hierarchical pathway by acting on different oscillations through the cholinergic and noradrenergic system. VNS may improve dysfunction of cortical auditory pathways by enhancing auditory processing.

MO196. Unexpected Suppression of Single-Neuron Responses to Natural Foreground Stimuli by Concurrent Backgrounds in Auditory Cortex

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Category: Primary Auditory Cortex
Background: In everyday hearing, listeners encounter complex auditory scenes containing spectrally overlapping sound sources. Accurate perception requires streaming, i.e., the grouping of sound features according to their source based on statistical regularities in the time and frequency domains. Numerous psychoacoustic studies have used auditory streaming to interrogate the perceptual limits of grouping cues, but less is known about its underlying neural basis, particularly for natural sounds.
Methods: The current study recorded single unit activity in the auditory cortex (AC) of awake ferrets using 64-channel laminar arrays. Passively listening ferrets were presented with natural sound excerpts from two broad, ethologically relevant categories: textures (backgrounds, BGs) and transients (foregrounds, FGs). BG and FG stimuli were presented individually and concurrently. To test the specific role of spectral and temporal sound statistics on streaming, synthetic versions of natural sounds, in which temporal anCd/or spectral modulation features were shuffled, were also presented individually and concurrently (Norman-Haignere and McDermott, 2018).
Results: Neural responses to concurrent sound pairs (BG+FG) were modeled as linear weighted combinations of responses to individual sounds. Model weights showed BG+FG responses consistently suppressed relative to individual sound responses. Perceptually, FG stimuli typically pop out from BGs. Surprisingly, the model weights showed stronger suppression of FG responses relative to BG responses. To investigate the sound features supporting FG suppression, spectral and temporal statistical features of each sound were regressed against the relative suppression for each sound. Sounds with low temporal stationarity, high spectral stationarity, and narrower spectral bandwidth, which are more prominent in FGs, were more likely to be suppressed and less likely to suppress the concurrent sound. When synthetic sounds with shuffled modulation statistics were presented, the relative suppression of FG responses incrementally decreased with removal of natural spectral and temporal modulations, but was not entirely eliminated. Effects were similar in primary and secondary auditory cortex.
Conclusions: While the generalized suppression of responses to concurrent sounds was expected, the relative suppression of FG responses was inconsistent with predictions from behavioral studies. Percepts typically emphasize temporally sparse and narrowband FGs, which predicts enhancement of their neural representation, relative to spectro-temporally dense BGs. Our investigation of synthetic stimuli revealed no single FG feature supporting their suppression. Instead, all the features that distinguished BG and FG contributed to this effect. Both feed-forward and lateral inhibition are known to shape evoked activity in AC. These pathways may be strongly activated by the dense BG stimuli, leading to widespread suppression. Studies in downstream auditory areas may reveal how this representation supports a preferential percept of FG stimuli. This result highlights the need for better basic models of auditory streaming, which could support advances in therapies for hearing loss, where streaming performance often suffers.

MO197. Cue-Independent Attentionally Modulated Cortical Signatures of the Auditory Looming Bias

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Category: Psychoacoustics

Background: Approaching sounds exhibit an increased perceptual salience compared to receding sounds. This effect, termed the auditory looming bias, emerges in behavioral as well as neurophysiological imaging data. It is assumed to be linked to evolutionary processes, meant to warn us of potential threats. Animal studies show that no attention is required for its appearance, supporting the theory of a reflexive mechanism. Human neuroimaging studies further suggest the looming bias to arise in a largely distributed network including both parietal and frontal regions while being inconclusive regarding the directionality and timing of their connections. However, comparability of those studies is limited by the different cues being used. Although previous studies of the phenomenon were mainly based on intensity changes, electrophysiological signals in those cases can be confounded by overall differences in sound intensity. To circumvent this issue, previous work varied the spectral magnitude profile of sounds to induce moving sensations: a sharpened magnitude spectrum led to a receding perception, while a flattened magnitude spectrum elicited a looming one.
Methods: In our current work we compared both types of manipulations independently and directly within one experiment, while looking into two attentional states. Stimuli were created by crosswise varying the intensity level and magnitude spectrum of sounds. As the direction-dependent filtering of sounds caused by the reflections on a listener's anatomy mediates the perceived distance, individual subject HRTFs were moreover used to filter the presented stimuli. To investigate the underlying cortical mechanisms of the looming bias, we recorded electrophysiological data of 28 participants using 128-channel scalp-EEG as well individual brain anatomies and electrode positions. For the experiment, subjects underwent a passive and an active listening condition. During passive listening, their top-down attention was directed away from the sounds by a muted and subtitled movie. In active listening participants had to judge the direction of the movement of each sound-pair by a keypress. We further employed directed functional connectivity measures and examined the interplay among previously identified frontoparietal regions.
Results: Behaviorally, the looming bias manifests in reaction times as well as accuracy scores of our subjects. Signatures of the bias are already present at around 80 ms and at stages as early as the primary auditory cortex. We find this to happen irrespective of attentional state or stimulus nature, while attention itself increases the amplitude of the phenomenon. Preliminary outcomes of our connectivity studies seem to further support a bottom-up connectivity pattern.
Conclusions: In combination, our results corroborate the claimed evolutionary origin of the looming bias, to be further discussed in the light of our ongoing studies on newborn participants. [Supported by FWF I 4294-B]

MO198. Open Board

MO199. Grouping and Segregating Sound Sources in Natural Environments
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Category: Psychoacoustics
Background: In natural environments, such as nature reserves and parks, the acoustic scene is formed by the landscape, vegetation, climate, and the animal species that inhabit the area. The variety and variability of animal species, referred to as biodiversity, create a complex acoustic scene with the sounds produced from multiple sources arriving at the ear simultaneously. These scenes vary in the diversity of species as well as the abundance of a given species. Recently, ecologists have developed computational methods to assess levels of biodiversity from acoustic recordings, raising the question of whether human listeners could solve a similar problem of estimating biodiversity from an acoustic scene.
Methods: First, we investigated whether listeners could discriminate the abundance of a given species. We began by collecting recordings from different bird species that inhabit the Parc Naturel de Chevreuse. With a large number of individual bird species recordings, we created single-species mixtures of varying sizes, from 1 to 32, and asked listeners to discriminate sound intervals of different sizes. Listeners could detect differences in abundance for small sizes (n<=8) but larger sizes (n>=16) were indistinguishable, suggesting listeners retain acoustic details when the abundance size is small but this capacity is lost when the size is large.
Results: Next, we investigated whether listeners group or segregate single-species mixtures presented concurrently with animal sounds (birds, amphibians and insects) or environmental sounds (rain and wind). We first measured statistics of our stimuli at the output of a standard auditory model and ranked the sounds

from most similar to least similar. We asked listeners to discriminate exemplars of single-species mixtures varying in size (1, 8 and 32) in the presence of concurrent sound sources as well as control conditions with the single-species mixtures presented in isolation. For the control conditions, we found that listener discrimination performance decreased with increasing abundance size. Listeners performance was comparable to the control conditions when the concurrent sound had different statistics, but performance decreased when the concurrent sound had similar statistics, suggesting listeners segregate sound sources that have different statistics but group sound sources that have similar statistics.
Conclusions: Our results reveal the capacities of auditory perception of sound sources common in natural environments, where listeners appear to retain acoustic details for small single-species mixtures and group sound sources with similar statistics. Overall, the findings suggest that auditory scene analysis principles previously demonstrated with artificial or speech stimuli apply to listening in natural environments.

MO200. Level-Dependent Responses to Speech in Noise Derived From a Nonlinear Cochlear Model

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Category: Psychoacoustics
Background: In a pilot experiment which we conducted in four normally hearing listeners, speech in noise perception improved with increasing stimulus level. We used short sentences as a speech signal and babble noise as a masker. We kept the signal to babble noise ratio constant (-5 dB) and changed the speech level from 40 to 70 dB SPL. In the presented study, we used a nonlinear cochlear model to investigate whether the auditory periphery is involved in the observed improvement of speech recognition with increasing level.
Methods: A cochlear model which simulates the basilar membrane as an array of fluid coupled oscillators is used. The model is nonlinear because the vibration of the oscillators is undamped with a feedback force which is at moderate and large levels limited by a sigmoidal function. The model predicts two-tone suppression and generates otoacoustic emissions. To predict how strongly the added babble noise distorts the response at the output of the model, cross-correlation is calculated between the model responses to speech and speech + babble noise. It is a variant of technique used to predict the quality of sound.
Results: Cross-correlation between the model responses was increased with increasing level, which should mean that the babble noise was less effective as a masker at higher intensities. In other words, the babble noise less distorted the signal at the output of the cochlear model as the level increased. This effect of stimulus level is in agreement with the experimental results showing improved speech recognition. Therefore, the simulation indicates that the auditory periphery is involved in the observed speech recognition improvement.
Conclusions: Experiments conducted in normally hearing human listeners showed that as the speech level increases, background babble noise is less effective as a masker. Because the cross-correlation between the model responses to the speech in noise stimuli and speech stimuli increased with increasing level, auditory periphery seems to be involved in the observed level effect. We can hypothesize that the improved recognition is due to the two-tone suppression phenomenon, which is important for perception of complex acoustic signals at moderate and high intensities.
Acknowledgement:
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MO201. Effects of Age, Hearing Loss, and Level on Auditory Context Effects

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Category: Psychoacoustics
Background: Auditory enhancement is the increase in our ability to hear out a target tone in the presence of spectrally flanking masking tones, when the masker and target are preceded by a copy of the masker. This effect is thought to reflect our ability to adapt to acoustic environments and achieve perceptual constancy in the face of varying talkers, reverberation, and background noise. Enhancement can be 20 dB in young normal-hearing listeners, and is reduced to near zero in people with cochlear implants. This study investigated the effect in people with hearing loss, age-matched people with normal hearing, and young normal-hearing listeners at different sound levels (N>=15 per group).

Methods: The stimuli were presented at 70 dB SPL per component to ensure audibility for the participants with hearing loss. The young normal-hearing listeners also heard the stimuli at 45 dB SPL per component. The 100-ms target tone was presented in the range between 1 and 2 kHz, selected randomly on each trial to avoid longer-term build-up effects of enhancement. Two flanking tonal masker components were placed on each side of the target, spaced 0.3 or 0.6 octaves apart from each other and the target, and were gated on and off with the target. The 300-ms precursor (when present) consisted of either just the masker components (enhancement condition) or both the target and masker components (control condition). A 100-ms probe tone was presented after the target and listeners judged whether the probe was presented at the same or different frequency relative to the target. The level of the target and probe was adaptively varied relative to the masker components to track threshold.
Results: Results from young normal-hearing listeners at the moderate (45 dB) level replicated earlier findings of enhancement near 20 dB. However, none of the conditions tested at 70 dB SPL showed as much enhancement. Although the hearing-impaired listeners showed very little enhancement overall, the amount was not significantly different from that found in the age-matched normal-hearing group, or the younger normal-hearing group when listening to the stimuli at 70 dB SPL.
Conclusions: The results reveal an unexpected effect of level on enhancement, with reduced enhancement at levels that hearing-impaired listeners must utilize in order for stimuli to be audible. Preliminary data on a new group of normal-hearing listeners are testing the hypothesis that differences in the slope of the psychometric function across conditions may affect the estimated amount of enhancement. The results so far suggest that enhancement is abnormal in people with hearing loss. However, they also suggest that the difference between normal-hearing and hearing-impaired listeners may be due at least in part to different levels, as opposed to the effects of hearing loss per se. [Supported by NIH grant R01DC012262.]

MO202. Streaming Sound Texture in Auditory Scenes

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Category: Psychoacoustics
Background: Sound textures are created by the superposition of many similar acoustic events (e.g., rain falling, birds chirping, or people clapping) and are thought to be represented in the auditory system by statistics that summarize acoustic information over time. Real-world auditory scenes frequently contain multiple concurrent textures (as when birds chatter next to a babbling brook), raising the question of whether listeners can "hear out" (i.e., stream) individual textures. We sought to characterize "texture streaming" by asking whether listeners can estimate the number of sound texture sources in an auditory scene.
Methods: In the first experiment ("streaming experiment"), participants heard auditory scenes composed of one or two real-world textures and judged the number of distinct sound sources. In a follow-up experiment ("similarity experiment"), we asked participants to rate the similarity of texture pairs from the streaming experiment on a 10 point scale.
Results: Listeners performed above chance in the streaming experiment, tending to correctly judge the number of sources in each auditory scene. Inspection of judgments for individual scenes revealed consistent patterns of errors across participants, indicating that particular combinations of textures tended to be mistakenly heard as a single stream. Additionally, we found similarity ratings to be partially predictive of streaming judgments. However, a substantial portion of the explainable variance in streaming judgments could not be predicted from perceptual similarity of the source textures, suggesting additional (as yet not understood) principles of perceptual organization.
Conclusions: Together, these experiments demonstrate the phenomenon of texture streaming—a neglected aspect of auditory scene analysis in which listeners are able to stream concurrent textures in auditory scenes.

MO203. Associations Between Misophonia and Musicality in Adults and Children

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Category: Psychoacoustics
Background: Misophonic experiences–characterized by intense negative reactions to sounds such as chewing and tapping–are surprisingly common in the general population. This may be due to early-developing characteristics of individual listeners which might lead some people to have more intense emotional auditory experiences than others, whether positively or negatively valenced. In this study, we

reasoned that both music and misophonia involve high-level affective responses to sound. We therefore asked whether everyday musicality can predict the extent to which children and adults experience misophonic reactions.
Methods: We administered surveys to adults (18+) and children (ages 6-15), assessing self-reported experience of misophonia, ASMR, and musical chills (frisson), severity of misophonia (using the Amsterdam Misophonia Scale or A-MISO-S and the Mini Sussex Misophonia Scale), musicality (using the Gold Musical Sophistication Index or Gold MSI), and musical training. We also measured real-time emotional responses to short videos that were intended to trigger misophonia, ASMR, or musical frisson by asking participants to press a button for every reaction they experienced while the video played (e.g., disgust, tingles) and rating overall valence and arousal for each video. We also measured participants' ability to perceive speech prosody, musical rhythm, harmony/tonality, and musical and vocal emotion.
Results: We found that cross-sectionally, musicality, frisson, and misophonic reactions tended to increase with age. Adults and children who had stronger misophonic reactions had higher self-reported misophonia severity, but they also experienced more musical frisson and had higher musicality scores (children) or more music training (adults).
Conclusions: To our knowledge, this is the first evidence to date that individuals who experience negative misophonic reactions may also tend to experience more positive musical reactions such as frisson.

MO204. Individual Differences in Absolute Pitch Possessors: Systematic Shifts and Contextual Effects
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Category: Psychoacoustics
Background: Absolute pitch (AP), colloquially known as "perfect pitch", refers to the rare ability to identify the note name of a given tone without external reference. It is known that AP is not an all-or-none ability, and different levels of AP have been previously proposed. However, no clear method to quantify individual differences in AP ability has yet been established. Another open question is the role of relative pitch (RP; the comparison of one pitch with another) between test tones and their context in AP tasks. This study aims to address both individual differences and contextual effects in AP possessors.
Methods: In this online study, AP possessors were presented with 300 piano tones within an octave at tunings of 0, ±20, and ±40 cents, relative to a standard tuning of A = 441.3Hz (5 cents higher than 440 Hz). For each tone, the participants reported the note name and rated the intonation of the tone on a five-point scale, where 1 = "very flat", 3 = "in tune", and 5 = "very sharp". Note naming performance as a function of intonation of the test tone was calculated and averaged across the 12 note names for each participant to form their response curve. To investigate potential contextual effects, note naming and intonation judgment performance was compared across different conditions, defined by intonation (in tune vs. out of tune) and size (large vs. small) of the musical interval between the test tone and its preceding tone.
Results: AP possessors showed a significant downward shift in their note-naming response curves, indicating that they tend to perceive tones as slightly sharper than they really are. Although participants had downward shifts of less than 20 cents on average, individual shifts were as large as 77 cents. In addition, participants rated test tones as more in tune when the interval between the test tone and its preceding tone were in tune, compared with when the interval was out of tune, regardless of the size of the interval. No such effect was observed for note-naming performance.
Conclusions: The results suggest that in AP possessors a) individual differences exist in downward shifts in note-naming and b) contextual RP information contributes to AP intonation rating, but not note-naming, of a given stimulus. Overall, the study provides new insights into the plasticity of pitch perception through the window of AP ability.

MO205. The Role of Temporal Coding in Real-World Hearing: Evidence From Machine Learning
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Category: Psychoacoustics
Background: Neurons can encode information in the timing of their spikes in addition to their firing rates. The fidelity of spike timing is arguably greatest in the auditory nerve, whose action potentials are phase-locked to the fine-grained temporal structure of sound with sub-millisecond precision. However, the role of this temporal coding in hearing remains controversial. Phase-locked spike timing has been proposed to

support hearing in noise, with recognition difficulties of hearing-impaired listeners potentially reflecting an inability to use temporal fine structure, but definitive evidence has remained elusive. We investigated the perceptual role of auditory nerve phase locking by optimizing models to perform real-world hearing tasks using simulated cochleae, asking whether phase locking in a model's cochlear input was necessary to obtain human-like behavior.
Methods: We trained deep artificial neural networks to recognize and localize words, voices, and environmental sounds using simulated auditory nerve representations of naturalistic auditory scenes. We manipulated the upper limit of phase locking via the lowpass cutoff in simulated inner hair cells, varying it between 3000 Hz (the presumptive upper limit in humans) and 50 Hz (eliminating virtually all phase locking to fine structure). Models were separately optimized with each cutoff and we measured the extent to which they replicated human behavior in a range of experimental conditions.
Results: Networks using high-frequency phase locking replicated human auditory behavior in all tested regimes. In particular, task performance was robust to sound level and background noise. Degrading phase locking reduced level robustness and impaired performance on some tasks more than others. Voice recognition and sound localization were most susceptible, with degraded phase locking leading to inhuman responses to pitch and localization cue manipulations. By contrast, degraded phase locking left word recognition largely intact, with models replicating human-level performance in many real-world noise conditions. Nonetheless, word recognition models with degraded phase locking failed to reproduce the fluctuating masker benefit and the effects of tone vocoding seen in normal-hearing humans.
Conclusions: Simulated auditory nerve phase locking is needed to reproduce human-like behavior in machine systems, in particular level-robust hearing and accurate sound localization and voice recognition. The results suggest phase-locked spike timing is used and therefore must be extracted by the auditory system. Our modeling approach links neural coding to real-world perception and clarifies conditions in which prostheses that fail to restore high-fidelity temporal coding (e.g., contemporary cochlear implants) could in principle restore near-normal hearing.

MO206. Lsd1 Knockout Promotes Atoh1-Mediated Transdifferentiation of Supporting Cells to Hair Cells in Mouse Cochleae

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Category: Regeneration
Background: Atoh1 is the master regulator dictating hair cell fate in the cochlea during development, and Atoh1 overexpression induces supporting cell transdifferentiation in mouse cochleae at neonatal ages. However, Atoh1 fails to exert fate conversion in mature supporting cells in terms of conversion rate and transcriptome profile, suggesting potential epigenetic barriers blocking the transcription activation of Atoh1 target genes. Lysine Specific Demethylase 1 (Lsd1) is a histone demethylase that is known to play a key role in cell fate determination by silencing lineage-specific genes. Previously, we knocked out Lsd1 while simultaneously overexpressing Atoh1 in Lgr5+ supporting cells at P0/P1 and found that Lsd1 KO led to significantly more induced hair cell-like cells compared to Atoh1 overexpression alone at P28. To confirm these results and investigate the mechanism behind them, we used Lgr5CreER;tdTomato reporter mice and performed lineage tracing, immunostaining, and CUT and RUN sequencing at P7.
Methods: P7 Immunostaining: Tamoxifen was injected at P0/P1 in four groups of compound transgenic mice: (1) Lgr5CreER, Atoh1-HA, Lsd1flox/flox, tdTomato (LALT); (2) Lgr5CreER, Atoh1-HA, tdTomato (LAT); (3) Lgr5CreER, Lsd1flox/flox; tdTomato (LLT), and (4) Lgr5CreER; tdTomato (LT) mice. At P7 the cochleae were harvested, fixed, and immunolabeled for hair cell specific markers.
P7 Cut and Run Sequencing: Tamoxifen was injected at P0/P1 in Lgr5CreER;tdTomato mice. At P7, the cochleae were dissected and Lgr5+ supporting cells were purified using Fluorescence Activated Cell Sorting to analyze Lsd1 binding sites and epigenetic modification changes.
Results: Lsd1 knockout with Atoh1 overexpression in Lgr5+ cochlear supporting cells at P0/P1 results in significantly more hair cell-like cells than with Atoh1 overexpression alone at P7, primarily in the inner hair cell (IHC) layer. Newly converted hair cell-like cells are derived from Lgr5+ supporting cells. Using Cut and Run, we expect 1) that in supporting cells, Lsd1 is enriched at hair cell gene enhancers which are recognized and bound by Atoh1 in hair cells and 2) that enhancer priming mark H3K4me1 at these enhancers is retained in supporting cells in the absence of Lsd1.

Conclusions: Lsd1 knockout at P0/P1 promotes Atoh1-mediated conversion of supporting cells to hair cells at P7 presumably by alleviating the epigenetic silencing of hair cell gene elements. This suggests that Lsd1 KO might help drive Atoh1-mediated conversion by opening up the chromatin and allowing Atoh1 to access its target genes more easily. These findings help shed light on the role of epigenetics in the reprogramming of differentiated sensory cells.

MO207. Determining the PK/PD Profiles of Niclosamide in the Cochlear Perilymph of Mice

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Category: Regeneration

Background: Noise-induced hearing loss (NIHL) is the second most common cause of hearing loss. While this form of hearing loss is preventable, it has been estimated that about 1 in 4 adults in the U.S exhibit a measurable form of hearing loss due to excessive exposure to loud noise. Recent evidence suggests that young adults are at an increased risk of developing NIHL from using personal music devices at unsafe sound levels for long periods of time. The damage caused by NIHL is irreversible and leads to permanent disability. Unfortunately, there are no FDA-approved drugs that can be clinically used to prevent or treat NIHL. Through our novel large-scale in silico transcriptome-based drug screens, we identified the otoprotective properties of Niclosamide, an FDA-approved antiparasitic drug. We have confirmed the ability of Niclosamide to protect cochlear hair cells through our in vivo studies in zebrafish lateral line neuromasts and adult mice. The primary aim of this study is to determine the necessary PK/PD profiles of Niclosamide in the inner ear perilymph of mice.

Methods: Intraperitoneal (I.P) injection of 10mg/kg Niclosamide was administered to twenty-one (3 per time point) 6–10 week-old FVB mice. Perilymph was collected from the inner ear of mice at 0, 30 min, 1 hour, 2 hours, 4 hours, and 6 hours after the injection of Niclosamide. Three control mice were injected with saline before perilymph collection.

A posterior auricular incision was made to access the posterior semicircular canal. 1ul of the perilymph was collected with hand-held capillary tubes, and the samples were diluted 1:50 with acetonitrile for further analysis. Blood samples were also collected for each time point.

A standard curve was constructed, and its validity was tested using known niclosamide concentrations. Matrix effect was also assessed. The perilymph and blood samples were then analyzed using the same conditions as the standard curve. Ibuprofen was used as an internal standard.

Results: Our preliminary results indicate that Niclosamide exhibits good PK/PD properties in the perilymph of the inner ear and peripheral serum when administered through I.P injections. The Cmax, AUC (Area under the curve), and t1/2 values obtained from this study will guide our future phase I/II clinical trials for repurposing Niclosamide as an otoprotective agent for NIHL.

Conclusions: Our study is the first to establish the PK/PD properties of Niclosamide in the perilymph of mouse inner ears after I.P injections. The results of this study validate Niclosamide's potential to enter the inner ear and serve as a potential agent to protect cochlear hair cells from noise- and cisplatin-induced damage.

MO208. Homotypic Versus Heterotypic Cellular Composition on the Differentiation of Lgr5+ Progenitor Cells Into Otic Organoids

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Category: Regeneration

Background: Cells expressing the leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5) protein has been established as tissue-resident stem cells. A subset of supporting cells expressing Lgr5 have been shown to be otic progenitors which are capable of differentiating into otic organoids bearing inner ear hair cells (HC). Harvesting LGR5+ cells for in vitro cell culture can be done by manual isolation (MI) via stripping of otic sensory epithelia or by magnetic-assisted cell sorting (MACS). MI limits the harvested population of cells into what is only present in the sensory epithelia making the isolated cells very heterotypic which includes LGR5+ cells plus other supporting cells. Meanwhile, MACS is capable of isolating a homotypic population of LGR5+ cells. This study aims to compare the effect of the two isolation

(homotypic versus heterotypic cellular composition) protocols on the differentiation of Lgr5+ progenitor cells into otic organoids.
Methods: Cochleae were harvested from postnatal day 1-4 C57BL/6 mice. For MI, the sensory epithelia were microdissected and manually stripped with forceps to isolate the HC and supporting cells from the mesenchyme. Conversely, whole cochleae were enzymatically dissociated into single cells and labeled with anti-Lgr5 magnetic microbeads and isolated via MACS. Spheroids from each group were induced to differentiate into 3D organoids via stimulation of Wnt signaling. Organoids were observed until 21 days and sampled for immunoflourescence and RNA sequencing analyses.
Results: Otic organoids were successfully generated from both isolation methods. Organoids developed HCs expressing molecular markers of the native HCs with stereocilia bundles. However, further observation showed that the heterotypic group showed more pronounced F-actin protrusions with clearer FM1-43 uptake indicating mechanotransduction channel functionality. These differences could be due to the scanty and dispersed expressions of Sox2 were observed in heterotypic organoids while relatively homogenous and regular expressions of Sox2 were observed in homotypic organoids. Heterotypic organoids also showed statistically higher expressions of Laminin, Pax8, and Atoh1 but lower expression of Pax2. RNA sequencing is currently underway to further investigate the differential gene expression.
Conclusions: Isolation of LGR5+ otic progenitor cells can be done using homotypic and heterotypic cellular composition. The both compositions are able to differentiate into otic organoids but the heterotypic cell composition of organoids from heterotypic group promoted a better niche for the maturation of more developed HCs. These findings can help promote a more suitable approach in generating otic organoids.

MO209. A Role for Galectins in Controlling Cultured Ger Cell Proliferation and Organoid Formation

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Category: Regeneration
Background: The adult mammalian cochlea lacks regenerative potential. The cells of the greater epithelial ridge (GER), a cell population that disappears postnatally, on the other hand, have robust proliferative capacity in vitro. This growth potential results in organoid formation. New hair cell-like cells differentiate in these organoids. We wondered how the growth-promoting cascade is triggered and sustained in GER cells.
Methods: GER cells were isolated at >90% purity from cochlear duct cells from postnatal day 2 (P2) Fgfr3-tdTomato/Sox2-GFP transgenic mice using fluorescence-activated cell sorting (FACS). They were cultured in media that efficiently support organoid growth. We harvested the organoids on days 1, 3, and 7, followed by dissociation for single-cell RNA-seq (SmartSeq2 protocol). Computational data analysis identified cell groups and associated gene expression patterns at the onset of proliferation. Curating the observed gene expression changes resulted in a priority list of putative growth-promoting signals, including extracellular matrix (ECM)- and cell adhesion-related genes. We then used inhibitors and activators for the prioritized pathways. Ligands and compounds that enhanced or inhibited GER cell growth and organoid formation were further analyzed using immunohistochemistry, western blot, and immunoprecipitation.
Results: Screened effectors included integrins, endogenous opioids, a cytoplasmic calcium-binding protein, and two galectins. Our results implicate specific galectins in GER cells contribute to organoid growth. Further analyses indicated the activation of retinoic acid signaling and extracellular signal-regulated kinases (ERKs) during the growth-promoting cascade.
Conclusions: We identified effectors that contribute to the proliferation of GER cells. We are in the process of functionally linking galectins with their potential binding partners and identifying the intracellular signaling cascade that results in GER cell proliferation. Identifying the mechanisms leading to robust organoid formation can provide clues for expanding organoid technology for cell-based assay development.

MO210. The Innervation of Regenerated Auditory Hair Cells in the Chicken Basilar Papilla is a Dynamic Process

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Category: Regeneration
Background: In contrast to mammals, which are incapable of functional repair of the organ of Corti, non-mammalian species can naturally regenerate hair cells. Newly regenerated hair cells subsequently become properly innervated. The lack of regenerative treatments motivates our investigation of how non-mammals

functionally restore hearing. Therefore, our goal is to investigate how newly regenerated chicken basilar papilla (BP) hair cells become innervated by cochlear ganglion (CG) neurons.
Methods: We used post-hatch day seven chickens. Sisomicin, an aminoglycoside antibiotic, was infused via the posterior semicircular canal. We have previously shown that this damage regimen causes rapid and complete hair cell loss within 24h. The BP was harvested at different timepoints post sisomicin treatment (PST) for immunohistochemistry.
Results: We noticed a retraction of CG neurites (dendrites) during the demise of hair cells. The neurites remained in recess near the basement membrane and did not appear to retract beyond the habenula perforata. The first regenerated hair cells appeared five days PST. We found that the regenerated hair cells extended a long basal protrusion towards the resting neurites near the basement membrane. Presynaptic specializations, revealed with CtBP2 immunohistochemistry, became evident from day 6 PST onwards. Synapse formation continued along the basal part of the hair cell protrusions for the following days. We noticed a dynamic in which the hair cell protrusions shortened from day 9 PST onwards, and the synaptic specializations gradually moved apically as hair cells matured and ultimately established their almost normal cytomorphology at day 14 PST. In accord with hair cell maturation and innervation, we found that high levels of calretinin, a hallmark of new hair cells, became lower and tapered off when hair cells appeared mature and properly innervated. During the process, we did not detect TUNEL-positive CG neurons or reduced CG neuron numbers.
Conclusions: The innervation of regenerated hair cells appeared to be a dynamic process where basal plasma protrusions seek CG neurites actively and establish synaptic contacts early on, followed by a week-long protrusion retraction and maturation process. Ca2+ signaling might play a role in this process.

MO211. Neural Tracking Measures of Speech Intelligibility: Manipulating Intelligibility While Keeping Acoustics Unchanged

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Category: Speech Perception
Background: Neural speech tracking has advanced our understanding of how our brains rapidly map an acoustic speech signal onto linguistic representations and ultimately meaning. However, it remains unclear how speech intelligibility is related to the corresponding neural responses. Many studies addressing this question have varied the level of intelligibility by manipulating the acoustic waveform, making it difficult to cleanly distinguish effects of intelligibility from the underlying acoustical confounds. Here, we study neural measures of speech intelligibility by manipulating intelligibility while keeping the acoustical structure unchanged.
Methods: In this study, acoustically identical degraded (three-band noise vocoded) speech stimuli (20 s duration) are presented twice, but the second presentation is preceded by the original (non-degraded) recording of the speech. This priming, also known as the pop out effect, changes the intelligibility of the second degraded speech passage while keeping the acoustics identical. We recorded magnetoencephalography (MEG) data from 20 younger adults and investigated how intelligibility affects acoustic and linguistic representations using multivariate Temporal Response Functions (mTRFs).
Results: As expected, behavioral results confirmed that speech intelligibility is improved by priming. TRF analysis revealed that acoustic (non-lexical) neural representations are not affected by priming. However, the later neural representation of the segmentation of sounds into language is strengthened by priming, suggesting that late neural encoding of linguistic features better reflects the level of speech intelligibility.
Conclusions: Taken together, our results show that acoustic and linguistic speech representations are differently affected by changes in intelligibility and linguistic representations may provide some objective measures of speech comprehension.

MO212. Oscillatory Pupil Dilation Responses to Multi-Talker Speech Predict Attention

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Category: Speech Perception

Background: Pupil dilation responses (PDRs) have been shown to index listening effort on a range of auditory tasks through a measure of cognitive resource allocation; however, most studies investigate the overall dilation during the course of entire stimuli. Nevertheless, speech contains natural rhythms that may require differing amounts of effort and cognitive resources from the listener. Additionally, attention is known to both modulate neural responses to speech and control cognitive resource allocation.
Methods: Therefore, we investigated PDRs to features of the low-frequency (0.1-1 Hz) speech envelope during speech perception to determine how PDRs to individual talkers are modulated by attention. We collected pupil dilation data while subjects were asked to attend to a single talker during two-talker speech with varying background noise. We also trained canonical correlation analysis models to decode the listener's attention by comparing PDRs with various low-frequency speech features of each talker.
Results: We found significant event-related PDRs to sound onset events for only the target talker during multi-talker speech perception. The decoding analysis revealed that the subject's focus of auditory attention is decodable from PDRs alone and decoding performance is similar between within-subject and between-subject models.
Conclusions: These results suggest that PDRs during multi-talker speech are sensitive to attention-specific effects of speech processing that influence the listener's internal state throughout each stimulus. The decoding results suggest that a listener's focus of auditory attention can be readily decoded from their pupil dilation without the need for subject-specific models, allowing a method of auditory attention decoding using a high-SNR biosignal with no individual setup required.

MO213. Predicting Speech Reception Threshold From Pure-Tone thresholds: A Big Data Analysis From 636,697 Patients.

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Category: Speech Perception
Background: Pure-tone and speech audiometry are quantitative methods in examining a patient's auditory function to quantify the level of hearing loss and provide clinicians with essential data for future treatment and rehabilitation. The speech reception threshold (SRT) is defined as the sound pressure level at which 50% of the words is repeated correctly. Generally, the SRT correlates with the pure-tone average (PTA) obtained at 0.5, 1, 2, 4 kHz. Sometimes a patient with a normal audiogram can complain of reduced speech understanding that is further aggravated by the environmental noise. In this work, we attempted to estimate the SRT of a patient, both in quiet and in noise, using their pure tone audiograms. For this purpose, we performed retrospective study from 636,710 impaired-hearing patients using a machine learning approach.
Methods: Data were collected between 2000 and 2021 in 700 Amplifon hearing care centers distributed all over France. Subjects from 0 to 110 years old (72 ± 15 years old) and both sex (51% females) were included in the study. For all the subjects, pure tone thresholds were measured from 125 Hz to 8 kHz (11 values) and both ears. The SRTs (disyllabic words presented in free field) in quiet and in noise (65 dB HL, adaptative procedure) were assessed in a subset of 83,981 patients. The aided speech performance provided by the hearing aids was measured in 14,218 patients.
Results: A principal component analysis was performed on left and right audiograms (1,273,420 row vectors in the data set, 11 dimensions). The first component was correlated to the PTA whereas the 2nd component reflects the shape of the audiogram. The audiograms were then classified in 100 clusters (k-means algorithm, Euclidean distance, 1000 iterations). Most of the clusters contain high-frequency hearing loss audiograms. In contrast, uncommon audiograms with U, reverse U, flat, rising or noise notched shapes were aggregated in smaller clusters. Interestingly, some clusters were mostly populated by audiograms collected in males (i.e. steep sloping audiograms) or females (i.e. flat audiograms). Significant relationships were found between SRT (in quiet and in noise) and the PTA. When evaluating the aided SRT in noise, the correlation was reduced, especially in patients with a PTA below 50 dB HL (i.e. mild and moderate hearing loss). Subjects with steeply sloping audiograms are particularly annoyed by noise, even wearing hearing aids.

Conclusions: Results provide potential feasibility in predicting the SRT in quiet and noise, without and with hearing aids, from the pure tone audiogram. For people with steeply sloping audiograms, hearing aids improved mostly the speech intelligibility in quiet, being much less effective in noise compared to other audiometric profiles.
This work is supported by Amplifon Group France (CIFRE PhD grant# 2018/0632)

MO214. Spatial Masking Release Under Congruent and Incongruent Visual and Auditory Spatial Locations

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Category: Speech Perception
Background: The spatial relation between target and masking speech plays an important role in speech perception. In general, listeners find it easier to track the target speech when it is separated from the masker speech in space. This phenomenon is known as spatial masking release. Recent advancements in 3D virtual reality (VR) and augmented reality applications have enabled the launch of metaverses that can deliver immersive experience for participants. They also allow us to examine the interaction of the visual and auditory inputs for spatial masking release.
Methods: In the present study, the multitalker maskers will always be presented at 0□ azimuth or in diffused locations. The target speech will be present at □45□, 0□, or +45□ azimuth. The visual cue of the target speech location will be realized by an avatar appearing in the virtual space. In the congruent conditions, the avatar will be at the same azimuth as the auditory target. In the incongruent conditions, the avatar will be at different azimuth from the auditory target. The experiments will be designed based on virtual interaction scenarios in both VR application in Meta Oculus Quest 2 and augmented reality applications in HoloLens 2. The matrix sentence test will be used to evaluate normal-hearing listeners' speech reception threshold in various hearing and visual conditions.
Results: Programming the VR application is underway. We anticipate having the VR application ready for demonstration at the poster with a set of perceptual data from human listeners.
Conclusions: The work will shed light on how the different sensory systems interact in the realm of spatial masking release.

MO215. Cortical Tracking of Continuous Speech-In-Noise: Children's Use of Linguistic and Acoustic Information

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Category: Speech Perception
Background: Speech perception in challenging listening conditions is a critical skill in everyday life and these skills are still developing in childhood. Children must often attend to speech amongst competing speech streams, like listening to a teacher's voice in a loud classroom. Cortical tracking of temporal speech envelopes is an emerging technique for examining the neural encoding of continuous speech in challenging listening settings. Prior work suggests that children have immature cortical tracking of speech acoustics, relative to adults, especially in noise. Thus, selectively poorer cortical tracking of speech acoustics, especially in multi-talker listening situations, may underlie listening challenges in children. Attentional control is required to successfully attend to a target speech stream, yet it is unclear the extent to which children employ different listening strategies to aid their speech perception. Our goal is to examine the extent to which children can strategically engage different hierarchical levels of sound-to-meaning processes during speech perception, as a function of multiple listening challenges.
Methods: In the current study, cortical tracking of continuous speech (temporal response function) was measured using electroencephalography (EEG) in children (n=8, two males, 6-12 years, average age=9;11). Children listened to an audiobook across three conditions: (1) in quiet, (2) in the presence of another talker (forward speech, masked by a different audiobook narrated by a distinct make speaker), and (3) in the presence of reverse speech (a different audiobook played in reverse). These conditions vary in listening complexity: reverse speech represents masking with sub-lexical competition, whereas the forward speech brings in lexical competition. Both noise conditions were presented binaurally at 0dB SNR to the target track. Two four-choice comprehension questions were presented after each one-minute block of story to ensure participants listened to the target story.

Results: Children had significantly higher behavioral performance in the quiet condition relative to both noise conditions, showing a group average of 90% accuracy on comprehension questions Behavioral performance was more accurate in the reverse speech condition than in the forward speech condition, with a 78% versus 63% average accuracy, respectively. However, EEG data showed a large range of individual differences in cortical tracking of linguistic features. Specifically, some children demonstrated enhanced linguistic tracking in the forward speech condition, where some showed enhanced tracking in the forward condition, even compared to in quiet.
Conclusions: These results suggest that while children may show immature tracking to continuous speech, individual variability in strategies employed for continuous tracking (e.g., focusing on acoustic or linguistic features) may relate to individual behavioral listening skills. Leveraging a large assessment battery of auditory, speech, and language tests, our current work is examining the sources of such individual differences.

MO216. Comparison of J-Hint and J-Matrix Test With Normal Hearing

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Category: Speech Perception
Background: It is known that people with hearing impaired have more difficulty hearing speech in noise than normal hearing. Therefore, it is necessary to assess speech audiometry in noise to confirm the effectiveness of hearing aids and for postoperative evaluation of cochlear implantation. The Japanese Hearing In Noise Test(J-HINT) (Shiroma., 2008) and the Japanese Matrix Test(J-Matrix Test) (Birger et al., 2015) are threshold tests for speech in noise using an adaptive method to control the speech level, and these results are represented as an signal to noise ratio. Both tests are similar in their test methods, but there are some differences between them. In this study, we compared the results of the J-HINT and the J-Matrix Test in some conditions.
Methods: The subjects were 30 males and 40 females with normal hearing (30-69 years). Both J-HINT and J-Matrix tests were conducted in a sound-proof room (RION AT-81). Speech signals were always presented from the subject's frontal loudspeaker. The three conditions were Noise Front (NF), where the noise signal was presented from the front (0°), Noise Right (NR), where the noise signal was presented from the subject's right (+90°), and Noise Left (NL), where the noise signal was presented from the subject's left (-90°). The Noise composite score was calculated from the thresholds of the three conditions by (NF×2+NR+NL)/4.
Results: The results of J-HINT were -3.9±0.2 dB(NF), -8.0±0.2 dB(NR), NL: -9.6±0.3 dB(NL), and -6.3±0.2 dB(Noise composite score). The J-Matrix test results were -6.6±0.1 dB(NF), -12.5±0.2 dB(NR), -13.7±0.2 dB(NL), and -9.8±0.1 dB(Noise composite score). The results obtained from J-HINT and J-Matrix test showed significantly high correlations: the correlation coefficients for NF, NR, NL and noise composite score were 0.52, 0.60, 0.58 and 0.69, respectively. The t-test results also showed a significant difference between the J-HINT and J-Matrix Test thresholds. This may be due to the influence of differences in the correctness of subject recapitulation. In the J-HINT, a sentence is considered correct when all 3 to 8 keywords in a sentence presented to the subject are answered correctly; in the J-Matrix Test, a sentence is considered correct when more than 50% (3/5 phrases) of a sentence consisting of 5 phrases are answered correctly. Since the J-Matrix Test has lower correctness criteria, the threshold converges to a condition with worse signal-to-noise ratio.
Conclusions: The J-Matrix Test had a lower threshold than J-HINT in all conditions. However, the results of both tests showed similar trends. Both tests are expected to be applied in clinical practice and may serve as an evaluation index for the effectiveness of hearing aids and cochlear implants in the future.

MO217. Open Board

MO218. Auditory Nerve Function Predicts Voicing Errors in Noise by Older Adults

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Category: Speech Perception
Background: Deficits in speech recognition in noise by older adults are well-documented, but the underlying mechanisms are multifaceted and incompletely understood. Age-related declines also occur in auditory nerve (AN) structure and function, a phenomenon referred to as neural presbyacusis, and have been

shown to adversely affect temporal processing and speech recognition. Impaired temporal processing may diminish listeners' ability to accurately encode voice-onset time (VOT), short gaps in speech that differentiate voiced and unvoiced stop consonants (e.g. /b/ from /p/). The present study tested the hypothesis that poorer AN function, estimated by reduced AN phase locking, predicts impaired perception of VOT that contributes to voicing errors (e.g., hearing initial-position /p/ for /b/ in monosyllabic words).
Methods: Older adults (55+ years) with clinically normal hearing or mild-to-moderate hearing loss performed a word-recognition-in-noise task. Stimuli were 52 monosyllabic words spoken by a male talker that differed by word-initial phoneme, beginning with either a /b/ or a /p/ (corresponding to a shorter and longer VOT, respectively). To assess the extent to which perception of VOT was affected by voice fundamental frequency, words were pitch shifted by +/- 3 semitones, yielding a high-pitch and low-pitch condition, for a total of 104 stimuli. Stimuli were presented at 88 dB SPL in speech-shaped noise (15 dB signal-to-noise ratio) with presentation levels selected to limit effects of audibility on performance, as estimated by the Articulation Index. Electrocochleography was performed on a subset of participants to estimate AN synchrony (phase locking value, PLV), from the N1 peak of the click-induced compound action potential (CAP). Logistic mixed-effects regressions were used to assess the extent to which PLV, pure-tone thresholds, word-initial phoneme, and voice pitch predicted the odds of making voicing errors, which was defined as reporting words that began with /b/ for stimuli beginning with /p/, and vice versa.
Results: The odds of making a voicing error was independently predicted by the word initial phoneme, the vocal pitch of the word, and PLV, but not by pure-tone thresholds. Specifically, listeners made significantly more voicing errors for words that began with /b/ (i.e. shorter VOT) than for those that began with /p/ (longer VOT), and for words presented in a higher pitch than in a lower pitch. Poorer AN synchrony (lower PLV) was significantly related to higher odds of voicing errors, and this effect was more pronounced for b-initial words than p-initial words.
Conclusions: Results indicate that older adults are more likely to make voicing errors during a word-recognition-in-noise task for stimuli with shorter VOT and with higher vocal pitch. Results also indicate that voicing errors, which may reflect diminished temporal processing, are associated with poorer AN PLV and suggest that mechanisms of AN decline are critical for understanding speech communication problems of older adults.

MO219. Effects of Preceding Vowels on Physiological Responses to Successive Consonants

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Category: Speech Perception
Background: Antecedent to speech understanding is speech perception, which relies on accurate information transmission in both ascending and descending auditory neural pathways. The auditory brainstem encodes the strength and fidelity of incoming signals as they travel to the auditory cortex afferently. Listeners need to detect, discriminate, then identify the critical characteristics of individual speech sounds before meaning is assigned. The least perceptible elements encoded as meaningful speech segments are phonemes. A syllable consists of two successive phonemes arranged as consonant-vowel(C-V) or V-C structures. Because the subcortical mechanisms for encoding time-varying speech sounds remain less understood, this experiment measured transient auditory brainstem responses(ABRs) and frequency-following responses(FFRs) to speech. The analyses of ABRs focused on the response to the transient onset portion of the stimulus, and FFRs targeted formant transition and several features of the periodic response, including timing, magnitude, and fidelity. This study aims to tap the subcortical mechanisms responsible for encoding speech sounds when there are effects of preceding sounds by using non-invasive means.
Methods: Synthesized C-V syllables of three stop consonants and low vowel, /ba/, /da/, and /ga/, were used to create syllabic context for "consonant leading"(C-V) and "consonant trailing"(V-C) conditions to measure the preceding phoneme effects. By abbreviating or extending the vowel duration, "abbreviated vowel" and "typical vowel" lengths were created to further test the effects. Speech-ABRs and FFRs were passively recorded from 20 participants. ABR wave V peak latency and peak-to-peak amplitude, FFR phase consistency, frequency-following amplitude, and stimulus-to-response correlation were used to interpret the response timing, magnitude, and fidelity to speech.
Results: Decreased amplitude and increased latency of wave V in the consonant trailing condition indicated the decreased subcortical neural response to transient onset when there is a preceding phoneme. Moreover, decreased FFRs analyses in the consonant trailing condition indicated that response timing, magnitude, and

fidelity to both formant transition and periodicity were negatively influenced by preceding phonemes. Besides, there was a trend of increased wave V amplitude when increased preceding vowel length showed a more robust response to transient onset. Consistently, an increased frequency-following amplitude showed vowel length positively strengthened the response magnitude. However, decreased phase consistency in typical vowel length showed that the timing of response is negatively influenced by a longer vowel. Overall, the preceding phoneme effects were consistent across stop consonants.
Conclusions: As the first study using non-invasive measurements of speech-ABRs and FFRs on preceding phonemes, we observed the negative effects of prior occurring vowels on successive stop consonants. More, the positive effects of increasing preceding vowels on strengthening the frequency-following responses to speech periodicity. The increment of response magnitude may relate to neural re-synchronization after a period of recovery. These results helped us understand how the subcortical mechanisms encode phonemes in speech sounds.

MO220. Do Hearing Aids Contribute to Speech Recognition in Older Adults?
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Category: Speech Perception
Background: An ever-increasing number of audiologists and researchers claim that hearing-aid use leads not only to improved audibility, but also to plastic changes in speech recognition and cognition. The evidence base for this claim is debatable. Although there is some consensus regarding the contribution of hearing aids to the recognition of speech in noise, other forms of challenging speech (e.g., rapid, dichotic) remain understudied in this context. In two recent studies in older adults with hearing loss, we found no contribution of hearing aids to the recognition of speech produced by a fast talker (Shechter Shvartzman et al., 2022; Rotman et al., 2020). Our aim here was to reanalyze the combined data from these two studies and determine whether a contribution of hearing aids emerges with a larger sample.
Methods: Data from 143 adults (age > 65) including 59 experienced hearing-aid (HA) users was submitted to mixed modelling. The data set included information on hearing (pure-tone thresholds at octave frequencies, 250-8000 Hz), cognition (vocabulary, working memory, attention), natural fast speech recognition and rapid perceptual learning (learning rates over 10 sentences of time-compressed speech).
Results: Hearing thresholds (PTAs) in this sample ranged from 16-75 dB-HL (HA users: M = 55, SD = 9; non-users: M = 40, SD = 12). Recognition accuracy of fast speech ranged from 0-98% (HA users: M = 31%, SD = 22; non-users: M = 45%, SD = 27). Consistent with the original studies, age and the degree of hearing loss had a negative impact on fast speech recognition (OR = 0.69 and 0.32 respectively). Likewise, working memory and perceptual learning rates had a positive impact (OR = 1.51 and 1.39). However, with this larger sample, hearing-aid use had a positive contribution to the recognition of natural-fast speech (OR = 1.25). In other words, although HA users had poorer fast-speech recognition than non-users, when hearing, cognition and rapid learning were held constant, modelled (predicted) scores of HA users were better than those of non-users.
Conclusions: Hearing-aid use seems to partially offset the effects of hearing loss on the recognition of fast speech. Large variability across hearing-aid users makes the relatively small contribution of hearing aids to speech recognition hard to recognize. Larger samples are needed to allow for the control of potential confounds with adequate statistical power. What this means for individual hearing-aid users remains to be determined by tracking fast speech recognition over time.

MO221. Effect of Sound Therapy on Oscillatory Brain Activity and Distress in Chronic Tinnitus Patients
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Category: Tinnitus
Background: Some tinnitus patients experience a decrease in tinnitus related distress after the use of sound therapy. However, despite the broad offer of sound therapy treatments and the frequent use of the treatment for patients, information is still missing about the effects and mechanisms influenced by sound therapy. The aim of this study was to investigate how sound therapy affected the oscillatory brain activity in chronic tinnitus patients and if these changes were related to changes in tinnitus related distress.
Methods: In this longitudinal study, the effect of sound therapy on oscillatory brain activity was investigated. 20 people suffering from tinnitus (Tinnitus Handicap Inventory score > 18) and 20 control

participants matched with gender, age and hearing thresholds took part in the study. The study consisted of 1) a baseline resting state electroencephalography (RS-EEG) measurement of all participants and 2) a follow-up RS-EEG measurement of the tinnitus group after 2 months of sound therapy usage. The baseline oscillatory brain activity was compared between the tinnitus and the control groups. Furthermore, to evaluate possible changes in the oscillatory activity caused by the sound therapy, the oscillatory activity before and after the use of sound therapy was compared in the tinnitus group. Finally, it was investigated whether the change in oscillatory activity with sound therapy was correlated to the tinnitus related distress.
Results: The results showed a reduction in the tinnitus related distress after long-term sound therapy treatment. Differences were found in the oscillatory activity for the alpha, delta, gamma and theta bands when comparing the tinnitus group with the matched control group. Moreover, the alpha power was increased in the tinnitus group compared to the control group, while the delta, gamma and theta power was decreased in the tinnitus group compared to the control group. After long-term sound therapy treatment, a reduction in the alpha power was found in the tinnitus group, while increases were found in the delta and gamma power. However, no correlation was found between the changes in the oscillatory activity and the reductions of the tinnitus-related distress.
Conclusions: Although the present study found differences in the oscillatory activity after long-term use of sound therapy, the changes were not correlated with the improvements in the tinnitus related distress that the participants experienced. This suggests that a better understanding of the sound therapy induced changes in the oscillatory activity and their relation to the tinnitus percept is needed before the oscillatory activity can be considered as a biomarker. Furthermore, the results also showed large individual differences in the effects of the treatment on the tinnitus related distress, emphasizing the need for further improvement and individualization of the treatment.

MO223. Identification of Tinnitus in Individual Animals Using Machine Learning

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Category: Tinnitus
Background: The subjective nature of tinnitus complicates animal physiology studies and necessitates time-consuming and error-prone behavioral testing. Over the past decade, studies across laboratories using diverse tinnitus induction and assessment methods have led to a consensus that neural signatures of tinnitus are present in the firing patterns of fusiform cells of the dorsal cochlear nucleus (DCN).
Methods: In this study, we developed two supervised machine learning algorithms based on previously published single-unit fusiform cell data (Wu et al., J Neurosci, 2016). The first method used a random forest classifier trained on user-extracted features including tonotopic location, burst pattern, and cross-neuron synchrony. The second method applied a neural network with principal components on unprocessed fusiform cell spike trains.
Results: We found that neural synchrony alone achieves 92% training accuracy, and that the spread of synchrony, more than the degree of synchrony, undergirds the tinnitus classification. We also established that 10-seconds spontaneous activity provides 70% training accuracy, which rose to close to 90% when the recording window is extended to 60 seconds. The two methods were cross-validated using new, unpublished test data.
Conclusions: The results suggest the possibility for near real-time tinnitus classification, demonstrating a proof-of-principle physiological identification of tinnitus in individual animal, and further showed that tinnitus-related changes in DCN are highly distinctive.

MO224. Ebselen Attenuates Noise-Induced Tinnitus in Mice

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Category: Tinnitus
Background: Tinnitus is a significant inner ear disorder with no FDA approved treatment. Exposure to loud sounds and noise-induced hearing loss (NIHL) is a major risk factor for noise-induced tinnitus. Prior studies have shown that ebselen treatment can prevent acute NIHL in young adults (with 4 days of ebselen treatment) with normal hearing and no prior tinnitus, and treats significant hearing loss and tinnitus in older adults with Meniere's disease (21 and 28 days ebselen treatment). Our recent preclinical work has utilized gap induced prepulse inhibition of the acoustic startle reflex (GPIAS) to assess tinnitus in two mouse models

of aminoglycoside (AG) ototoxicity. Here we found that tinnitus-percepts decreased as a function of time after AG treatment. The goals of this study were to develop a mouse model of noise-induced tinnitus (NIT) and to determine if ebselen treatment can prevent or reverse NIT.
Methods: CBA/CaJ mice were divided into three groups with Group 2 and 3 receiving a temporary unilateral occlusion of an external ear canal before a brief narrowband noise exposure: Group 1 served as an unexposed control with no occlusion; Group 2 received vehicle (DMSO/saline/d/p.o.); and Group 3 received ebselen (10 mg/kg/d p.o.). DMSO and ebselen receiving mice were treated for 3 days beginning one day before noise. Baseline ABRs and GPIASs were collected prior to, and three months after noise exposure. After three months, gap detection deficits for tinnitus were determined for each animal. Animals that developed NIT were treated with a 4-day course of ebselen and re-assessed. Hair cell counts were performed using immunofluorescence (phalloidin) microscopy.
Results: Three months after noise-exposure, permanent threshold shifts were observed in the open ear of all noise-exposed mice across the three tested frequencies (8-20 KHz). Cochlear histology revealed extensive IHCs and OHCs damage and loss in the open ear vs the closed ear. GPIAS showed behavioral evidence of NIT in 6 out of 27 noise-exposed mice. After 4 days of ebselen treatment in these six NIT mice, gap detection deficits were significantly reduced to pre-exposure levels. This reduction reversed over time, and the gap detection deficits returned to pre-treatment levels one week after treatment.
Conclusions: A 4-day course of ebselen treatment can temporarily reverse gap detection deficits to baseline, pre-noise levels in mice that developed NIT. This work is the first demonstration of an anti-inflammatory reversing NIT after the development of NIHL where OHC and IHC loss was significant. Longer treatment durations are being tested to determine if this reversal can be permanent and results in the repair of injured hair cells. These data provide promising implications for ebselen as a treatment for noise-induced tinnitus in adult humans.

MO225. Hearing Aid Amplification Schemes Adjusted to the Individual's Tinnitus Pitch, an RCT
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Category: Tinnitus
Background: Hearing aids can be used as a treatment for tinnitus. There are indications that this treatment is most effective when the hearing loss -and the tinnitus pitch- fall in the range of amplification of the hearing aid. Other models suggest that a gap in the amplification around the tinnitus pitch would enhance the lateral inhibition and thereby reduce the tinnitus.
Methods: We conducted a randomized controlled trial, designed as a Latin square balanced crossover study. Eighteen tinnitus patients with moderate hearing loss were included, all had been using hearing aids for at least 6 months. Patients were fitted with hearing aids using 3 different amplification schemes: (1) standard amplification according to the NAL-NL2 prescription procedure, (2) boosted amplification at the tinnitus frequency, and (3) notch filtered amplification at the tinnitus frequency. Amplification of the three settings was evaluated with real ear measurements. After two weeks of initial adaptation (during which the NAL-NL2 was used), the hearing aids were used for a period of twelve weeks, testing each setting for four weeks.
Results: Questionnaires and psychoacoustic measurements are used to assess the outcomes of each scheme. Comparisons will be drawn across schemes and correlations across measurements will be made within subjects.
Conclusions: This double-blind RCT assesses the efficacy of 3 different amplification schemes of hearing aids in tinnitus patients

MO226. Irregular Fiber Stimulation and Activation of Central Vestibular-Related Neurons
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Category: Vestibular: Basic Research and Clinical
Background: The distribution of neurons activated by irregular fibers throughout the vestibular pathway is not well delineated. Linear acceleration activates otolith organs which synapse with ganglion cells projecting to the vestibular nuclear complex (VNC) centrally. Jerk stimulation delivered in the naso-occipital plane has been demonstrated to activate irregular fibers originating in otolith organs. Using c-Fos as a marker of neuronal activity, we have begun to identify and examine nuclei in the VNC that respond to jerk stimulation

Methods: Male Sprague-Dawley rats were divided into two groups, sham (n=4) and stimulated (n=4). Sham rats were attached to a mechanical shaker arm, but were not stimulated. Stimulated rats were attached to a shaker and exposed to jerk stimulation in the naso-occipital plane (3,900 g/s). The stimulation paradigm consisted of 15 trials equally divided into three blocks. In each trial, 200-paired jerks were administered. During stimulation, vestibular short-latency evoked potentials (VsEPs) were recorded. Ninety minutes after the start of the stimulation, animals were perfused and the brains were collected, serially sectioned (40µm), and immunohistochemistry for c-Fos was performed on rostral and caudal VNC sections. c-Fos labeled nuclei were counted in five specific vestibular sub-nuclei (lateral – LVe, medial-parvocellular MVePC, medial-magnocellular - MVeMC, superior – SuVe and spinal – SpVe). Counts were normalized to 1mm2 between sham and stimulated groups.
Results: The number of cFos labeled nuclei is similar across brain regions in sham (76) and stimulated animals (84). In shams, rostral sections through the VNC contained c-Fos labeled nuclei in the LVe, MVeMC, MVePC, and SuVe. Caudal VNC sections showed a similar trend with c-Fos labeled nuclei in the MVeMC, MVePC and SpVe nuclei. In stimulated animals, c-Fos labeled nuclei significantly increased ($p = 0.0001$) compared to shams. Specifically, the caudal MVePC from stimulated animals had the greatest number of labeled nuclei per mm2 when compared to the MVeMC and the SpVe ($p = 0.0001$). The medial and lateral subdivisions of each vestibular nucleus showed a trend toward differential c-Fos labeled nuclei in stimulated animals while the sham group did not.
Conclusions: Activation of otolithic irregular fibers produced increases in neuronal activity throughout caudal VNC nuclei. These results correlate well with studies demonstrating wide spread distribution of irregular fiber afferents across the VNC. Calyx only afferents (irregular fibers) send projections to the uvula and flocculus of the cerebellum which in turn projects to the MVePC. Direct Calyx only afferent stimulation of MVePC neurons combined with stimulation from Calyx activation of MVePC projecting cerebellar neurons may account for the significant number of activate neurons in this region compared to other VNC nuclei. Future studies should examine the neurochemical phenotypes of activated nuclei in the VNC and the impact of peripheral trauma on their activation.

MO227. Transcription Factor Emx2 Mediates Vestibular Function by Regulating of Neuronal Innervation and Hair Bundle Orientation in the Otolith Organs

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Category: Vestibular: Basic Research and Clinical
Background: Afferent innervations to the otolith organs are segregated, distinguishable by their central projections to either the cerebellum or brainstem. Whether this segregation coincides with the line of polarity reversal (LPR), across which hair bundles are oriented in opposite orientation to each other, is not clear. Here, we investigated whether neuronal innervation segregates across the LPR and whether the transcription factor Emx2, required for changing hair bundle orientation and the establishment of the LPR, also regulates afferent segregation. Furthermore, we investigated the functional significance of this bidirectional sensitivity of the otolith organs.
Methods: Using lipophilic dye tracing technique, we investigated the neuronal innervation pattern in the otolith organs of several Emx2 mutant mouse strains that we generated. In addition, we measured vestibular evoked potential (VsEP) and conducted behavioral tests on two conditional mouse mutants (cKO) with loss of bidirectional sensitivity: 1) Emx2 cKO, in which the LPR is absent, and 2) Tmie cKO, in which the LPR is present but the mechanotransduction channels of hair cells normally expressing Emx2 are absent.
Results: Using an Emx2-lineage mouse strain, we demonstrated that the neuronal segregation occurs across the LPR in the otolith organs. In the Emx2 knockout utricles, afferents that normally innervate hair cells in the Emx2-positive domain which project to the cerebellum largely failed to reach their targets, whereas

afferents that normally innervate the Emx2-negative HCs which project to the brainstem were expanded across the entire otolith organ. Results from our gain-of-function Emx2 mouse mutants suggest that Emx2 expressed in both hair cells and supporting cells are involved in the neuronal selection. Together, these results indicate that the restricted Emx2 expression regulates the afferent innervation in the otolith organs. Behavioral analyses of Emx2 cKO and Tmie cKO mutant strains indicate that these mice are not hyperactive and show no difficulty in staying on a rotating rod, but they have difficulty in swimming comfortably and traversing a balance beam, when compared to their littermate controls.
Conclusions: Restricted Emx2 expression in the otolith organs mediates bidirectional sensitivity on two levels: hair bundle orientation and afferent neuronal segregation. Bidirectional sensitivity is required for specific vestibular functions in mice.

MO228. Role of Vestibular Hair Cell Input in the Upregulation of KCNQ Channels in Vestibular Ganglion Neurons During Rodent Development

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Category: Vestibular: Basic Research and Clinical
Background: Hair cells in the vestibular system transmit information about head positioning and movement to the brain via vestibular ganglion neurons (VGN). Ganglion neurons vary in resting membrane potential (RMP), current threshold, and in the number of action potentials produced by simple current injection (collectively termed 'excitability'). By the second postnatal week, ganglion neurons become less excitable, in part due to an upregulation of low voltage gated potassium channels like KCNQ. Potassium channels like KCNQ are known in some systems to be homeostatically controlled. For example, synaptic input from auditory hair cells is necessary for the normal acquisition of potassium channels and cell differentiation in spiral ganglion. We hypothesize that VGN maturation is similarly shaped by synaptic input. We expect that disruptions in hair-cell driven synaptic input will interfere with the natural maturation of KCNQ channels, leaving VGN hyperexcitable in comparison to neurons from age matched controls.
Methods: We use the VGLUT3 knock-out transgenic mouse, in which hair cells are unable to form glutamate-filled vesicles for release into the synaptic cleft. As a result, VGN do not receive normal input from vestibular hair cells. First, we use immunohistochemistry on vestibular tissue to look for expression of KCNQ4, a channel in the KCNQ family known to be upregulated during the second postnatal week. We perform patch-clamp recordings on disassociated, cultured VGN, and focus on ages P10 to P15. To measure cell excitability, we record RMP and firing pattern. We then compare results to the long studied Long-Evans rat, and to wild-type mice.
Results: Preliminary results suggest that VGN are more excitable in the VGlut3-KO compared to wild-type mice and rats, with the caveat that our VGLUT3-KO sample size is still small (N=12). Immunohistochemical experiments found essentially no KCNQ4 expression in calyxes in the central striolar zone of the VGlut3-KO at P14, compared to enriched expression in wild-type mice. This decreased expression of KCNQ4 is consistent with our finding that the proportion of neurons in the knockout mouse that have tonic firing patterns (33%, N=4 of 12) is higher than in rats at similar ages (5%, N=5 of 78). Phasic firing patterns are characteristic of neurons that have higher low-voltage gated potassium currents. Ongoing work aims to increase the number of cells recorded, and to use channel-specific pharmacology and immunohistochemistry to characterize the extent to which altered inputs impact the biophysical and morphological development of vestibular afferents.
Conclusions: Our preliminary data suggests that developmental upregulation of channel KCNQ is reliant on inputs from hair cells. Whether other qualities of vestibular afferent neurons such as expression of other ion channels and cell morphology are also under control of hair cell input remains to be seen.

MO229. New Techniques for Investigating Otoconia-Related Vestibular Pathologies in Histologically Processed Human Temporal Bones

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Category: Vestibular: Basic Research and Clinical
Background: Otoconia are composite (95% calcium carbonate, 5% protein) crystals that provide inertial forces to saccular and utricular hair cells for detecting gravitational and accelerational stimuli. Although otoconial pathologies are involved in some of the most frequent vestibular disorders, including benign paroxysmal positional vertigo, and progressive disequilibrium of aging, the pathomechanisms affecting otoconia remain unknown. Here, we leverage a new histological processing technique for human temporal bones (hTBs) that preserves the calcified tissue matrix, including otoconia, in combination with new analytical approaches that provide entirely new insights into the structure of human otoconia and their tissue environment.
Methods: Postmortem, formalin-fixed hTBs were embedded in a methyl methacrylate resin (Technovit-9100, Heraeus-Kulzer) without prior decalcification, and sectioned at 20 micrometer thickness using a laser microtome (TissueSurgeon, Rowiak LLS). Saccular and utricular otoconia within tissue sections were analyzed using Fourier-transform infrared (FTIR) microspectroscopy, polarized light microscopy, and covalent Schiff-type fluorescent labeling in combination with super-resolution microscopy. The data obtained on inorganic and organic matrix features was compared to that from formalin fixed, EDTA decalcified, celloidin embedded hTB tissue sections.
Results: The chemical signature of saccular and utricular otoconia, as determined by FTIR microspectroscopy, was heavily dominated by calcium carbonate, in line with previous reports. The inorganic matrix demonstrated polarized light dispersive (birefringent) features, characteristic of calcium carbonate crystals, and revealed a “core-and-shell”-like structure, which could previously only be visualized using transmission electron microscopy. The organic matrix, visualized for the first time by covalently binding fluorescent probes and super-resolution microscopy, consisted of glycoproteins organized in a complex three-dimensional pattern.
Conclusions: Sequentially applying polarized light microscopy, FTIR microspectroscopy, and covalent fluorescent protein labeling on non-decalcified, Technovit9100-embedded hTBs, allows, on a single tissue section, the differential analysis of inorganic and organic otoconial matrix features in correlation to associated tissue structures, such as the otoconial membrane and the macular neurosensory epithelia. Ongoing and future studies will leverage these new techniques to investigate otoconia-related pathologies in normal aging, and in otoconia-related vestibular disorders.

MO230. Clinical Gait Assessment in Patients With Unilateral and Bilateral Vestibulopathy

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Category: Vestibular: Basic Research and Clinical
Background: Gait requires the coordination of various sensory systems, especially the vestibular system. Vestibular deficits can induce postural instability and gait disorders due to the failure of vestibular reflexes which stabilize the gaze, the head and body. Chronic imbalance is amongst the main complaints of patients with bilateral vestibulopathy and causes a significantly higher risk of falls than in healthy populations. However, there is very limited quantitative data on the functional impact of vestibular deficits on gait patterns. The goal of this study was to determine objective and reliable metrics correlated with both clinical observations and patients' complaints regarding instabilities during walking.
Methods: Thirty gender and age-matched subjects were enrolled in the study: 10 patients with bilateral vestibulopathy (BV), 10 patients with unilateral vestibulopathy (UV), and 10 healthy subjects (HS). A 12-camera optoelectronic system (Oqus 7+, Qualisys, Göteborg, Sweden) was used to record the 3D trajectories of 35 reflective markers placed lower and upper body according to the Conventional Gait Model 1.0 and Plug in Gait model, respectively. Seventeen spatio-temporal parameters of gait and body kinematics were recorded at various self-selected walking speeds (usual, slow, fast) in an attempt to identify parameters that reliably distinguish healthy subjects (HS) from pathological patients (UVL and BVL).
Results: BV and UV showed a significantly lower walking speed, stride length, as well as a significantly higher step width, foot off time, and double support time compared to HS at usual walking speeds ($p<0.01$). The range of motion and speed of the head, neck and trunk were significantly lower for both pathological groups compared to HS at usual and faster speeds ($p<0.01$). Further analyses are currently ongoing to assess the potential differences of the full body kinematics between the three groups.

Conclusions: These findings indicate that various gait parameters including body kinematics are altered in patients with vestibular dysfunctions. These objective quantitative outcomes will be useful for clinical diagnosis and also to measure potential benefits of future rehabilitation interventions such as the vestibular implant.

MO231. Evidence of Vestibular and Balance Dysfunction in Patients With Mild Cognitive Impairment and Alzheimer's Disease

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Category: Vestibular: Basic Research and Clinical

Background: Given the expected rise in dementia prevalence, early diagnosis is vital. As a growing body of literature has identified a potential association between vestibular function and cognition, vestibular assessment may aid in early screening.

Methods: Cross-sectional analysis of the GECkO (Gehoor, Evenwicht, COgnitie) study, an ongoing prospective single-centre longitudinal cohort study. To untangle the proposed association between vestibular function and Alzheimer's disease (AD), vestibular parameters (including peripheral vestibular end-organ function and clinical balance assessments) between three groups of older adults (55-84 years) were compared: (1) healthy controls with age-normal cognition (n=50), (2) a group with Mild Cognitive Impairment (MCI; n=33), and (3) a group with AD (n=17).

Results: Regarding peripheral vestibular end-organ function, participants with AD demonstrated a delayed latency of the p13 component measured by cervical Vestibular-Evoked Myogenic Potentials (cVEMP) ($p = .001$, $\eta^2 = .13$) compared to healthy controls and participants with MCI. Other measures including n23 latency, presence of intact responses, rectified amplitude, mean rectified voltage (measured by cVEMP) and lateral vestibulo-ocular reflex gain (measured by video Head Impulse Test (vHIT)) did not differ between groups. Regarding clinical balance assessments, the Timed Up-and-Go (TUG), Performance-Oriented Mobility Assessment – Balance subscale (POMA-B), and Functional Gait Assessment (FGA) differed significantly between the three groups ($p < .001$, $\eta^2 = .26$; $p = .0031$, $\eta^2 = .12$; $p = .0001$, $\eta^2 = .18$; respectively). Here, more cognitively impaired groups were associated with worse clinical balance scores.

Conclusions: Vestibular deficits were more prevalent in groups with increasing cognitive decline. Regarding vestibular function testing, p13 latency as measured by cVEMP was delayed in participants with AD. Other cVEMP or vHIT measures did not differ between groups. All three clinical balance assessments (TUG, POMA-B, and FGA) resulted in worse scores along the AD continuum. However, included balance assessments converged from normal to abnormal at different rates, resulting in heterogeneous outcomes at the MCI stage. Future research integrating a complete assessment of otolith function testing, balance, and spatial cognition is recommended to really comprehend the gradual changes of the vestibular system and its associated regions in advancing degrees of cognitive decline. In addition, future research should focus less on semicircular canal function testing and therefore step away from redundant testing in this patient population.

MO232. Vestibular Phenotypes in Patients With Genetic Hearing Loss

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Category: Vestibular: Basic Research and Clinical

Background: As compared to hereditary hearing loss, relatively little is known about the genetic causes of vestibular disorders. We performed this study to determine the vestibular phenotypes in patients with hereditary hearing loss and identify the associated genetic factors.

Methods: This is a cross-sectional study that included a hereditary hearing loss cohort from a single institution. Patients with bilateral hearing loss with a definite family history and those who underwent genetic testing were enrolled in our cohort. From this cohort, patients who reported dizziness-related symptoms were selected for this study. The type, recurrence, and duration of dizziness were evaluated. Vestibular functions were evaluated using the bithermal caloric test, video head impulse test (vHIT), and cervical/ocular vestibular evoked myogenic potential (cVEMP and oVEMP). Genes important for vestibular functions were identified using the next-generation sequencing or whole-exome sequencing.

Results: Of 604 patients of cohort, 134 (22.2%) had vestibular symptoms. The data showed female preponderance (M:F = 50:84) and the most common inheritance pattern was autosomal dominant (AD) (n = 78, 58.2%). Genetic mutations confirmed by genetic tests were found in 35 patients. The most common symptoms were vertigo in confirmed and unidentified mutation groups. Vestibular symptoms mostly recurrent and persisted hours in the both groups. The symptom characteristics were not significantly different among the inheritance pattern of AD or autosomal recessive (AR) in the both groups. Caloric test, vHIT, cVEMP, and oVEMP showed decreased vestibular function in 41.3%, 17.1%, 65.0%, and 89.5% of patients in whom the tests were performed. Results of vestibular function tests were not significantly different between AD and AR groups, except caloric test which was found to be higher frequency of abnormal results in AR group of unidentified mutation group ($p = 0.006$). Nineteen genes were identified to be linked to vestibular symptoms; abnormal vestibular function test results were also identified to be related to several genes; some of which were identified for the first time. Several genes were identified, including COCH, KCNQ4, MYH9, NLRP3, SLC26A4, and WFS1, in the patients initially diagnosed with familial Meniere's disease. Most patients of familial Meniere's disease had unidentified mutations and AD inheritance pattern.
Conclusions: The vestibular phenotypes were found to be common in patients with hereditary hearing loss. This study promotes future work on understanding the mechanisms of identified genes that may reflect upon a common pathology in hearing loss and vestibular disorders.

MO233. Change in Head Orientation and Stability after Exposure to Intense Noise or Intratympanic Injection of Sodium Arsanilate in Rats

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Category: Vestibular: Basic Research and Clinical
Background: Exposure to intense noise is known to cause damage in the cochlea. Recent studies have shown that noise exposure also leads to changes in the vestibular periphery, as evidenced by reduced vestibular short-latency evoked potentials as well as reduced calretinin staining in calyx-only afferents of saccule (Stewart et al. 2020). Here, we study how noise exposure alters vestibular behavior, specifically head stability and head orientation with respect to gravity.
Methods: A motion sensor (Yost labs, 3-space LX Embedded) was attached to each rat's head via a surgically implanted head post. The animal was held in a restrainer that allowed free head movements and placed on a servo-controlled, horizontal turntable. We recorded head angular velocity and linear acceleration while the animal was subjected to abrupt whole-body or sinusoidal rotations about an earth vertical axis. We measured their head motions before and after 4-hour, 120 dB noise exposure or intratympanic injection of sodium arsanilate. Here, we report animals' head orientation and stability during the inter-trial-intervals of this behavioral experiment.
Results: All animals exposed to arsanilate (3 males) showed severe vestibular deficits. Head orientation was significantly more variable in both roll and pitch. The time the animal held its head motionless was also significantly reduced and head angular velocity during movement was significantly increased after the lesion for every animal tested ($p<0.001$). These findings indicate arsanilate exposure severely degrade animals' sense of gravity and head stability.
Animals exposed to noise (6 males and 3 females) showed subtle, but significant changes in head orientation and stability. In 2 out of 9 noise-exposed animals, head orientation was significantly more variable in both roll and pitch as in arsanilate animals. Most noise-exposed animals, however, exhibited significant increases in variance of head orientation in one direction (either pitch or roll), accompanied by significant decreases in variance in other directions. Furthermore, 6 out of 9 animals showed significantly reduced pitch angles (chins closer to chest) after noise exposure. In contrast to arsanilate exposed rats, noise exposed rats held their heads motionless during inter-trial intervals for significantly longer times (Signed rank test, $p < 0.01$). In all treated animals, enhanced head motion was significantly associated with greater variability in head orientation. Interestingly, the correlation was larger with roll variability for arsanilate-treated animals, but larger with pitch variability for 8 of 9 noise-treated animals. This finding suggests the saccule and utricle were differentially affected in arsanilate as compared to noise exposed animals.
Conclusions: Our findings show that noise exposure affects head orientation and stability but does so less severely compared to arsanilate exposure. We are currently conducting immunohistological studies to

determine which structures and/or cell types within the vestibular periphery are affected by noise or arsanilate exposure.

MO234. Investigation of Central Velocity Storage Using Sinusoidal Harmonic Acceleration and Velocity Step Testing

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Category: Vestibular: Basic Research and Clinical
Background: The vestibular ocular reflex (VOR) is a compensatory response that stabilizes vision during head movement. It is frequency-dependent and responds with linear efficiency during physiologic head movements between 1-5Hz. Below this frequency range and especially <0.08Hz, the VOR is non-linear and becomes dependent upon neural processes to effectively match the timing of the VOR to head velocity (VOR phase). This process is known as velocity storage (VS) and is fundamental to augmenting afferent vestibular drive during low-acceleration head movements. The VOR is frequently assessed during rotational vestibular testing and the characteristics of VS can be quantified from VOR phase and decay of the VOR following sustained rotations.
Velocity storage is often deleteriously impacted with a reduction of VOR gain through peripheral vestibulopathies and less often through central pathologies. For example, abnormally prolonged VOR phase, particularly <0.08Hz where VS is requisite, is a known byproduct of peripheral vestibulopathies. However, there are infrequent clinical cases where normal VOR phase is preserved in the presence of abnormal VOR gain. We investigated the prevalence and phenotypes of such cases in patients undergoing rotational testing.
Finally, we investigated the dependency of the VS mechanism on low-frequency stimuli. Because VS is known to govern both VOR phase and propagation of the VOR time constant (TC), the two parameters are mathematically related. As such, we investigated the dependency of VS to low-frequency stimuli by correlating VOR phase from sinusoidal acceleration stimuli from 0.01-0.64Hz with velocity step sizes (VSS) of 60°/sec, 100°/sec, and 240°/sec.
Methods: Sinusoidal harmonic acceleration (0.01 – 0.64Hz) and velocity step test results (60, 100, 240°/sec) from a total of 535 visits from 297 patients seen at the NIH for various treatment protocols were retrospectively extracted. Sinusoidal harmonic acceleration VOR gains and phase leads were analyzed and compared to velocity step TCs using descriptive and inferential statistics.
Results: Significant correlations were identified between VOR phase and VOR TCs for sinusoidal frequencies between 0.01-0.04Hz, suggesting a low-frequency dependency of VS. While no significant relationships exist between VOR phase and 240°/sec, except at 0.01Hz, moderate-to-strong correlations were present between VOR phase and 60°/sec VSS from 0.01-0-04Hz. While the correlations observed for 100°/sec VSS were comparable to those for 60°/sec, multiple regression analyses consistently identified 60°/sec VSS to be a more robust stimulus than 100°/sec.
There exists a small cohort of patients with reduced VOR gains and normal phase leads (4-8%). While central pathology was common within this cohort, it was not a determinant factor. Additional phenotypic characteristics will be presented.
Conclusions: These results provide clear evidence supporting a low-frequency preference when recruiting the VS mechanism. Further investigation is needed to better understand the VS mechanism in patients with abnormally reduced gains and preserved normal phase leads.

MO235. A Novel Method for Measuring Ocular Torsional Responses to Head Tilt in Rats and Mice

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Category: Vestibular: Basic Research and Clinical
Background: The otolith organs of the peripheral vestibular system detect linear and gravitational acceleration. To maintain stable retinal images against head tilt with respect to gravity, the otolith-ocular reflex induces compensatory ocular counter rotation (OCR). While efforts have been made to develop non-invasive methods to measure torsional eye movements in rodents, implementation is technically challenging. We recently found that different from the smooth boundary of the human pupil, the pupils of mice and rats exhibit irregularly jagged edges. Taking advantage of the irregularity of pupil edges, we developed a novel method of measuring OCR during head tilt in mice and rats.

Methods: Videos of the pupils of Long-Evans rats (N=5) and C57BL/6 mice (N=5) were recorded using an ISCAN video-based eye tracker while the animals were subjected to a 45-degree downward head tilt that took about 16 seconds. Videos of each animal was captured for 20 seconds pre-tilt and 60 seconds post-tilt. Two distinguished features of an animal's pupillary boundary were selected as landmarks for tracking torsional eye movements. Using a custom MATLAB application, orientations of the lines connecting the features and the pupil center as well as the location, size, and orientation of the pupil were calculated at an interval of 0.5 seconds during tilt and 1.0 second pre-tilt and post-tilt.
Results: Three experiments were conducted to validate the approach of using pupil edge features as landmarks to measure torsional eye movements in rats and mice. First, we compared the torsional eye movements measured with the two distinguished features of the pupil edge. Linear regression analysis found that the two measurements were highly correlated with a unitary slope ($R^2=0.991$ for mice and $R^2=0.973$ for rats). Second, we measured the time course of OCR in response to head tilt, which exhibited robust compensatory torsional responses featuring an overshoot and an exponential decline to a stable position, consistent with the published OCR responses established by other methods. Third, we measured the gains of OCR, which exhibited a value of 0.33 ± 0.03 and 0.36 ± 0.04 in mice and rats, respectively, again consistent with previous studies.
Conclusions: In this study, we developed and validated a novel method of measuring torsional eye movements in mice and rats by taking advantage of their irregular pupil edges. As a new non-invasive approach for measuring torsion and OCR gains, this method provides a useful tool to assess otolith functions in mice and rats experiencing genetic deficits, traumatic brain injuries, or therapeutic treatments.

MO236. Fibrosis of the Semicircular Canals and Vestibular Implant Surgery

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Category: Vestibular: Basic Research and Clinical
Background: Bilateral vestibulopathy is a heterogenous clinical condition in which both vestibular systems are impaired, leading to disabling complaints such as imbalance and oscillopsia. Various etiologies can underly the disease, including genetic diseases, auto-immune diseases, infections, and toxicity. Some of these conditions can lead to fibrosis or ossification of the inner ear, including the semicircular canals.
As no cure is available for bilateral vestibulopathy, several research groups are developing a vestibular implant; a neuroprosthesis that aims to restore missing information by providing electrical stimulation of the vestibular nerve afferents. These are often designed to be placed inside the semicircular canals, to stimulate the ampullary nerves.
The aim of this study was to correlate preoperative imaging with intraoperative findings and to evaluate methods to handle fibrosis of the semicircular canals during vestibular implantation and apply these when implanting patients with fibrosis of the semicircular canals.
Methods: Patients with bilateral vestibulopathy that underwent vestibulocochlear implantation and had fibrosis of the semicircular canals were included. Pre-operative imaging consisted of CT and T1 and T2 weighted MRI images. The fluid signal on MRI was analyzed and compared with the same location on CT imaging. During surgery, images from the surgical microscope were recorded. Techniques to implant the electrodes in the semicircular canal ampullae were developed. Pre-operative imaging and the surgical video were compared.
Results: Vestibulocochlear implantation was performed in three patients with bilateral vestibulopathy and fibrosis, caused by DFNA9. In four semicircular canals, a low or absent fluid signal was found on T2-weighted MRI images, which correlated with the areas of fibrosis found intraoperatively. After meticulous skeletonizing of the semicircular canals, the areas of fibrosis were identified as a 'whiteline', instead of a 'blueline'. Subsequently, different surgical strategies, such as removing, probing, and bypassing the fibrosis were applied. All three patients were implanted successfully with electrodes near the ampullary nerves of all three semicircular canals. A step-by-step approach was developed to deal with different locations of fibrosis and ossification.
Conclusions: Pre-operative imaging using both MRI and CT can identify fibrosis and differentiate from ossification, when considering semicircular canal vestibular implantation. The identified areas correlated well with the surgical findings. These preparations aid the surgical procedure. During surgery, meticulous

skeletonizing of the semicircular canals can identify the areas of fibrosis and thereafter various techniques can be applied to facilitate correct electrode placement.
Due to the still limited number of patients undergoing vestibular implantation, a case series approach was chosen. However, as etiologies causing fibrosis are ample, strategies to deal with these obstacles are important in the development of vestibular implantation procedures.
To our knowledge, this is the first time pre-operative imaging findings of semicircular canal fibrosis are correlated with surgical findings and ensuing management strategies were developed.

MO237. Acetylcholine-Mediated Responses in Type I Vestibular Hair Cells

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Background: In the mammalian peripheral vestibular system, efferent fibers originating in the brainstem innervate type II hair cells (HC-II) and afferents with bouton and calyx terminals. Since mature HC-I are covered by calyx afferent terminals, it is believed that very few of them receive direct efferent contacts (Wackym et al. 1991, Li et al. 2007). However, ACh evoked currents have been demonstrated in HC-I in pigeons (Li and Correia 2011) and a non-α9 mediated response was reported in about 7% of HC-I in guinea pigs (Guo 2018). Here, we describe an α9-mediated effect in all HC-I in the rat crista, similar to that observed in vestibular HC-II and cochlear hair cells.
Methods: Whole-cell patch clamp recordings from HC-I and calyx terminals were performed in excised preparations of rat cristae, at postnatal days 13-17. We used heterozygote hemagglutinin (HA) tagged-α9 mice (Vyas et al. 2020) to immunolocalize α9 protein throughout the vestibular epithelia.
Results: Application of 1 mM Ach resulted in an inward current in all HC-I (375 +/- 24 pA, n = 39, holding potential of -98 mV). The inward current was blocked by either 10 μM tubocurarine (n = 14; 84%), 10 μM strychnine (n = 2; 90%), or 400 nM α-BTX (n = 3; 67%), suggesting the presence of α7- and/or α9-containing nAChRs. The reversal potential of the ACh-induced current was ~-60 mV (n = 12) and the outward current at -30 mV was blocked by blockers of SK and BK channels (n = 4).). Immunodetection of α9HA in mouse vestibular epithelia revealed strong immunolabeling clustered on HC-I. Next, we applied ACh during current clamp recording from a calyx terminal (n = 7). Application of ACh to HC-I and HC-II at rest (i.e., membrane potential ~-70 mV) results in depolarization, which resulted in an increase in the firing rate of the calyx. We then applied ATP to depolarize the hair cells through P2X receptors and then applied ACh. In this case, the firing rate of the calyx decreased due to hyperpolarization of HC-I and HC-II (i.e., membrane potential higher than reversal potential of response to ACh).
Conclusions: We found that almost all vestibular HC-I of the rat crista responded to ACh application in a manner similar to vestibular HC-II and cochlear HCs. This effect was mediated by an α9-containing receptor and was associated with calcium sensitive potassium channels. Possible roles can be envisioned, like a cholinergic feedback from the calyx onto the HC-I; however further experiments are needed to test for and demonstrate possible functional roles.

MO238. Bone Conducted Vibration for the Symptomatic Relief of Vertigo - Clinical Trials Phase 1 and Phase 2 Results

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Category: Vestibular: Basic Research and Clinical
Background: Vertigo – the false perception of motion or rotation – is often the result of damage or dysfunction of the vestibular otoliths and/or labyrinths. Despite the high incidence of vestibular vertigo in the adult population (~7.4%, Agrawal et al, 2013), there is currently no treatment available that does not require pharmaceuticals, surgical intervention, or extensive exercise therapy. The OtoBand, developed by Otolith Labs, is a novel device that rapidly reduces or relieves vestibular vertigo by sending a single low frequency (~50 Hz) vibration to the vestibular end organ through bone conduction. The OtoBand is a light weight, battery powered device worn over the mastoid with a headband.
Methods: Here we report results from a phase 1 dosing study and a double-blind counterbalanced sham-controlled phase 2 study. Both studies were carried out remotely in order to accommodate COVID restrictions, as well as to test the effectiveness of the device in a real-world setting. Inclusionary criteria

included a Dizziness Handicap Inventory (DHI) score > 35, chronic vertigo (episodes for at least 90 days) with at least one episode of vertigo per week, and provided a self-reported diagnosis of their vestibular pathology (benign paroxysmal positional vertigo, Meniere's, vertiginous migraine, or vestibular neuritis/labyrinthitis).
Results: In the phase 1 study (N = 40), we found that the OtoBand significantly improved participant ratings of vertigo severity, as well as walking and standing instability ratings, within five minutes of applying the device during an episode of vertigo. The most common transducer force setting used was the highest available (~0.794 N RMS) for all participants, except for those with self-reported vestibular migraine who most often chose the lowest power setting (~0.398 N).
In the phase 2 study (N = 27), the effectiveness of a sham device operating at a different vibration frequency (100 Hz) and at lower force levels (< 0.04 N) was compared to the OtoBand device. Despite the differences in frequency and power, vibrations emitted by both devices were easily detectable. Participants received an OtoBand device and an identical looking sham for two weeks. Devices were sent in a counterbalanced order across participants. A responder was defined as a participant who rated the device as helping on at least half of uses. A mixed-effects logistic regression model identified a significant effect of OtoBand use over the sham device (odds ratio = 3.59 [1.04 - 12.39], p = .043). A significant, albeit small effect of the "Emotional" factor of the DHI was also identified (odds ratio = 1.21 [1.03 1.42], p = .022).
Conclusions: Here we show evidence that the OtoBand can serve as an important treatment option for those suffering from chronic vestibular vertigo. Safety and pivotal studies are in the planning phase.

MO239. The Effect of CD4 and CD8 T-Cells on Vestibular Organs in a Mouse Model of Lassa Fever
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Category: Vestibular: Basic Research and Clinical
Background: Lassa Fever (LF) is caused by infection of Lassa virus (LASV), an arenavirus endemic to West Africa that is associated with a high prevalence of sensorineural hearing loss and vertigo. Using our mouse model of LASV infection which induces symptoms similar to LF in humans, we investigated the mechanisms of LASV induced vestibular damage.
Methods: In study 1, inner ears of LASV infected STAT1-/- and IFN -/- mice were compared. In study 2, inner ears of STAT1-/- mice in CD4 and/or CD 8 (T-cell) depleted groups were compared. The temporal bones were harvested, embedded in paraffin and processed for H and E and immunohistochemistry. Vestibular sensory organs were assessed for structural damage, hemorrhage, lymphocyte infiltration, presence of CD3+ lymphocytes, and LASV antigen. The mice were observed daily for signs of gross behavior associated with imbalance such as head tilting and/or head bobbing.
Results: Study1: LASV-infected STAT1-/- mice displayed structural damage and hemorrhagic changes around the vestibular nerve (VN), sensory epithelia, and stroma underneath maculae of saccule and utricle and cristae of all three semicircular canals (SC). Hair cells were largely preserved. LASV antigen staining and CD3+ lymphocytes overlapped the damaged areas of infected STAT1-/- mice. LASV Infected-IFN-/- mice showed less damage and LASV antigen labeling and fewer CD3+ lymphocytes.
Study 2: CD4 and CD8 double depletion in infected mice caused less damages than infected mice in study 1 and CD4 or CD8 single depletion in study 2. The vestibular nerve displayed less damage than cochlear nerve. Compared to CD8 depletion, infected mice with CD4 depletion showed more hemorrhagic changes and structural damage, in form of vacuolization, to the sensory epithelial and vestibular nerve, but without displaying imbalance. Within the CD4 depletion group, posterior SC displayed worse vacuolization than lateral and anterior SC, superior VN showed more vacuolization than inferior VN. Compared to CD4 depletion, mice with CD8 depletion showed more infiltration of CD3- lymphocytes, especially at the periphery of vestibular nerve ganglions, and more stroma damage underneath the maculae of saccule and utricle and crista of posterior SC. Mice with CD8 only depletion displayed physical signs of imbalance earlier than mice with CD4 and CD8 double depletion.
Conclusions: The results suggest that immune-mediated response is necessary for the damage of the vestibular organs observed in the partial immune activity model, STAT1-/- mice, as opposed to no damage observed in the IFN-/- model with much less immune activity. In STAT1-/-mouse model, vestibular organs showed less damage than cochlear organs and superior VN was more susceptible to damage than inferior

VN overall. Vestibular organs of infected STAT1-/- mice with uninhibited CD8 T-cells are more prone to structural damage while CD4 T cell-mediated cellular injury is one of the mechanisms causing signs of vestibular dysfunction induced by LASV.

MO240. Open Board

MO241. Pupil Responses Associated With the Perception of Gravitational Vertical Under Directional Optic Flows

Joo Hyun Park[1], Sung Ik Cho[2], Sangeun Lee[1], June Choi[3], JungHyun Han[2], Yoon Chan Rah*[3]
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Category: Vestibular: Basic Research and Clinical

Background: In addition to the information from the otolith organs, accurate visual information plays an important role in calculating the internal verticality. The determination of subjective verticality could require a considerable amount of cognitive resources as the process has to evaluate and integrate multiple sensory information. This study assessed the pupil responses in the sensory integration of various directional optic flows during the perception of gravitational vertical.

Methods: A total of 30 healthy participants with normal responses to conventional subjective visual vertical (SVV) were enrolled in this study. The SVV is determined by measuring the difference (error angles) between the luminous line adjusted by the participants and the true vertical. SVV was performed under various types of rotational (5º/s, 10º/s, and 50°/s) and straight (5º/s and 10º/s) optic flows presented via a head-mounted display. Error angles (º) of the SVV and changes in pupil diameters (mm) were measured to evaluate the changes in the visually measured subjective verticality and related cognitive demands.

Results: Significantly larger error angles were measured under rotational optic flows than under straight flows ($p<0.001$). The error angles also significantly increased as the velocity of the rotational optic flow increased. The pupil diameter increased after starting the test, demonstrating the largest diameter during the final fine-tuning around the vertical. Significantly larger pupil changes were identified under rotational flows than in straight flows. Pupil changes were significantly correlated with error angles and the visual analog scale representing subjective difficulties during each test.

Conclusions: These results suggest increased pupil changes for integrating more challenging visual sensory inputs in the process of gravity perception.

MO242. Open Board

TUESDAY, FEBRUARY 14, 2023

POSTER SESSION 4

TU1. Characterizing Clinical Auditory Physiological Measures in Old Macaque Monkeys

Karina Jirik*[1], Amy Stahl[1], Jane Mondul[1], Catherine Alek[2], Leslie Liberman[3], M. Charles Liberman[3], Troy A Hackett[2], Ramnarayan Ramachandran[2]
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Category: Aging

Background: Age-related hearing loss (ARHL) can elevate pure-tone thresholds and impair spectral, temporal, and spatial auditory processing. The mechanisms underlying these age-related perceptual deficits are thought to involve both peripheral and central changes in the auditory pathway. However, the foundational studies for these speculations largely involve the use of rodents, which have a much shorter life span than humans. To better understand the effects of aging in the auditory system, we recorded auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) in old macaque monkeys, which are phylogenetically closer to humans and which have a longer life span, to document

changes relative to younger subjects. Future work will involve correlating these response changes to cochlear pathology and to perceptual changes.
Methods: Subjects were macaque monkeys (Macaca mulatta, 2 male, 1 female) aged 29 - 30 years. ABRs (vertex-to-mastoid) were recorded in response clicks (100 us), click pairs at varying interclick intervals (ICIs; 1-10 ms), clicks at varying presentation rates (27.7/s-200/s), chirps (1.6 ms), and tone pip stimuli (0.5-32 kHz in octave steps). ABR waves I, II, and IV were analyzed for amplitudes and latencies. Distortion product otoacoustic emissions (DPOAEs) were measured from 1-32 kHz (f2/f1=1.22, L1-L2=10 dB, 4 frequencies per octave). Data were compared to published data from young adult macaques. Subjects were then euthanized, and the cochleas were perfused for analyses of hair cells, synapses, nerve fibers, and other indicators of cochlear integrity.
Results: DPOAE thresholds were higher and DPOAE amplitudes were lower in older compared to younger monkeys. ABR thresholds to tonebursts, clicks, and chirps were also higher, wave I latencies to clicks and chirps were longer, and click-evoked amplitudes of waves I, II, and IV were smaller. Preliminary analysis indicates that ABR Wave amplitudes decreased more quickly with duration in older monkeys than in younger monkeys. Preliminary histological analysis in one macaque showed scattered outer hair cell loss and increased stereocilia pathology on inner hair cells throughout the cochlea. However, the damage in this one case was less than that seen in older humans with comparable threshold elevations .
Conclusions: Preliminary results suggest that additional histological analyses, e.g. of the stria vascularis, may be needed to better explain the physiological changes. These results are the first step towards understanding the degradation of hearing performance and its structural and functional correlates across the life span in a non-human primate.

TU2. Age-Related Differences in Neural and Perceptual Signatures of Temporal Fine Structure Processing Underlying Multi-Talker Speech Intelligibility

Leslie Zhen*[1], Jacie R. McHaney[1], Maggie E. Zink[1], Claire Mitchell[1], Satyabrata Parida[1], Sarah Anthony[1], Megan Hallihan[1], Christopher A. Brown[1], Bharath Chandrasekaran[1], Aravindakshan Parthasarathy[1]
[1]University of Pittsburgh
Category: Aging
Background: Aging is associated with declines in multi-talker speech intelligibility that persist despite normal audiometric thresholds. One hitherto understudied mechanism could be degraded processing of temporal fine structure (TFS) cues, culminating in reduced neural representations of the input speech signal. Recruitment of additional cognitive resources and listening effort can occur concomitantly with TFS encoding deficits to compensate for reduced multi-talker speech intelligibility. Prior research suggests that multi-talker speech processing can be explained via variability in TFS encoding abilities and compensatory increases in listening effort in young adults. However, age-related differences in these bottom-up and top-down contributions to multi-talker speech processing remain poorly understood. Here, we extended this prior work in young adults to middle-aged adults to investigate how the interplay between bottom-up and top-down contributions on multi-talker speech intelligibility changes with age.
Methods: Young (18-25 years) and middle-aged (40-55 years) adults with normal audiometric thresholds completed a battery of behavioral and neurophysiological assessments designed to probe neural sources of variability in multi-talker speech intelligibility. This battery included behavioral frequency modulation (FM) detection to measure the fidelity of TFS coding; frequency modulation following responses (FMFRs), which are phased-locked neural responses to the FM stimulus from the peripheral auditory system; and pupillometry to measure listening effort, while subjects performed a digits-comprehension task that assessed multi-talker speech intelligibility.
Results: Preliminary results suggest that performance in young and middle-aged adults are comparable on behavioral FM detection thresholds. Multi-talker speech intelligibility, assessed using the digits-comprehension task, was also comparable between age groups. However, middle-aged adults showed a greater increase in listening effort with challenging SNRs during the digits-comprehension task compared to young adults. This suggests that middle-aged adults require greater listening effort to achieve the same behavioral accuracy in multi-talker speech compared to young adults. Ongoing analyses using FMFRs are aimed at elucidating the age-related differences in bottom-up neural coding of stimulus TFS cues, and their relationship to compensatory increases in listening effort and behavioral performance in multi-talker speech.
Conclusions: Overall, the indices of TFS processing and effortful listening examined here can provide novel insights into the bottom-up and top-down factors that contribute to poor multi-talker speech

intelligibility as a function of age. Poor encoding of TFS cues and over-reliance on top-down resources towards effortful listening may underlie age-related listening difficulties in multi-talker speech. Understanding how these bottom-up and top-down contributors to hearing in noise change with age has important clinical implications for emerging interventional strategies that aim to either enhance sensory representations of the signal or maximize efficiency of cognitive resources used for active, effortful listening.

TU3. Exposure to a Signal-In-Noise Augmented Acoustic Environment Improves Tone-In-Noise Detection in a Mouse Model of Age-Related Hearing Loss

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Category: Aging

Background: A major complaint in patients with age-related hearing loss (ARHL) is the decreased ability to understand speech in noisy environments. This decline stems from a combination of a loss of peripheral function and deficits in central auditory processing. We have reported (Dziorny et al. 2021) that passive exposure to an augmented acoustic environment (AAE) containing short silent gaps improved gap detection in a mouse model of congenital sensorineural hearing loss. Here we hypothesized that an AAE comprised of tone bursts embedded fluctuating background noise will improve the ability for neurons to encode tones in noise. Old mice were exposed to TiN AAE for 2 months then neural responses were measured from the auditory midbrain and compared to that of an unexposed control group of similarly aged mice.

Methods: CBA/CaJ mice, aged 22-24 months, were exposed to TiN AAE for 12 hours per day, for 2 months. Control mice were housed in exact conditions but were not exposed to the stimuli. Designed to mimic a naturally noisy environment, the AAE is comprised of a continuous background noise (CBN) varying between 2 levels, 50 dB SPL for 3sec followed by 65 dBSPL for 6sec. Tone bursts were presented at random signal-to-noise ratios ranging from 0 to +12 dB SPL in 3 dB steps during the 65 dB SPL portion of the CBN. Multi-channel recordings using a 16-channel vertical electrode array were acquired from the inferior colliculus. Frequency response areas were measured using 25 ms tone bursts from 4 to 64 kHz at 0 to 80 dB SPL. Neural correlates of TiN detection were measured at two CBN levels, 40 or 60 dB SPL and quiet using a 50 ms tone burst of 8, 12, 16, 20, or 24kHz . Temporal response patterns were grouped into onset and sustained responses and only sustained units were considered for this presentation.

Results: Distribution of the best frequencies and minimum thresholds for both the AAE and control groups were similar, with no changes due to AAE exposure. Sustained responses were objectively quantified as having at least 50% sustained driven activity. Tone detection was characterized as either excitatory or inhibitory responses. AAE exposed units had an increased proportion of excitatory tone detection at several SNRs across all frequencies and an increased strength of detection, via sensitivity index, for several SNRs at 12, 16, and 20 kHz, showing that AAE improves tone detection in noise with a generalized effect for frequencies near the 16 kHz AAE frequency.

Conclusions: Results suggest that exposure to a TiN AAE improves neural correlates of tone detection in background noise in the inferior colliculus of old mice. This passive treatment may provide a novel intervention to mitigate common auditory processing deficits found in aged listeners.

TU4. Single-Nucleus RNA Sequencing With Spatial Transcriptomics Revealed Unique Cell Types Corresponding to Age-Related Hearing Loss in Carworth Farms White Mice

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Category: Aging

Background: Age-related hearing loss (ARHL) is the most common cause of hearing loss and is one of the most prevalent conditions affecting the elderly globally. Despite its polygenic nature, little is known about the genes and pathways involved, preventing the development of therapeutic intervention. To understand the molecular detail of the observed pathophysiology within the structural complexity of the cochlea, a cell-type-specific approach, such as single-cell RNA sequencing (scRNA seq), is might be of essence. Single-cell transcriptomic profiling has emerged as a powerful tool to characterize the cellular heterogeneity, however, intact whole cell extraction from the adult cochlea is still difficult due to the robust actin-cytoskeleton in the sensory epithelium, the tight junctions and the entanglement of capillaries in the stria

vascularis. To overcome these obstacles, we performed single-nucleus RNA sequencing (snRNA seq) on the 48 aged CFW mouse cochleae and characterized the transcriptional profile of the cell types corresponding to age-related hearing loss. Additionally, we performed cell-mapping within the cochlea using multiplexed single molecule Fluorescence in-situ hybridization (smFISH).
Methods: We selected a group of 48 mice to represent different auditory brain stem response (ABR) patterns on their proportion in the general Carworth Farms White (CFW) cohort. 10-month-old mice cochleae were quickly dissected, followed by single-nucleus isolation by Dounce homogenization. A total of 36,000 nuclei were sorted from each mouse cochleae by fluorescent automated cell sorting, then isolated nuclei were subjected to Chromium Next GEM chip cDNA synthesis. A total of 48 single nuclei barcoded RNA-seq libraries were generated from the cDNAs and subsequently sequenced by NovaSeq 6000. Nuclei were first clustered using the Leiden community detection algorithm, followed by silhouette analysis and manual inspection to be sub-clustered. Necessary and Sufficient Forest was used to identify the minimum number of necessary and sufficient DEGs for each cluster and Friedman-Rafsky non-parametric test was utilized for cross-platform cluster-level comparison (FR-match). Post-neonatal cochleae at day5 were subjected to the smFISH and a total of 124 of DEGs were utilized for the DNA probe.
Results: We successfully isolated nuclei and characterized the cellular transcriptional profile in the aged CFW cochleae by snRNAseq. The initial clustering yielded 26 clusters, subsequently divided into 60 sub-clusters. The FR-match algorithm annotated a total of 44 sub-clusters, and we identified unique cell types showing negative and positive correlation with ABR threshold shift in the high frequency rage. DEGs with the smFISH revealed the relative location of cells in the cochlear tissue, demonstrating validity of the clustering and providing complementary cell-cluster annotation.
Conclusions: snRNAseq with spatial transcriptomics identified unique cell clusters related to ARHL. Our findings could serve as basis for further studies to develop treatment strategies for ARHL.

TU5. Associations Among Sensory Impairments, Cognition, and Psychological Functioning
Jennifer Coto*[1], Ivette Cejas[1], Alexia Pavlovic[2], Molly Smeal[1], Aria Nawab[1], Susan Blanton[1], David Loewenstein[1], Rosie Curiel Cid[1], Xue Liu[1]
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Category: Aging
Background: Previous research has identified associations between hearing, visual, and olfactory impairments, and cognitive decline. These sensory disorders often manifest as early signs of neurological diseases, such as Alzheimer's disease. There is a need for sensitive tests to detect mild cognitive impairment (MCI) that are easy to administer in clinical settings and can be incorporated into longitudinal population-based studies. This study aimed to examine sensory and cognitive associations in older adults.
Methods: Enrollment is ongoing with an expected n=50-100 at the time of the conference among 500 participants being longitudinally followed in NIH-funded projects from the Center for Cognitive Neuroscience and Aging and the University of Miami Ear Institute. To date, 14 participants have been recruited as part of an ongoing study identifying a sensory impairment battery for the early detection of MCI. Participants completed the Mini-Mental State Exam (MMSE), Geriatric Depression Scale (GDS), University of Pennsylvania Smell Identification Test (UPSIT), Pittsburgh Sleep Quality Index (PSQI), the Loewenstein-Acevedo Scales for Semantic Interference and Learning (LASSI-L), a vision screening, and an audiological evaluation. The majority of participants were male (57.1%), Non-Hispanic White (64.3%) (78.6%), and married (57.1%). Most participants completed college/graduate degree (64.3%) and had a mean age of 65.14 (SD = 8.46). Twenty-eight percent of the sample reported a household income of $99,999/year or less, 28.6% reported $100,000 or more, and 21.4% did not know,. Over half of participants reported full-time employment, 7.1% part-time employment, and 35.7% were not employed.
Results: While the current n is too modest to address associations between vision, smell, and cognitive functioning, our projected n of 50-100 will enable examination of the association between hearing sensitivity and cognition, and other sensory impairments. Interestingly, linear regressions revealed significant relationships between cognition and psychological well-being. The GDS was significantly associated with the MMSE, with participants who reported more symptoms of depression having a lower MMSE score [$F(1,12)=5.50$, $p<.05$, $B=-.30$]. Additionally, there was a significant association with the PSQI subjective sleep component and the MMSE, indicating that those with more difficulty with subjective sleep had lower global cognition scores measured by the MMSE [$F(1,10)=5.12$, $p<.05$, $B=-1.09$]. Moreover, there was a significant association with the PSQI daytime dysfunction component and the MMSE, where those

who reported more difficulty with daytime dysfunction also had lower MMSE scores [F(1,10)=5.76, p<.05, B=-1.70].
Conclusions: These preliminary findings highlight the need for further investigation of the associations among sensory disorders and mental health with cognition. Future analyses will expand on the current findings by examining the association between hearing, cognition, and mental health. This is an important first step in helping to identify early makers of cognitive impairment with the ultimate goal of improving patient outcomes.

TU6. The Curvature Quantification of Wave I in Auditory Brainstem Responses Detects Cochlear Synaptopathy in the Elderly

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Category: Aging
Background: Age-related hearing loss is the most common sensory disorder in the elderly. During the early-stage elderly listeners oft complain of degraded speech perception in adverse listening environment. Animal studies suggested that a cochlear synaptopathy might be one of the main mechanisms. A decreased Wave I amplitude in supra-threshold auditory brainstem response (ABR) could diagnose this pathology non-invasively. However, the interpretation of the Wave I amplitude in humans is controversial. Recent work has established a robust and reliable mathematic algorithm, i.e. curve curvature quantification, in mice with promising results. The current study aimed to determine whether the curve curvature has also sufficient test-retest reliability to detect cochlear synaptopathy in aging human.
Methods: Twenty-nine subjects with normal hearing were included into this study. All of them accepted an extended pure tone audiogram examination ranged from 0,125 to 16 kHz and an ABR with a stimulus of 80 db nHL click. The amplitude, curvature at the peak and the area under the curve (AUC) of Wave I were calculated and analyzed.
Results: The Pearson correlation analyses clearly demonstrated a significant negative correlation between age and curvature (R= -0,33, p= 0,015), as well as between curvature and high-frequency thresholds (R= -0,36, p= 0,009). Additionally, there is also a negative correlation between the high-frequencies thresholds and AUC of the Wave I (R= -0,32, p= 0,02).
Conclusions: Thus, these results suggest that curvature quantification and AUC of Wave I can be reliably used to diagnose a cochlear synapthopathy in aging human. It may be applied in the daily routine to diagnose early degenerations of the auditory nerve.

TU7. Age-Related Hearing Deficits Correlate With Diminished Central Inhibition but Not With Cochlear Synaptopathy

Marcelo Gómez-Álvarez*[1], Sónia L. Coelho-de-Sousa[1], Georg M. Klump[2], Enrique A. Lopez-Poveda[1]
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Category: Aging
Background: Older listeners often suffer from temporal resolution and speech-in-noise intelligibility impairments, even when their hearing is clinically normal, and the causes for such problems are not well understood. Some studies have suggested that those age-related hearing deficits may be due to cognitive decline, while others have suggested that they may be caused by cochlear synaptopathy and/or primary deafferentation. However, the evidence in support of these ideas is inconclusive. Here, we explore an alternative hypothesis: age-related hearing impairment is related to decreased central inhibition.
Methods: For adults (N=30) without cognitive impairment and clinically normal audiometric thresholds (≤20 dB HL), we measured speech reception thresholds for sentences masked by an international female fluctuating masker (SRTN), gap detection thresholds (GDT), and frequency modulation detection thresholds (FMDT). We also measured the growth rate (slope) of the wave-I amplitude of the auditory brainstem response with an increasing level as a proxy for cochlear synaptopathy and the Stroop Color and Word Test (SWCT) score as a proxy indicator of central inhibition.
Results: As expected, we found that SRTN, GDT, and FMDT increased (worsen) significantly with aging [r(28)=.502, p=.005; r(28)=.456, p=.011; and r(28)=.455, p=.011, respectively], and wave-I slope and SWCT scores decreased (worsen) with age [r(28)=-.423, p=.020; r(28)=-.489, p=.006, respectively]. Importantly, we also found that SRTN, GDT, and FMDT were not correlated with wave-I slope [r(28)=.043, p=.820; r(28)=.241, p=.199; and r=(28)=.029, p=.880, respectively], but in contrast, they were negatively

correlated with SWCT scores [r=(28)=-.361, p=.049; r=(28)=-.402, p=.027; and r=(28)=-.646, p=<.001 respectively]. The correlation between FMDTs and SWCT scores remained significant [r(28)=-.54, p=<.001], even after partialling out the effect of age.
Conclusions: It is more likely that age-related speech-in-noise and auditory temporal processing deficits be caused by diminished central inhibition than by cochlear synaptopathy. [Work supported by Junta de Castilla y León (grant SA0252P20), Ministerio de Ciencia e Innovación (grant PID2019-108985GB-I00), and the European Regional Development Fund].

TU8. The Stria Vascularis in Mice and Humans is an Early Site of Age-Related Cochlear Degeneration, Macrophage Dysfunction, and Inflammation

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Category: Aging
Background: Age-related hearing loss, or presbyacusis, is a common degenerative disorder affecting communication and quality of life for millions of older adults. Multiple pathophysiologic manifestations, along with many cellular and molecular alterations, have been linked to presbyacusis, however, the early events and causal factors have not been clearly established.
The strial vascularis (SV) of the cochlear lateral wall is a highly metabolically active epithelium with complex functional features critical for supporting hair cell function. This elegantly designed cytoarchitecture may make the SV vulnerable to stress and age-associated pathological conditions. One of these pathological conditions is inflammaging, which is a condition of chronic low-grade inflammation that is seen increasingly with aging. Using a well-established mouse model of "normal" age-related hearing loss and postmortem human temporal bones, this study tested the hypothesis that pathological alterations (such as cellular and molecular dysfunction related to inflammaging) in the SV is an early site of age-related cochlear degeneration.
Methods: Young adult (1.5-3 months; n=46), middle-aged (12-18 months; n=23), and aged (>24 months; n=62) CBA/CaJ mice (both sexes) were used to examine auditory function (via ABR measurements), and age-dependent cellular and molecular changes in the SV and non-SV regions (via transcriptome profiling and high-resolution imaging analysis). Age-dependent structural and transcriptomic changes were also evaluated in macrophages isolated from young adult (1.5-2 months; n=44) and aged (10-14 months; n=22) Cx3cr1GFP/+ mice (both sexes). Further, comprehensive morphological and histochemical examinations were completed on human temporal bones from young (20 to 42 years; n=6), middle-aged (55 to 65 years; n=5), and aged (68 to>89 years; n=12) donors (both sexes) for comparison with our findings in mice.
Results: Cochlear structural and transcriptomic analysis of middle-aged and aged mice revealed that early pathophysiological alterations in the SV were associated with increased macrophage activation and may be a molecular signature indicative of inflammaging. The increase in macrophage activation observed in the SV in both mice and humans is a result of cochlear aging and macrophage dysfunction/dysregulation. Structural-functional correlation analyses showed that an age-dependent increase in macrophage activation, but not changes in SV thickness, was associated with an elevation of ABR wave I thresholds.
Conclusions: The application of cellular, molecular, and novel imaging approaches has allowed us, for the first time, to compare age-related macrophage and SV alterations in both mice and humans. This study highlights 1) the SV of the lateral wall as an early site of age-related cochlear degeneration, and 2) aberrant macrophage activity as a biomarker of age-related hearing loss. Methodological improvements for the quantitative analysis of human cochlear tissues provide a means for future otopathological investigations that scrutinize the role of immune cell malfunction in age-related and other forms of sensorineural hearing loss.

TU9. Investigating Normal Hearing Listeners' Variance in Speech-In-Noise Understanding: Difficulties and Resolutions

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Category: Auditory Cortex and Thalamus: Human Studies
Background: Many studies in the last decade contributed to explaining the large variance in normal hearing listeners' abilities to understand speech in noise. Those studies revealed several factors associated with the speech-in-noise variance such as outer hair cell damages, distorted cochlear tonotopy, the loss of auditory

nerve fibers, poorer auditory grouping, and attention and memory deficits. However, a clear diagnostic method that identifies the source of speech-in-noise variance is yet to be established.
Current limitation of the model that explains normal hearing listeners' speech-in-noise variance is due to several difficulties. First, "normal hearing listeners" do not yield consistent variance across different bivariate studies. Second, the outcome variable (abilities to understand speech in noise) is multi-dimensional and not well defined. Third, the hierarchy and redundancy of the factors that impact the outcome variable is unknown, which prevents selecting the right modeling equation.
Methods: We tried to resolve the above difficulties. First, we setup clear criteria for recruiting three different "normal hearing" cohorts: musicians, noise exposed non-musicians, and non-noise exposed individuals (projecting 30 individuals in each group for a N=90). All participants had normal standard audiograms with thresholds at or below 20dB HL from 250-8,000Hz. These cohorts were recruited separately but treated as a single continuous group in the analyses. Through this divided recruitment and merging, we obtained a considerable variance in the degree of auditory skills and peripheral damages even in age-controlled subjects with normal hearing thresholds. Second, we captured a holistic measure speech-in-noise ability by combining a single word-based speech-in-noise test, a sentence-based test, and a self-report of speech-in-noise difficulties. Third, we conducted extensive tests of subcortical and cortical auditory functions including extended high-frequency audiometry, distortion product otoacoustic emissions, middle ear muscle reflex, medial olivo-cochlear reflex, auditory brainstem responses, and cortical activities during auditory working memory and attention tasks.
Results: Although most bivariate correlation analyses failed showing significant association between a single independent and predictor variable, a mediation effect has been found when the cortical measures of auditory functions were used as a mediator.
Conclusions: This ambitious ongoing study is expected to reveal the hierarch and relative contributions of subcortical and cortical auditory functions to normal hearing listeners' speech-in-noise variance. Preliminary findings from 40 subjects will be reported.

TU10. Pre-Stimulus EEG as an Indicator of Perceptual Difficulties in Noise

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Category: Auditory Cortex and Thalamus: Human Studies
Background: In the cochlea, the synapses between inner hair cells and auditory nerve fibers are the most vulnerable structures with aging or noise exposure. The death of these synapses (cochlear synaptopathy) is thought to be one of the causes of hearing difficulties in noisy environments. The past decade has seen numerous studies investigating the diagnosis, consequences, and possible treatments for cochlear synaptopathy. However, it remains unclear how the auditory cortex reacts to this change at the periphery. A recent study found that, in mice with about 90% synapse loss, the auditory cortex shows hyper-synchronized neuronal activity only in the pre-stimulus period in missed tone detection trials in noise (Resnik and Polley, 2021). This suggests that such 'internal noise' in the auditory cortex following cochlear synaptopathy might be responsible for degraded behavioral performance in noisy listening conditions. In this study, we investigated whether this 'internal noise' can also be captured in humans with EEG. Correlation between the likelihood of cochlear synaptopathy and the degree of 'internal noise' will be presented when internal noise is observed.
Methods: Participants with normal hearing or mild hearing loss performed a monaural tone detection task while their EEG was recorded. A target tone (100 ms) with a frequency drawn between [800, 1250] Hz appeared randomly in either a quiet or a noisy (white noise) background, with an inter-target-interval uniformly drawn between [1.75, 4] seconds. Participants were asked to respond (via key press) as fast as possible whenever they heard a target. For each ear, in each background (quiet vs. noisy), data from 100 missed trials were collected. Lifetime noise exposure was assessed for each participant using a noise exposure questionnaire (Beach et al., 2013; Valderrama et al., 2018; Yeend et al., 2017).
Results: The analysis of EEG data tested whether baseline activity for missed trials in a noisy background was correlated with (1) lifetime noise exposure level, (2) speech comprehension masking release (threshold of speech perception in speech-shaped noise minus threshold of speech comprehension in silence), and (3) pure tone threshold in each ear.

Conclusions: To summarize, this study investigates (1) if EEG can capture cortical hyper-synchronized baseline activity (depicted as 'internal noise') and (2) whether humans with a higher risk of cochlear synaptopathy have stronger 'internal noise', particularly in noisy listening conditions.

TU11. Investigating the Neural Correlates of Harmonicity in Auditory Cortex in Humans

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Category: Auditory Cortex and Thalamus: Human Studies

Background: Pitch and harmonicity are central perceptual properties of real-world sounds, including speech and music, and are crucial for the perceptual organization of such sounds in an auditory scene. However, there are still significant gaps in our basic understanding of how pitch is represented in auditory cortex. Previous work has suggested that regions near the anterolateral end of Heschl's gyrus are sensitive to pitch, as they show greater activation to resolved harmonic complex tones than unresolved harmonic complex tones and frequency-matched noise. However, it is not clear whether this differential activation truly reflects the stronger pitch salience elicited by harmonically related tones, or whether these cortical regions are sensitive to other spectro-temporal differences between pitch-evoking complex tones and frequency-matched broadband noise, such as spectral density.

Methods: In this study, we compared 3T fMRI responses for harmonic complex tones (resolved and unresolved) to inharmonic complex tones, which were matched to the harmonic tones in terms of spectral density and frequency range but did not elicit a clear unambiguous pitch. Additionally, we measured responses to frequency-matched broadband noise, which has traditionally been used as a reference condition for localizing pitch sensitive regions. Lastly, we examined the effect of phase manipulation in the unresolved harmonics in order to investigate the responses driven by envelope fluctuations.

Results: We observed robust, bilateral, stimulus-driven activity in Heschl's gyrus and surrounding auditory regions for all stimuli. For complex tones with resolved components, preliminary univariate analyses indicated no systematic difference in activation within previously reported pitch sensitive regions for stimuli with and without salient pitch percepts, when controlling for spectral density and bandwidth. For stimuli with only unresolved harmonics, strong activation was observed based on amplitude modulation strength, which was not restricted to the putative pitch-sensitive regions. Our findings indicate that the regions near the anterolateral end of Heschl's gyrus may not be sensitive to F0 or pitch per se, but might be sensitive to specific spectro-temporal properties that covary with sounds traditionally used as pitch stimuli. Additional multivariate pattern analysis (MVPA) were conducted to investigate differences in brain activation between harmonic and inharmonic tones that were not seen in univariate analyses. MVPA results indicate that although the responses to harmonic and inharmonic tones can be decoded with moderate accuracy, they are not focused within the anterolateral region of Heschl's gyrus.

Conclusions: Results indicate that although responses to harmonic and inharmonic tones can be decoded with moderate accuracy, these differential responses are distributed throughout Heschl's gyrus, rather than being localized to a particular region.

Acknowledgments: NIH grant K99DC017472 (Mehta) and NIH grant R01DC005216 (Oxenham)

TU12. Adaptation to Noise for Amplitude Modulation Detection Measured With Electroencephalography in Normal-Hearing Listeners and Cochlear Implant Users

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Category: Auditory Cortex and Thalamus: Human Studies

Background: Normal-hearing (NH) listeners show better amplitude modulation (AM) detection thresholds in noise as the AM probe delay from the noise onset increases. This 'adaptation to noise' can be as large as 10 dB and it might occur because auditory neurons shift (adapt) their dynamic range towards the most common level in the noise. However, higher order processes, such as listener's expectations about the upcoming stimulus and predictive coding could also be involved. This study aims at investigating whether adaptation to noise occurs and is consistent with the characteristics of neural dynamic range adaptation when factors related to the listener's decision making are not involved. On the other hand, we know that cochlear implant (CI) users show behavioural adaptation, but it is uncertain if auditory neurons adapt to sound level

statistics when stimulated with electric current. The second aim was to compare adaptation to noise for NH and CI users measured with electroencephalography (EEG) to investigate if the same mechanisms are involved.
Methods: EEG recordings were obtained using a 64-channel Biosemi system (16 kHz sampling). Participants were presented with AM sinusoidal probes (250 ms, 2.5 kHz carrier, 40 or 96 Hz modulation rate, 50% modulation depth) delayed 50 or 500-ms from the onset of a broadband noise (0.1-10 kHz), referred to as 'early' and 'late' conditions, respectively. The AM probe level was 70 dB SPL and the overall noise level was 70-, 65-, 55-, or 45-dB SPL. If neural dynamic range adaptation enhances AM detection, we expect a change (early vs late) in the amplitude of the auditory steady-state responses (ASSR) and auditory change complex (ACC), and this change to be larger when the AM probe and noise are closer in level. 'Higher order processes' such as attention to the stimulus will be controlled for by asking the participants to watch a film with subtitles as well as with the highest AM rate (96 Hz), which is considered to evoke mainly brainstem activity. Behavioural AM detection thresholds were measured using a two-alternative, forced-choice paradigm.
Results: Research is ongoing. Preliminary results for four NH listeners suggest that behavioural adaptation is similar across noise levels (2.7 dB on average) and that the amplitude of the ASSR is larger when delaying the probe in the noise (average difference = 0.11 μV) only when the noise level was 55 or 45 dB SPL.
Conclusions: Behavioural and physiological measures of adaptation were not consistent across all conditions (although the sample is small). [Supported by the Spanish Ministry of Universities, Unión Europea NextGenerationEU/PRTR, University of Salamanca, Spanish Ministry of Science and Innovation (grant PID2019-108985GB-I00), the European Regional Development Fund, and the MRC Senior Fellowship in Hearing Research (MR/S002537/1)].

TU13. Neural Signatures of Adaptive Plasticity in Speech Perception as Measured With Human Intracranial Stereo-Electroencephalography

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Category: Auditory Cortex and Thalamus: Human Studies
Background: The ease with which we understand speech belies the complexity of mapping acoustics to native-language speech representations acquired across experience. Even simple distinctions that change meaning, as from bear to pear, are signaled by multiple acoustic dimensions [e.g., voice-onset time (VOT) and fundamental frequency (F0)] that do not stably map to phonemes like /b/ and /p/. Rather, the perceptual weight of an acoustic dimension—its effectiveness in signaling a phoneme category—flexibly shifts when short-term acoustics depart from the norm, like an unfamiliar accent. These adjustments are acknowledged to be important in accommodating everyday variation in speech input. Yet, we know little of the neural mechanisms underlying this adaptive plasticity in speech perception.
Methods: To address this gap, we used a well-established behavioral paradigm that invokes adaptive plasticity (Idemaru and Holt, J Exp Psychol Hum Percept Perform, 2011) simultaneously with human intracerebral recordings (stereo-electroencephalography; sEEG) in the context of epilepsy surgery. Stimuli consisted of the words bee and pea presented in two contexts, each of which exposed listeners to a different short-term speech-input regularity. This regularity either matched a typical pattern of VOT and F0 covariation in English (Canonical; higher F0s and longer VOTs for /p/) or introduced an 'accent' (Reverse; lower F0s with longer VOTs for /p/). Upon introduction of the accent, listeners down-weight F0 in /b/-/p/ categorization of 'Test' stimuli with perceptually ambiguous VOT (which removes this dominant dimension from adjudicating a category-identity decision) and highly distinct F0. The proportion of Test stimuli categorized as /p/ quantifies the perceptual weight of F0.
Results: Preliminary results (N=4 patients) show the predicted behavioral adaptive plasticity effect. We hypothesized that the neural basis of this effect may be distributed across time and brain regions. Accordingly, we used a linear classifier to decode low-versus-high F0 from the sEEG high-gamma-band (75-150 Hz) envelope (HGE) in different brain regions and time points for ambiguous-VOT stimuli. When the classifier was trained with Canonical examples, it performed well on unseen Canonical data but significantly worse on Reverse data. Moreover, the performance on Reverse data did not improve even with training using Reverse examples. These preliminary results suggest that the neural encoding that distinguishes low-versus-high F0 in Canonical context provides a weaker contrast in Reverse context. This

is in line with behavioral alteration of F0 perceptual weight. To examine network-level changes as a function of short-term input regularities, we computed across-region functional connectivity using the phase-locking-value measure applied to the HGEs, contrasting Canonical and Reverse contexts. Preliminary results revealed a functional brain network—spanning regions in auditory, parietal, and frontal cortex—that is implicated in F0 downweighting in Reverse context.
Conclusions: This study was supported by National Institutes of Health grants R21DC019217 (to LLH and TJA) and T32DC11499 (to VV).

TU14. Neurophysiological Measures of Salience-Dependent Disruption of Sustained Auditory Attention
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Category: Auditory Cortex and Thalamus: Human Studies
Background: Being distractible is a vital feature of auditory attention, since relevant, or even dangerous information may be found outside of our current focus of attention. On the other hand, irrelevant sounds can disrupt sustained attention and hinder focus on a task. “Salience” is the degree to which a sound stands out, such that it captures attention and is distracting. Salience effects on bottom-up attention, measured behaviorally, have been captured by a computational model (Huang and Elhilali, 2018). While behavioral validation is key to understanding which sound features drive salience, a physiological measure of distraction would allow to monitor attention during continuous listening tasks. Here, we examine whether the existing model for salience captures attention measured neurophysiologically (EEG and pupillometry), as a first step to developing neurophysiological indices of distraction for use in applied contexts.
Methods: We asked if neurophysiological responses to to-be-ignored distractors during sustained auditory attention correlate with modelled salience and how age and hearing loss affect these responses. To this end, we asked N = 47 participants (Age range: 24 – 82; 24 participants with sensorineural hearing loss compensated with linear gain) to listen to continuous speech while we recorded their pupil size and EEG. Meanwhile, one-second-long distractor sounds of varying modelled salience were presented at random points in time, which participants were asked to ignore. We extracted the distractor-evoked mean pupil dilation and the neural tracking of both target and distractor stimuli from EEG.
Results: To-be-ignored distractor sounds lead to pupil dilation and strong neural tracking. Both the distractor-evoked mean pupil dilation and neural tracking of the distractor correlate positively with modelled salience. Distractors disrupt neural tracking of the target talker, but this disruption was not found to be directly related to modelled salience. While both traits age and hearing loss lead to stronger neural tracking in general, these traits did not affect the dependence on the modelled salience of the responses.
Conclusions: This study may help to identify sensitive neurophysiological measures of distraction and to overcome challenges related to behavioral indices of salience measured in sustained attention tasks, such as interrupting a continuous listening task for behavioral feedback.

TU15. Open Board

TU16. Layer 6 is a Primary Nexus for Cholinergic Modulation of Columnar Sound Processing
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Category: Auditory Cortex and Thalamus: Structure and Function
Background: Basal forebrain cholinergic neurons (BFCNs) regulate arousal, stimulus salience, plasticity, and learning at time scales spanning milliseconds to hours. At faster timescales, BFCN-mediated acetylcholine (ACh) release modulates auditory cortex (ACtx) sound processing via nicotinic (n) receptor (r) subtypes. Layer (L) 1 GABAergic neurons are highly enriched for nAChRs, and have been identified as a key nexus in ACtx microcircuits for plasticity and learning. L6 corticothalamic neurons also have strong direct inputs from BFCNs (Clayton et al., Current Biology 2021), though nAChR and muscarinic (m) AChR expression profiles have not been characterized as well in deeper cortical layers. Here, we performed 9-plex fluorescence in situ hybridization (FISH) to document AChR mRNA expression across all layers of the mouse primary ACtx (A1). Laminar mRNA expression profiles were then compared to a functional analysis

of cholinergic modulation based on experiments that combined optogenetic BFCN activation with columnar single unit recordings.
Methods: For FISH studies, mice were injected with the retrograde tracer Cholera Toxin B in the medial geniculate body to label corticothalamic neurons (N=3). Multiplex FISH was used detect nicotinic and muscarinic receptor transcripts in 3357 A1 neurons. For single unit electrophysiology, a virus was used to express ChrimsonR or ChR2 in BFCNs of Chat-Cre mice (N=5). High-density A1 columnar single unit recordings were performed in awake, head-fixed mice using optogenetic protocols that characterized real-time modulation of frequency tuning or short-term associative plasticity in frequency tuning (n =111 single units).
Results: Among inhibitory neurons, we confirmed prior reports that nAChR transcripts were particularly abundant in L1 inhibitory neurons, including α4, β2, and α7. Among excitatory neurons, nAChR subunits were highly and specifically enriched in L6, where expression levels of α4 and α7 met or exceeded levels measured in L1. MAChR transcripts were rarely found in L1, while M2 and M4 were enriched in L4. Optogenetic activation of BFCNs reduced frequency tuning precision and unsystematically changed best frequency by an average of 0.25 octaves. Repeatedly pairing BFCN stimulation with a fixed tone frequency induced a specific enhancement of the paired frequency assessed up to 1h post-pairing that was most prominent in L6 putative excitatory neurons.
Conclusions: Enriched nAChR expression identifies neural subtypes that interface with BFCNs to modulate sensory processing and plasticity in local cortical circuits. L1 GABA neurons are one well-established nexus for BFCN-mediated plasticity and here we combine evidence based on gene expression and single unit recordings that L6 is another primary nexus for cholinergic modulation. Our ongoing and future work will characterize BFCN-evoked synaptic currents in L6 excitatory neurons and develop a model for how two key neural subtypes that sit at opposite ends of the cortical column work cooperatively to adaptively shape sound processing during active listening and learning.

TU17. Multi-Scale Modeling and Visualization of Neuronal Networks With the Brain Modeling Toolkit (BMTK), Sonata Data Format, and Visual Neuronal Dynamics (VND)

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Category: Auditory Cortex and Thalamus: Structure and Function
Background: Models of cortical networks provide a framework for testing and understanding the mechanisms underlying cortical processing, dynamics, and disease.
Methods: BMTK, SONATA, and VND provide a set of integrated tools and standardizations to facilitate model building, sharing, and visualization.
Results: The BMTK software (https://alleninstitute.github.io/bmtk/) provides an interface for building network models using customizable connection rules and realistic neuronal types, and allows users to deliver sensory stimulus inputs to the network while recording spiking activity and extracellular potentials. Models can be built at a variety of scales depending on the application, for instance, a network of biophysically and morphologically detailed neurons versus a network of simpler point neurons with interesting synaptic dynamics. The roles of individual cell types can be investigated by introducing perturbations to subpopulations of model neurons. BMTK was used to produce a large-scale biologically realistic model of primary visual cortex that is freely available to the scientific community (Billeh et al., 2020). SONATA (https://github.com/AllenInstitute/sonata) is a standardized and shareable file format that stores the model structure, components, inputs, and simulation outputs, with support in a variety of simulation programs in addition to BMTK. The VND visualization tool (https://www.ks.uiuc.edu/Research/vnd/) enables exploratory 3D visualization of model network morphology, connectivity, and activity, which is useful for validation during model building, as well as for model analysis and rendering publication-quality images and videos.
Conclusions: Together, these tools can be used to explore a variety of questions purely in silico or in conjunction with experimental data.

TU18. The Central Auditory Alteration in Animal Models Induced by Noise-Induced Hearing Loss (NIHL) and Photothrombotic Hearing Loss Model (PT)

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Category: Auditory Cortex and Thalamus: Structure and Function
Background: Sound stimuli are converted into electrical signals by hair cells and transmitted to the central nervous system (CNS). Irreversible damage to the hair cells in the auditory peripheral organ, which is prone to permanent hearing loss, is considered the main cause of peripheral nervous system (PNS) damage. Damage to the PNS in the auditory area induces secondary degeneration in the neuronal cells in the auditory pathway of CNS. The alternation of auditory cortex neuronal cell population after auditory sensory deprivation has been reported. However, there are many different pathomechanisms of hearing loss such as ototoxicity, excitotoxicity or ischemic toxicity. It is not clear whether these different type of PNS damage results in CNS alteration.
The purpose of this study is to verify and compare changes in the central auditory pathway in animal models induced by noise-induced hearing loss (NIHL) or photothrombotic (PT) hearing loss.
Methods: We used male Sprague-Dawley rats (7 weeks old) for NIHL and PT models. All NIHL rats were exposed to a narrow band of noise (16kHz) for 5hr at a sound pressure level (SPL) of 105dB. The rats of the PT model were injected with RB in the femoral vein followed by an occlusion of the vessel with a photochemically induced thrombus upon laser irradiation (532nm, 175mW, 15 min). Auditory brainstem response (ABR) was measured to confirm hearing loss and brains were sampled at 1 week after noise exposure and surgery. Immunohistochemistry (IHC) was performed for neuronal apoptosis and neuroinflammation.
Results: In the NIHL model, mean values of the threshold measured by auditory brainstem response (ABR) were increased after noise exposure. IHC of the brain sections revealed that mature neurons expressing the neuronal Nuclear (NeuN) protein in the auditory cortex decreased in number compared to the control group. However, astrocytes expressing glial fibrillary acidic protein (GFAP) and ionized calcium-binding adapter molecule 1 (Iba1) increased compared to the control group. These results show an increase in neuronal cell death and an increase in neuroinflammation in the NIHL model's auditory cortex region. In the PT model, hearing loss after surgery was confirmed using ABR. Deterioration of cochlear structure as well as alternation of synaptic connections in the cochlear nucleus were observed.
Conclusions: In this study, HIHL and PT animal models both resulted to hearing loss. The NIHL model presented an increase in cell death and neuroinflammation in the auditory cortex region while the PT model showed deterioration of the cochlear structure.
Comparison of auditory cortex and cochlear nucleus after both NIHL and PT models are in progress.

TU19. Behavioral and Neural Correlates of Early Music Exposure in Mice

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Category: Auditory Cortex and Thalamus: Structure and Function
Background: The mammalian auditory system has evolved to process behaviorally important sounds, which are most often complex. For animals to survive in nature, their ability to perceive these sounds is essential. We studied the representation of one specific family of complex sounds in the mouse brain: human music, a remarkably complex acoustic set of stimuli with dynamic spectrotemporal structure. Music has been extensively studied in humans and it has been shown that music preference is associated with activity in brain areas that are linked to emotions. Moreover, several studies have shown the effect of music on non-human mammals. In a recent study, mice showed idiosyncratic preferences for human music. This provides a good opportunity to use mice as models to investigate the brain areas that facilitate music preferences.
Methods: We conducted behavioral experiments on mice to explore this observation. We subjected young mice to a musical environment made up of Beethoven's Symphony No. 9 excerpts during their critical development period (P7–P40). We evaluated their musical preferences when they were young adults (P60–P90). Furthermore, we examined the neural responses to various sounds in the auditory cortex using wide-field calcium imaging, including snippets from the music the animals were first exposed to.
Results: Our findings revealed that in comparison to control and naïve mice, exposed mice significantly preferred music to silence. These effects showed sex effects, with males showing stronger preferences for

the exposed music than females. Furthermore, when given the option between exposed and unexposed music, females expressed an interest in the latter.
The neurophysiology results indicated the suppression of the overall evoked activity in the auditory cortex of exposed animals compared to control or naïve animals. The magnitude of the auditory responses in the auditory cortex was correlated with behavioral readouts in females but not in males.
Conclusions: Music exposure during critical period modified the behavioral and neural responses of mice. Moreover, these responses showed significant sex effects.

TU20. Characterization of Neural Responses to Intraneural Stimulation of the Auditory Nerve in Guinea Pig

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Category: Auditory Nerve
Background: Direct stimulation of the auditory nerve with a penetrating electrode array can provide more selective activation of auditory nerve fiber groups compared with scala tympani stimulation via a traditional cochlear implant (CI). Intraneural stimulation also enables access to low frequency fibers that are difficult to stimulate using a CI. Thus, direct nerve stimulation can potentially provide more accurate transmission or a greater extent of sound information, especially spectro-temporally complex signals such as human speech or music. As auditory nerve implants (ANIs) are developed for use in humans, further studies are needed in animal models to inform stimulation strategies and identify differences in auditory system activation by ANIs versus conventional CIs.
Methods: A single-shank 16-site electrode array was implanted at the modiolar section of the auditory nerve in ketamine-anesthetized guinea pigs. Responses to electrical stimulation (charge-balanced, biphasic pulses) were recorded in the central nucleus of the inferior colliculus (ICC), with a 32-site electrode array oriented along the tonotopic axis. For each stimulation site, current values up to 120uA (in steps of 20uA) were presented to generate an electrical tuning curve, which was used to determine the frequency region in ICC that was preferentially activated by each stimulation site. Pulse duration was also varied to analyze its effects on tuning curve characteristics. To serve as a comparison benchmark, tuning curves were also generated (before implantation of the auditory nerve) using acoustic stimuli (pure tones, 1-40kHz, 0-70dB SPL).
Results: Electrical tuning curves demonstrated a tonotopic organization of the nerve that was similar to that seen in past studies. The shallowest stimulation sites activated high frequency regions while deeper shanks targeted more middle and low frequency fibers. The activated frequency regions going from shallow to deeper sites did not strictly follow a monotonically progressing pattern; characteristic “jumps” were evident, most often from very high frequency regions to very low frequency regions. Comparisons were made to analogous tuning curves generated via acoustic stimulation, showing similar activation patterns and tonotopic spread of activation. Changing the electrical stimulus pulse duration resulted in variations in the activation patterns across the range of activated frequencies.
Conclusions: Intraneural stimulation in the guinea pig is capable of activation of neural fibers in a manner similar to acoustic stimulation. This indicates that a potential ANI would allow for tone-like perceptions across the entire hearing range. To study efficacy of transmission for more complex speech-like signals, follow up studies will be performed using guinea pig vocalizations. In order to reduce differences in response patterns due to electrical versus acoustic activation, optimization of the electrical stimulation parameters will also be performed.

TU21. Fabrication and Biocompatibility of Photoreactive Nanocapsules Delivery of Neural Differentiation Factors to the Cochlear Modiolus

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Category: Auditory Nerve
Background: The current rehabilitation strategy for patients with age-related hearing loss involves cochlear implant (CI) surgery which transmits electrical signals directly to the spiral ganglion neurons (SGN) to restore hearing. However the success of CI depends on the status of remaining SGNs due to secondary degenerative hearing loss or sensorineural hearing loss.

Stem cell therapy is being studied as a method for auditory nerve regeneration, but the low survival rate and differentiation capacity in the cochlea do not guarantee successful treatment. As a way to overcome this, the development of a hydrogel nanocapsules capable of carrying singlet neural progenitors as well as differentiation factors for auditory nerve differentiation and be released by light irradiation is considered to be a very promising approach to increase the treatment efficiency.
This preliminary study aims to fabricate a photoreactive nanocapsule that is able to release the loaded growth factors after light irradiation.
Methods: The photoreactive amphipilic polymer was designed with an near infrared (NIR) photocleavable hydrophobic dye molecule and a biocompatible polyethylene glycol (PEG)-based polymer modified with a long hydrophilic amine group capable of forming particles through self-assembly encapsulating either a neural progenitor cell or growth factor or both. These nanocapsules were tested in vitro for their biocompatibility and photoreactive release of loaded growth factors (NT-3 and BDNF). In vivo test was performed by injection inner ear via the round window membrane.
Results: A photosensitive polymer designed with polyethylene glycol (polyethylenglycol, PEG)-based growth factor (GF) particles and a growth factor secreted in response to red laser was prepared. It was confirmed that PEG-octamethylene diamine hydrophilic polymer was prepared through 1HNMR analysis. In addition, by utilizing the properties of photocleavable Leucomethylene blue (LMB), it was confirmed that nanocapsules capable of selectively secreting substances (BDNF, NT3) by photostimulation were possible. Stained sectioned tissues from the implanted cochlea confirmed the presence of the nanocapsules in the modiolus and release of the growth factors after light irradiation.
Conclusions: A photoreactive nanocapsule was successfully fabricated that is capable of encapsulation and photo-triggered release of BDNF/NT3 growth factors into neural progenitor cells in vitro and successful in vivo delivery into the cochlear modiolus. This study can help improve the efficiency of cochlear implants and stem cell therapies for the restoration of hearing.

TU22. Loss of Limbic Mineralocorticoid or Glucocorticoid Receptors Impacts Auditory Processing in the Cochlea

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Category: Auditory Nerve
Background: Emerging evidence for associations between hearing impairment and cognitive decline implies that hearing is dependent on the formation and storage of auditory memories in the limbic system in a mood- and arousal-related manner. Considering glucocorticoid release upon stressful and exciting situations that result in altered auditory perception, we were interested in the contribution of mineralocorticoid- (MR) and glucocorticoid receptors (GR) on hearing function.
Methods: We used a tamoxifen-inducible CreERT2/loxP system to generate single or double deletion of MR and GR in limbic brain regions of adult mice in combination with auditory measurements that include ABR wave analysis, CAP latencies, ASSR).
Results: While threshold sensitivity in MRGR conditional knockout (cKO) double mutants were unchanged, early and late ABR waves, CAP latencies, and ASSR, suggested a direct beneficial effect of limbic MR/GR function on auditory-nerve processing. Analysis of single MR or GR cKO revealed that the phenotype of MRGR cKO mice resulted from opposing influences on auditory fiber responses, i.e. stimulating and inhibiting action: Limbic MR deletion reduced IHC ribbon numbers and ABR wave I responses, leaving later waves, and synchronization to amplitude-modulated tones, unchanged. This indicates that limbic MR activation may alter auditory nerve fiber discharge rates. In contrast, limbic GR deletion improved early and late ABR waves without reducing IHC ribbon numbers. CAP thresholds, latency, and synchronization to amplitude-modulated tones were improved. This suggests that limbic GR activation affects neural response synchrony, thus influencing temporal auditory processing
Conclusions: Our findings suggest that MR/GR stress hormone receptors are candidate factors for positive- and negative cochlear pre-cognitive processing during auditory cue perception and auditory cognitive dysfunction.
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TU23. Males are More Susceptible to Obesity Related Hearing Loss Than Females in CBA/Ca Mice

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Category: Auditory Nerve

Background: Obesity is an independent risk factor for hearing loss. Although attention has focused on major obesity comorbidities such as cardiovascular disease, stroke, and type 2 diabetes, the impact of obesity on sensorineural organs, including the auditory system, is unclear. Using a high-fat diet (HFD)-induced obese mouse model, we investigated the impact of diet-induced obesity on sexual dimorphism in metabolic alterations and hearing sensitivity.
Methods: Male and female CBA/Ca mice were randomly assigned to three diet groups and fed, from weaning (at 28 days) to 14 weeks of age, a sucrose-matched control diet (10 kcal% fat content diet), or one of two HFDs (45 or 60 kcal% fat content diets). Auditory sensitivity was evaluated based on the auditory brainstem response (ABR), distortion product otoacoustic emission (DPOAE), and ABR wave 1 amplitude at 14 weeks of age, followed by biochemical analyses.
Results: We found significant sexual dimorphism in HFD-induced metabolic alterations and obesity-related hearing loss. Male mice exhibited greater weight gain, hyperglycemia, increased ABR thresholds at low frequencies, elevated DPOAE, and lower ABR wave 1 amplitude compared to female mice. Hair cell (HC) ribbon synapses (CtBP2) and postsynaptic density protein (PSD-95) puncta showed significant sex differences. A heat shock protein that protects HCs against major stresses, heme oxygenase-1 (Hmox1; also known as HSP 32), was significantly increased by the HFD in females, but not in males. Cochlear cytochrome c oxidase expression was significantly elevated in HFD-fed females, but not in males. Also, the concentration of adiponectin, an otoprotective adipokine, was significantly higher in female compared to male mice. Finally, adiponectin receptors were widely expressed in the inner ear.
Conclusions: Female mice are more resistant to the negative effects of an HFD on body weight, metabolism, and hearing. Females showed increased cochlear cytochrome c oxidase and Hmox1 mRNA levels, circulating and intra-cochlear adiponectin levels, and HC ribbon synapses and PSD-95. These changes may be mediate the resistance to HFD-induced hearing loss seen in female mice.

TU24. Expansion of a Gaussian Model of Auditory Evoked Potentials

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Category: Auditory Nerve

Background: The standard diagnostic battery for hearing loss is limited in determining site-of-lesion of a sensorineural hearing loss. Early auditory evoked potentials (AEPs) are clinically available and capable of assessing hair cell, auditory nerve, and auditory brainstem function; however, they are rarely used in adult diagnosis. While recording AEPs may only take ten minutes, the analysis of the response is time-consuming, requires extensive training, and the interpretation is poorly understood. The standard of extracting data from the auditory brainstem response (ABR) and electrocochleography (ECochG) is by visual determination of peaks and troughs to calculate latencies and amplitudes. Automation of this process, by finding local maxima and minima, is fairly simple and is used my many researchers. Nonetheless, both automation and visual-determination perform poorly in noisy data and can over- or underestimate amplitudes when waves overlap in time. In a prior study, we found that a Gaussian-model was a valid approach to extracting data from the wave I complex of adult ABRs.
Methods: The present study expands the model to waves III and V of the ABR, and tests the performance of the model on the ABR waveforms of 350 adults with normal hearing and hearing loss, collected with tone-burst and click stimuli. Furthermore, the model is tested on ECochG waveforms of 100 adults with normal hearing and mild hearing loss, collected with tympanic membrane electrodes.
Results: Performance of the model is measured in two ways. First the modeled response waveform is compared to the observed waveform via RMS error. Second, model-estimated peak latency and amplitude are compared to two other methods (1) visual-determination by expert audiologists and (2) a peak-picking algorithm.
Conclusions: The Gaussian model of waveform morphology is a promising method of extracting data from AEPs. The Gaussian model can easily be implemented into existing practice as it is simple, reliable, and

metrics are calculated instantly. Another benefit of a model approach over automated peak-picking is the addition of metrics such as wave width that may be a proxy measure of neural synchrony, and first-spike latency that may classify the responding fibers by spontaneous rate.

TU25. An Open-Source Scalable Cochlear Implant With Multisegmented Electrodes

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Category: Auditory Prostheses

Background: An advanced cochlear implant (CI) that focuses on the challenges of surgical positioning, safe insertion depth, crosstalk, low spatial resolution and high device cost could significantly improve the effectiveness and adoption of this successful neuroprosthetic. We propose a custom, microfabricated and scalable electrode array with higher channel counts and custom circuitry to help address these challenges. In addition to the technical challenges, the high costs of developing hardware, software, and regulatory burdens have slowed advancement in auditory prostheses. The entry barrier is even more challenging for new companies, academic institutions, and independent research groups that don't already have the infrastructure and necessary resources, preventing or slowing the adoption of technological improvements in the clinic. An openly-developed and open-sourced CI with customizable functionality connected to a reconfigurable electrode array would enable these entities to develop and contribute new ideas and technologies to the CI industry.

Methods: We propose an improved cochlear implant system described in an open-source framework including design plan, manufacturing method, and operating software. The implant overcomes the existing issues of resolution and surgical variability through an expanded and reconfigurable electrode array consisting of 32 tetra-segmented electrode groups. Each of the electrodes can be arbitrarily selected to supply or subtract stimulation current for more targeted stimulation and can be independently controlled by up to four different current stimulators. The entire system is designed for scalability, including custom electronics that can support a future higher channel count while enforcing charge-balanced stimulation without a dedicated discrete capacitor for each electrode element.

Results: We have designed and microfabricated prototype samples of a 32 channel multi-segmented cochlear electrode array using the Lawrence Livermore National Laboratory Biomedical Foundry. In addition, a machined mold and molding process used to encapsulate the array and form it into a cylindrical yet flexible shape has been tested. Alongside the electrode array, a custom-designed ASIC (application specific integrated circuit) is being designed and will be built to connect to this array and provide four independently controlled stimulators. The design of the controller has been completed and simplifies the operation of the system yet allows future expandability.

Conclusions: The early cochlear implant components described here demonstrates two primary objectives. The first is the ability to microfabricate the electrode array which should enable lower cost manufacturing as well as enable scalability to higher channel counts. The second is a proposed implementation of the custom ASIC that will provide expanded and enhanced capabilities over existing commercial arrays. Finally, this work is another step towards developing an openly-developed CI benefitting from community input.

TU26. Finite Element Optimization of Piezoelectric Accelerometer Designs for Totally Implantable Auditory Prostheses

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Category: Auditory Prostheses

Background: Moderate to severe hearing loss is a debilitating condition that affects over 5% of the world's population. Hearing aids (HAs) and cochlear implants (CIs) positively impact patients' lives who suffer from sensorineural hearing loss. However, both HAs and CIs display numerous limitations that affect their adoption and use rates. Several key limitations are associated with the external elements of these devices (e.g., microphone and signal processor). These limitations impact device safety, appearance, acoustic performance, and ease-of-use (Calero, et al. (BioMedical Engineering OnLine 17, 23 (2018)). A totally implantable auditory prosthesis would help to address these issues by eliminating external components. A major barrier to progress toward this goal is the lack of a completely implantable acoustic sensor capable of matching or exceeding the performance of commercial external microphones. Our previous studies have indicated that piezoelectric microelectromechanical systems (MEMS) accelerometers have the potential to

function as implantable sensors within the middle ear meeting a 20-phon noise floor over a 100Hz – 8kHz range. These designs have used simple plate geometries for the sensing elements and satisfy the previous specifications. In the current study, we explore variations of these geometries to improve the noise floor for the same operational bandwidth.
Methods: Our ultraminiature accelerometer designs consist of proof-mass-loaded piezoelectric cantilever bimorph beams in either single or dual bandwidth configurations. To validate our analytical model, we test our sensors in benchtop experiments using a laser doppler vibrometer and determine their sensitivity with respect to frequency. We also conduct finite element simulations utilizing COMSOL Multiphysics to investigate the effect of beam and proof-mass shape on the sensor performance. An optimization that is constrained by manufacturing realities is performed to maximize that performance while minimizing sensor volume.
Results: Previous experimental results have validated the analytical model for transverse and longitudinal excitations. We used our model to determine a dual bandwidth sensor design that meets low noise and low mass specifications. This design utilizes a rectangular plate area and proof-mass configurations. With the numerical optimization, a better and more compact design (not necessarily rectangular) can be identified. Preliminary results show that changing the geometry of the bimorph beams can have a positive impact on sensor performance by lowering the minimum detectable acceleration while the sensitivity remains the same.
Conclusions: Our validated modeling and testing show that a small packaged piezoelectric MEMS accelerometer can meet the 20-phon threshold using rectangular sensing geometries. Our finite element simulations of configurations that do not have closed-form solutions indicate that changing the sensing element geometry can lower the noise of the device. Investigations of these geometries will inform our finalized prototype designs that will eventually be manufactured and tested. We would like to acknowledge the NIH training grant (T32 DC00011) that funded this research.

TU27. A Computational Modeling Framework for Auditory Nerve Stimulation With a Cochlear Implant and the Novel Auditory Nerve Implant

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Category: Auditory Prostheses
Background: This work presents a computational modeling framework incorporating a 3D model of a human cochlea and auditory nerve. The framework compares neural activation from a conventional cochlear implant (CI) with that from a novel auditory prosthesis for direct stimulation of the auditory nerve, the auditory nerve implant (ANI). The ANI that is currently under development targets the auditory nerve between the cochlea and the brainstem with a 3x5 array with penetrating electrodes. The computational framework offers the possibility to investigate ANI stimulation prior to the first implantations in human subjects. In this context, it is important to estimate the amount of current to elicit threshold and comfort levels with ANI, as the ANI electrodes will likely have higher impedances than the CI electrodes. In this study, we present updated results based on a refined 3D model of the cochlea, a more detailed geometry of the auditory nerve, and optimized parameters for the 3D finite element model (3D-FEM) stimulation.
Methods: A 3D-FEM model of the cochlea and the auditory nerve including auditory nerve fiber (ANF) pathways was created based on histological data. The 3D-FEM model contains a CI array inserted into the scala tympani and an ANI placed in the auditory nerve. The 3D-FEM model is surrounded by a sphere with bone material. In the new version of the 3D-FEM model the conductivity of the bone material has been fit to transimpedances recorded from human CI users. The 3D-FEM model was used to simulate the voltage distribution along the ANFs when stimulating with the CI or the ANI. A phenomenological stochastic neuron model was applied to simulate excitation of the ANFs, resulting in excitation profiles that show the activation of the ANFs over their tonotopic frequency. Excitation profiles derived at different stimulation levels were concatenated to spatial tuning curves (STCs). Based on the STCs, we estimated thresholds, dynamic ranges, and specificity of stimulation with the ANI and the CI.
Results: For the CI, the STCs had a single peak whereas for the ANI, the STCs varied from single frequency peaks with a narrow spread of activation to multimodal profiles with multiple peak frequencies or very broad excitations. The number of peaks in the STCs were sensitive to the placement of the stimulating electrode and the anatomic and tonotopic organization of ANFs. The computational model predicted that the

ANI requires significant less current than the CI to elicit thresholds. This result is consistent with previous studies in animals (Middlebrooks and Snyder; 2008).
Conclusions: The results of this project will be used to understand the basic mechanisms of auditory nerve activation with the CI and for the future development of fitting and speech coding strategies for the ANI clinical trial.

TU28. Within and Between-Channel Amplitude Modulation Processing in Cochlear Implant Users
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Category: Auditory Prostheses
Background: Cochlear Implants (CIs) work by dividing the incoming acoustic signal into a limited number of frequency channels, extracting the slowly varying amplitude envelope in each channel, and using this to modulate the level of electrical pulses delivered to the auditory nerve. Speech information transmission is reliant upon the ability to detect, track, discriminate and process the amplitude-modulated (AM) envelope of speech sounds independently in different channels. Information transmission can be hindered at many stages in the auditory pathway due to, for example, spread of electrical current, survival of inner-ear neurons and the neural representation of dynamic spectro-temporal cues.
Methods: In this research we employed a psychoacoustic task to explore within- and between-channel AM processing in adults with CIs. We recruited a group of normal hearing adults and a group of adult CI listeners using either Nucleus or Advanced Bionics devices. Acoustic sinusoids of two different rates (4 versus 8 Hz, 13 versus 40 Hz and 40 versus 95 Hz) were discriminated in a three-interval two-alternative forced choice task, where the modulation depth of both modulated sinusoids was adjusted adaptively to derive an AM discrimination threshold. Testing was conducted in quiet (within channel) and in the presence of speech envelope interferers on adjacent, or adjacent +1 channels (between channel). Stimuli were delivered through headphones (HD600s) placed over the sound processor of the cochlear implants. All front-end noise reduction features were de-activated during the experiment. A series of experiments were conducted iteratively to optimise stimuli and to explore limits of AM processing across the frequency range. We compared findings between normal hearing and cochlear implanted adults using the same acoustic stimuli.
Results: This research is ongoing but findings from the initial stages suggest, as expected, that the AM depth at threshold was larger in the presence of the interferers for both normal hearing and cochlear implanted listeners. However, performance for the CI users was more similar to the normal hearing listeners than expected. We observed a degradation in performance for higher AM frequencies for both groups but it was more pronounced for the CI listeners. The goal of developing this task is to determine if it can be used to identify poor functioning channels for consideration for re-mapping.
Conclusions: This research will help us to understand how these AM processing skills vary across CI listeners, how they relate to speech perception and if re-mapping can improve the delivery of AM cues.
Supported by UK Medical Research Council Senior Fellowship (MR/S002537/1)

TU29. Relationship Between Electrode Position, Electrode Impedance and Electrophysiological Thresholds in an Animal Model of Cochlear Implantation
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Category: Auditory Prostheses
Background: It was earlier described that perimodiolar electrode arrays induce lower mean stimulation threshold levels and reduces the spread of excitation (Hughes, 2006, 2010; Frijns, 2002). However, it is a matter of debate how accurate impedance measures can reflect the electrode position.
Methods: To investigate this, adult guinea pigs (Dunkin Hartley) were mechanically deafened just before the implantation of a guinea pig scala tympani electrode array. The first six electrode contacts were used for a short time stimulation of 8 hours and therefore adjusted to a mean stimulation level of approx. 40 CL above eCAP thresholds. A CP810 sound processor was used to provide the stimulation under anesthesia in a standardized acoustic environment (radio play at 65 dB SPL). Electrode impedances were determined in the

monopolar (MP) and the common ground (CG) mode just after the electrode insertion and after 8h of stimulation. Thresholds of electrically evoked compound action potentials from the auditory nerve (eCAPs) and the auditory brainstem (eABR) where determined at the same time points. µCT-scans of all cochleae were performed with an isotropic voxel size of 10.5 µm after the histological tissue fixation. The µCT used a high voltage of 70 kVp, a current of 114 µA, and an integration time of 381 ms to minimize electrode artifacts. The position of the electrode contacts in relation to the center of the modiolus was determined.
Results: The results showed a strong positive correlation between the electrode impedances measured in the CG-mode and the distance of the electrode contacts to the modiolus (r=0.8). However, this correlation was poor if impedances were determined in the MP-mode (r=0.1). Interestingly, the correlation between eCAP/eABR-thresholds and the distance of the electrode contacts to the modiolus was weak. The correlation coefficient was only 0.3 or 0.4, respectively.
Conclusions: The results suggest that the position of the electrode could be determined precisely by the measurement of electrode contact impedances in the CG-mode. There is a trend to the reduction of electrophysiological thresholds by a perimodiolar electrode position.
This study was supported by Cochlear Research and Development Ltd., Addlestone, UK.

TU30. Relating Electrophysiological (Auditory Chance Complex (ACC)) Measures of Amplitude Modulation (AM) Rate Discrimination to Am Discrimination and Speech Perception in Cochlear Implant Users

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Category: Auditory Prostheses
Background: When listening to speech, cochlear implant (CI) users predominantly make use of envelope cues (amplitude modulations (AMs)) within channels and comparisons of these cues across channels. It is known that for CI users that there is a relationship between speech perception abilities and the ability to detect and discriminate AM cues. For individuals with poorer perception of AM cues (or not able to give subjective feedback) re-mapping approaches could improve AM delivery and in turn improve speech perception. In this work our goal is to develop both psychophysical and electrophysiological measures of AM frequency discrimination and relate them to speech perception abilities.
The objective measure that we use captures the neural response to a perceived change in an auditory stimulus such as different AM frequencies (the so-called auditory change complex (ACC)). The amplitude of the ACC is related to the size of the perceived change in the stimulus.
Methods: A group of adult Cochlear device CI users participated in this experiment. An alternating ACC paradigm with continuous switching between two AM frequencies (13 and 40 Hz, both fully modulated) was used at three switching rates (0.5, 4.5 and 6.5 Hz) and two pulse rates (128 and 800pps). The 0.5 Hz switching rate was also measured at different sites along the CI to assess the differences in response across electrodes. For the ACC measurements participants passively listened to the alternating AM stimuli. The electroencephalology (EEG) responses were measured with a 64 channel Biosemi (16 kHz) Active Two system. Artefacts caused by CI processing are attenuated using interpolation and spatial filters.
Behaviourally, the same two AM frequencies were discriminated in a three-interval two-alternative forced choice task to determine if stimuli were discriminable. Speech-in-noise perception was measured using an adapted version of the coordinated response measure with multi-talker-babble as masker.
It is hypothesized, that CI listeners with higher N1-P2 amplitudes (characterising the ACC) across the electrode channels are better able to behaviourally discriminate between different AM frequencies and that this is related to better speech perception abilities.
Results: This work is ongoing, preliminary results suggest that the alternating ACC paradigm for continuously changing AM frequency provides an objective measure of AM discrimination that is related to the behavioural discrimination of the same stimuli. The ACC N1-P2 response is better defined with a higher pulse rate (800 pps). Our ongoing work on measuring responses on different channels and analyses for relationship with speech in noise perception will be presented additionally.
Conclusions: Our early findings suggest that the alternating ACC to AM frequency change provides a meaningful discrimination measure that has the potential to be used to guide mapping to improve AM discrimination.

TU31. Decline and Recovery of Functional Responses to Electrical Stimulation Following Cochlear Implantation in Human CI Users – Comparison to Data From Guinea Pigs

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Category: Auditory Prostheses

Background: In previous studies in animals, we have documented progressive reduction and subsequent recovery of responsiveness to cochlear implant (CI) stimulation following surgical insertion of the implant. Details of these decline-and-recovery patterns in guinea pigs are documented in a companion poster. The objective of this pilot study was to determine if comparable patterns of decline and recovery of function occur in human subjects.

Methods: Three adult human CI users (ages 74 to 82) participated. All three subjects met traditional FDA-guidelines for cochlear implantation. Electrically-evoked compound action potential (ECAP) amplitude growth functions (AGFs) were recorded from four electrodes distributed along the electrode array (Electrodes 5, 10, 16, 22). Recordings were made in the operating room on the day of surgery, via remote in-home testing daily for 14 days post-implantation (DPIM), and then three times per week (in-home) up to 90 DPIM. One subject had a previously implanted contralateral ear that served as a control.

Results: Prior to implantation, all three subjects had residual low-frequency acoustic hearing. Subject 1 had the best hearing, followed by Subject 3, and then 2. However, postoperatively, none of the subjects had measurable acoustic thresholds.

ECAP AGFs showed various patterns comparable to those seen in guinea pigs. Postsurgical patterns fell into one of two categories: 1) electrodes that showed decline and recovery over time and 2) electrodes that showed minimal to no change over time. Across all three subjects, the most apical electrodes displayed steeper AGF slopes with an initial decline of slope over time reaching a minimum between 0 to 23 DPIM, and then recovering, reaching a long term relatively stable level within 70 days. Results seen in most apical electrodes, especially in Subject 1 and 3, were comparable to trends seen in guinea pigs with moderate to good spiral ganglion neuron (SGN) survival. ECAP AGFs on most of the more basal electrodes displayed shallower AGF slopes and showed minimal to no decline and recovery (except in Subject 1 who had decline and recovery on all electrodes). Results seen in most basal electrodes were comparable to results in guinea pigs with poor SGN survival.

Conclusions: Human subjects show patterns of decline and recovery of ECAP AGFs after cochlear implantation that are comparable to those seen in guinea pigs. Better recovery at the more apical sites and in subjects with better preoperative acoustic hearing is consistent with the observation from animal studies, that better recovery is associated with better cochlear health. The combined animal and human studies suggest that ECAP AGFs are a useful measure of cochlear health near the individual stimulation sites, but only after stabilization, which can take several months after implantation.

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TU32. Evaluation of the Audiological Results of the Active Middle Ear Implant Codacs With Regard to Pre-Existing Stapes Fixation

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Category: Auditory Prostheses

Background: The Codacs™ active middle ear implant has proven itself for hearing rehabilitation in patients with both conductive and mixed hearing loss. Intraoperatively, patients with a fixed footplate and often also patients with a mobile footplate were found during the implantation. The aim of this study was to compare these two patient collectives with regard to the audiological outcome.

Methods: The study included data from patients who received a Codacs™ Direct Acoustic Cochlear Implant between September 2009 and February 2018 at Hannover Medical School. The patients suffered from a severe combined hearing loss as a result of advanced otosclerosis. The implantation was carried out in a total of 106 ears in the period under investigation. A fixed stapes footplate was present in 53 patients and a physiologically mobile stapes footplate was present in 53 patients. The puretone- and speech-audiometric data were analyzed retrospectively.

Results: The results revealed in terms of tone audiometry, that the mean bone conduction threshold in patients with a fixed stapes footplate did not change significantly after the implantation of the Codacs™. In

patients with a physiologically mobile stapes footplate, a significant deterioration in the mean bone conduction threshold was detected. In terms of speech audiometry, the Codacs™ enabled a significant improvement in speech understanding in both groups.
Conclusions: In the field of active middle ear implants for treatment of severe hearing loss, the Codacs™ leads to a significant improvement also in cases of a mobile footplate. Despite promising audiological results, which have been confirmed in several clinical studies, the manufacturer has discontinued production of the implant.

TU33. Reducing the Foreign Body Response on Human Cochlear Implant Electrode Materials Subcutaneously Implanted in Vivo With Thin, Photograftable, Zwitterionic Hydrogel Coatings
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Category: Auditory Prostheses
Background: The foreign body response can be detrimental to the function of many medical implants. In the case of cochlear implants, the foreign body response can not only hamper the performance of the stimulating electrode array, but it can damage the cochlea with neo-ossification and cause residual hearing loss. Biomaterial solutions that can address these problems are sorely needed. To this end, our research group has engineered a thin, zwitterionic surface film that can be grafted onto the surface of medical implants by photopolymerization, that reduces adhesion of agents of the foreign body response like fibrinogen, macrophages, and fibroblasts in vitro. The focus of this work is to determine the effect of this zwitterionic thin film on the foreign body response to cochlear implants materials in vivo, with readouts of capsule thickness and cellular histology.
Methods: Cochlear implant housing material polydimethyl siloxane (PDMS) and human cochlear implant mid-scala electrode arrays were prepared with thin film coatings by soaking the surface with a photografting-agent in acetone, dispersing a monomer solution with cross-linker over the surface, and photopolymerizing. Several monomer types were prepared for the PDMS sheet experiments to compare the effects of zwitterionic coatings against other common biopolymers like (hydroxyethyl)methacrylate and (polyethyleneglycol)dimethacrylate. After washing and sterilizing, the substrates were implanted subcutaneously in mice for either 6 weeks (PDMS sheets) or 1 year (cochlear implants). After flash freezing, sectioning of implanted PDMS sheets was performed by CryoJane microtomy and histologically prepared by H and E stain. Upon removal, implanted electrode arrays were embedded in epoxy, sectioned with a diamond knife, and stained with Toluidine Blue.
Results: The fibrotic capsule on PDMS sheets was significantly reduced when coated with a zwitterionic thin film. Coatings of other biopolymers caused either significant ulceration or were less effective than zwitterionic thin films at reducing capsule thickness. The least inflammation is seen in the tissue morphology of the zwitterionic coated PDMS. Similarly, zwitterionic thin films on cochlear implants were not only intact and present after 1 year in vivo, but they demonstrated a potent anti-fibrotic effect. Capsule thickness was significantly reduced on coated electrode arrays, including both the platinum-iridium electrode face and the PDMS housing.
Conclusions: These results demonstrate that zwitterionic thin films can reduce the foreign body response and the associated fibrotic capsule on human cochlear implant electrode arrays and related materials in vivo. Such a reduction in capsule thickness has the potential to improve cochlear implant performance by reducing power consumption and signal spread, while also preventing complications like residual hearing loss and neo-ossification.

TU34. Does Central Gain Compensation Exist in Electrical Hearing?
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Category: Auditory Prostheses
Background: In acoustic hearing, desynchronized and reduced afferent inputs from subcortical neural structures result in declined inhibition in the central auditory system, which leads to amplified cortical

representation of auditory inputs (i.e., central gain compensation) (Presacco et al., 2016, 2019; Perry et al., 2019; Harris et al., 2021, 2022; Rumschlag et al., 2022). Unfortunately, increased central gain does not restore neural synchrony (Rumschlag et al., 2022), and is associated with declined speech perception performance in noise in human listeners with acoustic hearing (Harris et al., 2022). Whether central gain compensation exists in electrical hearing or its relationship with speech perception outcomes in cochlear implant (CI) users remains completely unknown. This study aimed to address this critical knowledge gap in our field.
Methods: To date, 8 post-lingually deafened adult CI users, ranging in age between 36.8 and 69.0 years (mean: 67.3 yrs, SD: 12.7 yrs), have been recruited and tested for this study. In each participant, the amplitude of the electrically evoked compound action potential (eCAP) and the electrically evoked auditory event-related potential (eERP) measured at the maximum comfortable level was used to quantify the level of neural activity of the cochlear nerve and the auditory cortex, respectively. Phase locking value calculated based on eCAP results was used to quantify neural synchrony in the cochlear nerve to electrical stimulation. Speech perception scores were measured using Consonant-Nucleus-Consonant (CNC) words and AzBio sentences presented in quiet, as well as in noise at signal-to-noise ratios (SNRs) of +10 and +5 dB.
Results: Our preliminary data showed that larger eERPs were associated with greater eCAP amplitudes and higher phase locking values. Higher phase locking values were associated with better speech perception scores measured using both CNC words and AzBio sentences. The association appeared to be stronger for more challenging listening conditions.
Conclusions: Our preliminary data indicated a lack of central gain compensation in electrical hearing. Knowledge gained from human listeners and animal models with acoustic hearing cannot be simply generalized to human CI users.

TU35. The Effect of Presentation Rate on Stream Segregation in Cochlear Implant Listeners

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Category: Auditory Prostheses
Background: Several studies have investigated stream segregation in cochlear implant (CI) listeners, but certain aspects of the phenomenon remain poorly understood. In normal hearing (NH), the tendency to hear segregation increases as the frequency separation (ΔF) between alternating tones is increased and/or the interstimulus interval (ISI) between tones is decreased (faster presentation rate). Both effects can be accounted for by the tonotopic representation of alternating sounds in the auditory cortex. However, whilst stream segregation in CI listening appears to be influenced by ΔF, ISI has little apparent effect on segregation judgments (Cooper and Roberts, 2007). Undurraga et al. (2021) showed CI listeners exhibited reduced neural encoding for spectral and temporal auditory changes occurring at relatively rapid, near-speech-like, rates (6-7 Hz) – a finding attributed to increased patterns of cortical adaptation. We speculated that increased cortical adaptation may influence the perception of stream segregation, such that any effect of ISI may be apparent only at rates slower than those tested previously (i.e., >200 ms ISI).
Methods: Experimental stimuli comprised sequences of 16 low-frequency 'A' tones and 4 higher-frequency 'B' tones. Stimuli were presented acoustically, and tone frequencies were set to the center-frequencies of given electrodes. The 'B' tones occurred only in the second half of the sequence (e.g., 'A-A-A-A-A-A-A-A-ABA-ABA-ABA-ABA-'). Listeners used a seven-point scale to report the ease of following the isochronous rhythm of the 'A' tones during the final portion of the sequence. The ISI was varied between 100-400 ms.
Results: In an initial dataset, three CI listeners reported a greater ease of hearing segregation at larger A-B frequency separations. However, only one listener reported a greater ease of segregation at shorter ISIs – the judgments of the other two listeners were largely uninfluenced by ISI.
Conclusions: Despite testing an expanded range of ISIs, these preliminary results are largely consistent with Cooper and Roberts (2007) and suggest ISI does not consistently influence segregation judgments in CI listening. We are currently continuing data collection for this experiment, and piloting 'objective', threshold-based measures of stream segregation to further explore the role of ISI. We will report any relevant additional findings. This research is funded by an MRC Senior Fellowship in Hearing Research (MR/S002537/1).
Cooper, H. R., and Roberts, B. (2007). Auditory stream segregation of tone sequences in cochlear implant listeners. Hearing Research, 225(1-2), 11-24.

Undurraga, J. A., Van Yper, L., Bance, M., McAlpine, D., and Vickers, D. (2021). Neural encoding of spectro-temporal cues at slow and near speech-rate in cochlear implant users. Hearing Research, 403, 108160.

TU36. Scalp-Recorded Correlates of Temporal Pitch Processing in an Animal Model of Cochlear Electric Stimulation

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Category: Auditory Prostheses

Background: Most cochlear-implant (CI) users show poor sensitivity to pitch conveyed by the periodicity of electric stimulation, contributing to impaired perception of music and of speech amid competing sounds. To investigate the neural basis of this temporal pitch limitation, we are developing non-invasive EEG measures of temporal processing in a feline animal model. We showed recently that normal-hearing cats are sensitive to the rates of pulse trains that are bandpass filtered to target only the basal cochlear regions stimulated by CIs. In parallel, a scalp-recorded measure, the cortical Acoustic Change Complex (ACC) was elicited by changes in pulse rates. Also, the scalp-recorded Frequency Following Response (FFR) showed robust neural phase locking across a range of pulse rates relevant to pitch perception. Here, we adapt these measures for cats chronically implanted with a CI and compare the electric-hearing results to the normal-hearing baseline.

Methods: Cats were deafened bilaterally and implanted in one ear with an 8-channel intracochlear electrode array (Advanced Bionics). Every two weeks thereafter, cats were sedated and the electric eACC and eFFR were measured from the scalp. The eACC was recorded for changes in amplitude-modulated (AM) pulse trains (4-kpps carrier rate, 30% depth) that alternated every second between a base and a higher AM frequency. The eFFR measured phase locking to trains of fixed-amplitude pulses that varied in rate from 40 to 640 pulses per second (pps).

Results: CI stimulation elicited robust eACC and eFFR that were still present at 6-months post implantation. Like the normal-hearing ACC, eACC magnitudes were sensitive to the size of the AM frequency change (~36 vs. 66%) and were maximal at intermediate AM base frequencies (~300 Hz) but declined at lower and higher frequencies. Opposite from the normal-hearing ACC, eACCs were stronger for decreases in AM frequencies compared to frequency increases. eFFR spectral amplitudes tended to be larger than normal-hearing FFR but showed a similar decline with increasing pulse rate up to ~600 pps. Group delay, computed for eFFR phase values progressing across rates, revealed latencies corresponding to sources at the cortical level for lower rates (<200 pps) and the brainstem level at higher rates (>300 pps).

Conclusions: These results demonstrate that the scalp-recorded ACC and FFR can be adapted for the study of electric hearing in the cat animal model. These measures will permit longitudinal studies of putative neural limitations on temporal processing with a CI or novel stimulating devices. The eACC may also serve as a surrogate measure of the cat's perceptual acuity, based on the sensitivity of normal-hearing ACC that corresponded well with psychophysical measures in the same cats. These results are being compared to parallel experiments in human CI users to achieve a combined physiological and psychophysical understanding of temporal acuity by electric stimulation

TU37. Decline and Recovery of Functional Responses to Electrical Stimulation Following Cochlear Implantation in Guinea Pigs – Relation to Long-Term Cochlear Health

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Category: Auditory Prostheses

Background: In previous studies in animals, we have documented progressive reductions in responsiveness to electrical stimulation during the first few days or weeks following insertion of a cochlear implant (CI). Subsequently, the functional responses to electrical stimulation often recover in the weeks or months following the initial loss, and may reach a level of responsiveness equal to, or higher than that recorded a few hours after surgery.

The objective of this study was to determine the across-subject variation in patterns of decline and recovery of cochlear implant function and determine the relationship between these patterns and the long-term health of the implanted cochleae as assessed by spiral ganglion neuron (SGN) density and inner hair cell (IHC)

survival. In a companion study we compare the patterns seen in guinea pigs to those seen in a pilot study using humans receiving CIs.
Methods: Thirty guinea pigs in two treatment groups were studied: 1) Implanted in a hearing ear (N=18), and 2) Deafened by injection of neomycin into the scala tympani and implanted (N=12). Nine of the deafened animals were treated with neurotrophin, intended to retard nerve loss. Electrically-evoked compound action potential (ECAP) amplitude growth functions (AGFs) and other data were collected over time. Animals were then euthanized, and the cochleae extracted for histological analysis. For this study, we focused on changes that occurred in the slopes of the ECAP AGFs from the day of implantation until they stabilized.
Results: The hearing group had long-term measurable acoustic thresholds, near complete IHC survival, high ensemble spontaneous activity (ESA) (except two), and high SGN densities. The deafened group had no measurable acoustic hearing, no IHCs (except one), low ESA levels and low SGN densities.
ECAP AGF slopes measured on the day of surgery did not differ significantly between groups. Then, AGF slopes declined, reaching a minimum 2 to 63 days post implantation (DPIM). Minimum AGF slopes were not significantly different between groups. AGF slopes then increased by various amounts (-0.08 to 12.36 μV/μA) reaching a long-term relatively stable level within 91 DPIM (mean = 45 DPIM). The amount of recovery and steepness of stable AGF slopes were significantly different between the groups, but the time required for stabilization was not. The amount of slope recovery and the stable AGF slopes were significantly related to cochlear health.
Conclusions: Cochlear implant function is unstable for weeks to months after cochlear implant insertion. The length of this unstable period varies across individuals and is independent of the health of the cochlea. However, greater recovery occurs in healthier ears. Long-term SGN density accounts for about 40% of the across-subject variance in the amount of recovery from insertion trauma.
Support: NIH R01 DC015809.

TU38. Background Noise in Early Auditory Environments of Children With Cochlear Implants is Detrimental to Quantity and Quality of Language Input

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Category: Auditory Prostheses
Background: Cochlear implants (CI) provide auditory input to children with severe to profound sensorineural hearing loss. However, speech and language outcomes vary largely among children after implantation. Speech heard by children in their early natural auditory environments is a major source for language learning. Exposure to more words (quantity) enhance language and cognitive development in typically developing children. Language input directed to children (child-directed words; quality), rather than simply overheard speech, is highly important for language learning. The quantity and quality of language input could be negatively affected by the complexity of auditory environments, particularly due to background noise, which makes speech perception for CI listeners extremely challenging. We examined the effects of background noise on the quantity and quality of language input experienced by children with CIs during the first year after cochlear implantation.
Methods: Home environments of 14 early implanted children (mean age at CI activation=15 mo ± 4.1 mo) were recorded using Language ENvironment Analysis (LENA) devices. Five percent of audio were randomly sampled from each day-long LENA recording and analyzed perceptually. Speech from adult talkers was coded for whether it was child-directed speech and whether it involved perceptual interference from any overlapping noise. When words co-occurred with an overlapping background noise, the perceived level of noise interference on understanding the words (i.e., low, medium, or high) was identified based on the judged relative loudness and masking by the competing sound source(s). Inter-rater reliability analyses indicated a substantial agreement among coders for three categorizations of speech versus non-speech, child-directed versus adult-directed speech, and level of noise interference (Cohen's Kappa ≥ 0.67). The degree of noise interference was used to update the estimated number of words experienced by each child in a day.
Results: Approximately 48% of the total words and 47% of the child-directed words in a day overlapped with a source of noise. Our estimates showed that children heard around 3,857 fewer total words per day (~15.3% word loss) and roughly 1,638 fewer child-directed words per day (~15.1% word loss), after adjusting for background noise effects. The estimates of the number of words were significantly different before versus after noise-adjustment (total words: $t(13) = 6.49$, $p < 0.0001$, child-directed words: $t(13) =$

4.38, $p < 0.0001$). Moreover, results showed the wide range of individual differences across children in exposure to words (total and child-directed) persisted after considering the effects of background noise.
Conclusions: Findings support raw word counts do not appropriately represent the actual quantity and quality of language input and the individual differences in children with CIs. Further studies are warranted to investigate how the differential effect of environmental noise on the quantity and quality of language input may drive variability in language outcomes across children with CIs.

TU39. Open Board

TU40. Optimizing Ossiculoplasty: In-Vivo Anatomical Measurement of the Human Middle Ear With Hand-Held Optical Coherence Tomography

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Category: Auditory Prostheses
Background: Imaging the middle ear can aid in the surgical management of conditions disrupting the ossicular chain. For example, predicting the prosthesis fit, such as the angle of contact between the prothesis head and tympanic membrane (TM), is crucial as this affects how well ossicular chain reconstruction will restore hearing. Revision surgeries, needed due to shifts in prosthesis placement and for revision ossiculoplasty following cholesteatoma removal, would also benefit. However, no established imaging modalities exist that provide reliable and effective pre- and intra- operative guidance in ossiculoplasty due to the limited resolution of existing techniques like CT and MRI. Thus, developing an imaging modality able to pre-operatively measure the angle of the TM and distances between middle ear structures would provide information on optimal prosthesis fit and better identify patients who would benefit from revision surgery. Recent advancements in optical coherence tomography (OCT) imaging allow for the spatial and temporal resolution to measure the anatomy and physiology of the living cochlea and hence can be applied to the structures of the middle ear. Thus, this study aims to evaluate the ability of a hand-held OCT (HHOCT) device to quantitatively measure the middle ear in healthy volunteers.
Methods: We imaged the middle ear of 11 healthy volunteers with no reported pathology or hearing deficits using our custom-built, HHOCT device with integrated live video feed for identification of anatomical landmarks (e.g. umbo). OCT volumes in each ear were taken (<0.5 s per volume) in the superior-posterior quadrant of the TM to measure the incus diameter, distance from the distal incus above the capitulum to the overlying TM, and angles in the longitudinal and transverse axis of the incus from images post-processed using Amira (Thermofisher Scientific) and Fiji (NIH).
Results: Based on orthogonal slices generated from 3D OCT volumes the mean incus diameter was 0.73 ± 0.09 mm. The mean predicted prothesis was 3.99 ± 0.60 mm. The mean longitudinal and transverse axis TM angles were 20.11 ± 7.77 degrees and 32.72 ± 9.86 degrees, respectively. The similarity of our measurements with those reported in cadaveric studies demonstrates the accuracy of HHOCT. The measurement variability across subjects highlights the importance of individualized measurements for ossiculoplasty.
Conclusions: To our knowledge, our HHOCT device is the first to quickly obtain in-vivo middle ear anatomical information necessary for determining the optimal prosthesis fit. This is critical for improving hearing outcomes and reducing the need for revision surgery in patients. Additionally, this device could be used to identify postoperative complications once the TM has healed. Future studies should characterize pathological ears within a clinic setting to further demonstrate OCTs potential as a diagnostic tool.

TU41. Understanding How Cortex Supports Flexible Sensory Representations

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Category: Binaural Hearing and Sound Localization
Background: Learning is a fundamental function of the brain: sensory representations must be flexible to adjust to environmental changes, thus allowing us to adapt to the world. Understanding the mechanisms of learning are important not only for normal function of the brain but also in disease, for example, after

hearing loss. To study the role of feedback during learning. Sound localization provides a precise measure of learning. Unilateral hearing loss severely disrupts sound localization ability. Re-learning to localize sounds after unilateral loss requires corticofugal feedback projections from the auditory cortex (ACx) to the inferior colliculus (IC)2–6. This suggests a potential mechanism for learning involves cortico-collicular feedback. I hypothesize that the function of cortico-collicular feedback is to provide contextual cues about present sensory conditions. i.e. that the feedback from auditory cortex indicates that the encoding in the upstream brain area no longer matches other perceptual cues that the brain is receiving during behaviour.
Methods: To test this hypothesis, we trained water-deprived mice in a head-fixed Go No-go behavioural task in which the stimulus was a wideband noise burst, the Go stimulus was presented from ±90°, and the no-go stimulus was presented from the opposite side (counterbalanced). Mice were rewarded with water for correctly licking on Go stimuli and received a timeout for licking on No-go stimuli. Once mice reach a performance criterion (d'>1) they will be tested with unrewarded probe trials from locations between ±90° to obtain a psychometric curve. To change the sensory conditions, one ear will be plugged, and mice will be trained and tested. We expect that the mice will initially have disrupted ability to discriminate the stimuli but over training, will recover this ability.
To test the function of feedback neurons from AC to IC during this process, I will use a retrograde adeno-associated virus to express an inhibitory light-activated channel (GtACR2) in ACx neurons that project to IC. I will use chronically implanted optical cannulas to inactivate these neurons while mice perform the sound localization task with a unilateral earplug. Prediction: Mice will be unable to re-learn to localize stimuli where inactivation of feedback neurons occurs. Inactivation of feedback neurons after training in normal hearing conditions will not affect sound localization ability.
Results: Preliminary findings using acute electrophysiology recordings in the auditory cortex of awake, passively listening mice showed effective retrograde transmission of the virus and expression of the opsin as evidenced by a reduction in responses in a subset of cells.
Conclusions: These findings will demonstrate the significance of the cortico-collicular feedback in sound localization in the mouse and form the base to investigate the neuronal plasticity in the IC underlying behavioural adaptation after unilateral hearing loss.

TU42. The Binaural Interaction Component of the Auditory Brainstem Response is Enhanced by the Use of Chirp Stimuli

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Category: Binaural Hearing and Sound Localization
Background: Chirp stimuli enhance temporal synchrony in the auditory nerve and brainstem by accounting and compensating for the frequency- and level-dependent delays accrued by the traveling wave in the cochlea. In monaural measurements, chirps have been shown to produce larger responses in waves I and V of the auditory brainstem response (ABR). Here, we test the hypothesis that chirp stimuli also increase the amplitude of the binaural interaction component (BIC) of the ABR. The BIC is a derived component of the ABR that shows promise as a potential biomarker for binaural hearing. However, clinical use of the BIC has been limited because it is sometimes unreliably measured using traditional click stimuli in humans. Here we generate and compare different chirp stimuli methods to maximize the BIC.
Methods: ABR peak latencies were measured in response to tone bursts over a range of stimulus frequencies and intensity levels in nine chinchillas. Stimuli consisted of 5-ms tone bursts (1-16kHz in one-octave increments) presented via sealed and calibrated insert earphones from ~10 dB below to 50 dB above ABR detection threshold. Latencies of ABR waves I-IV and the BIC DN1 wave (the largest amplitude peak in the BIC) were plotted against frequency and then fit to a power function: Tau=k*f^(-d), where tau is latency in seconds, f is frequency, and k and d are constants. The values of k and d were then used to construct a series of sound level-specific chirps based on 1) monaural ABR waves I and IV and 2) binaural BIC DN1. ABR measurements were then made to compare the chirp-evoked BIC with traditional click-evoked BIC.
Results: Consistent with previous reports, we show that chirps evoked significantly larger monaural ABR amplitudes compared to clicks at low-to-moderate stimulation levels (Student's t-test with bonferroni correction, $p<0.01$). Here, we report that the amplitude of the BIC is also enhanced by using chirp stimuli. At 50-70 dB, both monaural-based chirps as well as the BIC-based chirp evoked significantly larger BIC amplitudes over the click (Student's t-test, $p<0.01$).

Conclusions: Chirps designed to optimize monaural ABR peak amplitudes appeared to produce similar gains in BIC DN1 amplitude as chirps designed to optimize DN1 directly. This is likely explained by the observation that upward spread of excitation in the cochlea is limited at lower sound levels, and that the cochlear-neural delay, which we exploit here in our chirp design, is more pronounced at lower stimulation levels as well. Surprisingly, we find no statistical difference in BIC amplitude between monaural and binaural chirps, suggesting that any chirp which compensates for cochlear delay may be sufficient to evoke optimal binaural BIC responses.

TU43. Fast or Slow Rates? A Comparison of Two Electrophysiological Measures of Binaural Processing

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Category: Binaural Hearing and Sound Localization

Background: The ability to process interaural time differences (ITDs) underpins sound source localization and speech perception in noise. Given its importance, many studies have investigated how the human brain processes ITD. To this end, studies have recorded auditory evoked potentials to changes in interaural phase differences (IPDs), presented at either at slow or fast rates, thereby eliciting transient cortical responses (acoustic change complex, ACC) or steady-state responses (interaural phase modulation following response, IPM-FR). To date, it remains unclear how these responses relate to each other. Here, ACC and IPM-FR are recorded from the same individuals, using a paradigm that is identical, except for the rate at which the changes in IPD are presented.

Methods: Twenty-six normal-hearing adults participated in this study. Stimuli were 500-Hz carrier tone, 100% amplitude-modulated at 40-Hz or 80-Hz. The carrier contained an IPD, which periodically changed from +90° to -90°, or, from 0° to 180°, either at a fast (6.7-Hz) or slow (0.6-Hz) rate, eliciting IPM-FR or ACC, respectively.

Results: For both measures, the results reveal larger responses when using 80-Hz amplitude-modulated tones. IPM-FR – but not ACC – was significantly larger for IPDs changing from 0° to 180°.

Conclusions: Here, we show that ACC and IPM-FR can be reliably obtained from normal hearing adults. Unlike the effects of AM, IPD seems to affect only IPM-FR. We propose that this is attributed to the faster rate used to elicit IPM-FR, inducing neural adaptation.

TU44. Predicting the Auditory Motion Tracking Abilities of Bilateral Cochlear Implant Users and Typical Hearing Listeners Using Binaural Cues

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Category: Binaural Hearing and Sound Localization

Background: Auditory motion perception is the ability to discriminate and track moving sound sources. The cues used to perceive auditory motion are poorly understood as compared to the cues used for stationary sound localization. In particular, the abilities of people with cochlear implants (CIs) to track auditory motion are poorly understood. Typical hearing (TH) listeners use the binaural cues arising from differences between the two ears in time of arrival of sounds (interaural time differences; ITDs), and intensity at the ears (interaural level differences; ILDs) to accurately localize stationary sounds. When using their clinical processors in the sound field, CI listeners have less access to ITDs than TH listeners, leading to much greater localization errors than TH individuals. Prior studies suggest that ILDs generally predict the stationary localization abilities of CI listeners, but it is still unclear whether and how CI listeners use binaural cues for auditory motion perception. It was hypothesized that if listeners use binaural cues to track auditory motion, the auditory motion tracking responses of CI and TH listeners could be predicted by ILD and ITD cues in the stimuli, respectively, because CI and TH listeners primarily depend on these cues for stationary sound localization. Alternatively, if binaural cues are not used for auditory motion detection, it was predicted that listener responses would appear unrelated to the cues derived from the stimulus.

Methods: Auditory motion responses for ten TH and ten CI listeners were modeled using acoustic binaural cues as predictors. ITDs and ILDs were derived from stimuli recorded with a KEMAR mannikin. Both response start location and response range of motion were considered. Normalized response data for each listener were fit with a linear regression and the resulting model error was used to evaluate how well the binaural cues predicted auditory motion responses.

Results: TH listener responses for start location and range of motion were well predicted by the ITDs measured in the stimulus, as hypothesized. CI listener start responses were well predicted by ILDs but were not as consistent as TH responses. However, CI listener range of motion responses were distorted and not well predicted by either cue. These results suggest that ILDs alone are not sufficient for following the location of a moving sound stimulus, leading to poor tracking responses from CI listeners.
Conclusions: The binaural cues available in a stimulus can predict the auditory motion perception of TH listeners, but can only partially explain the perception of auditory motion by CI listeners. It is possible that encoding the ITDs from the stimulus could improve the accuracy of CI listener tracking of auditory motion. Future research can investigate which cues unrelated to those measured here impact the perception of auditory motion by CI listeners.

TU45. Sound Localization is Impaired by Concurrent Sources Even When We Know Where to Listen

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Category: Binaural Hearing and Sound Localization
Background: Sound localization is critical to understanding the surrounding environment. Localization of single sound sources has been extensively studied, but less is known about localization in scenes with multiple sources, particularly realistic scenes with natural sounds. A better understanding of the perceptual limits of sound localization in such settings could inform the design of physical and virtual human spaces. Here, we investigate the ability of humans to localize natural sounds in the presence of concurrent distractor sounds, and whether this ability is aided by knowing where to listen.
Methods: We presented different numbers of natural sounds using a speaker array with 133 high-definition speakers (19 azimuth positions x 7 elevation positions). A participant heard a burst of white noise from one speaker either before ("pre-cue") or after ("post-cue") a scene containing 1 to 6 environmental sounds (the "auditory scene") playing concurrently from different speakers. The participant reported whether the location of the noise burst coincided with any of the speaker locations used in the auditory scene.
Results: Performance decreased with the number of sources in the scene, regardless of whether participants were pre-cued to a source location. However, participants showed higher perceptual sensitivity in the pre-cue condition than the post-cue condition.
Conclusions: Our findings suggest that there is some benefit to knowing where to listen when localizing sounds in auditory scenes. However, localization is limited by the number of concurrent sound sources.

TU46. Sound Localization Training During Earplug Use: Effects of Training Space and Individual Auditory Factors

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Category: Binaural Hearing and Sound Localization
Background: Spatial hearing supports communication and navigation in everyday environments, as well as accurate and timely responses to important safety signals. Human spatial hearing depends on three main localization cues and may be degraded when one or more of these cues is disrupted. Notably, perturbation of the spectral shape cue – a monaural cue generated by the filtering of sound by the head and pinna – leads to marked increases in large localization errors, including up-down and front-back confusions. However, the adult auditory system appears able to robustly accommodate altered spectral shape cues; with sufficient time, listeners gain near-normal localization performance, learning to localize with "new ears" while retaining normal localization ability with their non-distorted (original) cues. Many questions remain regarding the time course and completeness of such learning. The present study builds on our previous work in this area, in which spectral shape cue distortions are produced using real-world hearing devices – earplugs, which offer hearing protection but also notoriously compromise sound localization ability. We evaluate the effectiveness of an abbreviated training protocol and the extent of generalization to untrained locations after training with two different loudspeaker distributions.
Methods: Twelve participants, split into 2 groups, performed ~45 minutes of daily sound localization training for 2 weeks (excluding weekends) while wearing Combat Arms Earplugs (Gen 4.1), which provide minimal attenuation when worn in 'open' mode. Stimuli were brief (100 ms) bursts of broadband noise presented at an average level of 70 dBA. Each group was trained on a different set of 12 source locations (loudspeakers) positioned on a 360˚ speaker array inside a darkened hemianechoic chamber. Group 1 was

presented with training speakers at 2 elevations (-30˚ and +30˚) across 6 azimuths (±45˚,0˚,180˚, and ±135˚). Group 2 was presented with training speakers at 6 elevations (-30˚, -15˚, 0˚, 15˚, 30˚, and 45˚) across 2 azimuths (0˚, and 180˚). All subjects completed pre- and post- testing on a more extensive set of 24 speakers. Finally, pinna morphology (via 3D scan) and head-related transfer functions were measured both with and without the earplugs in place to relate behavioral performance to individual auditory factors.
Results: Following initially marked increases in localization errors with the use of earplugs, subject response accuracy improved across the duration of the 2-week training protocol. Differences in the form and extent of improvement are evaluated with respect to the spatial distribution of training speakers and subject factors including the severity of spectral shape cue degradation.
Conclusions: Auditory localization is degraded during the use of devices that disrupt natural spectral shape cues, but improves with behavioral training. This study is part of an ongoing effort to learn more about the breadth and depth of adaptation to changes in spectral localization cues.

TU47. The Effect of Velocity, Duration, and Direction on Spatial Localization of Moving Sounds

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Category: Binaural Hearing and Sound Localization
Background: Moving sounds are common, and often important when operating as signals of impending danger or unexpected opportunity. Spatial and temporal properties of motion trajectories provide information regarding the location of the trajectory's offset. Although offsets are typically overestimated in the direction of the trajectory (representational momentum "RM"), they can also be underestimated (representational lag "RL"). Our previous work found that slower velocities (20 °/s) resulted in RM while faster velocities (40 °/sec) resulted in RL. However, it was unclear whether this effect could be explained by velocity or the duration component of velocity. Furthermore, RL was larger with rightward moving sounds than leftward. The current experiment aimed to replicate velocity and direction from our previous experiment while exploring the duration component of velocity.
Methods: Participants (n=30) heard eccentric virtual sounds moving towards midline ending at one of 7 offset locations (0° midline, ±4°, ±8°, ±16°). Participants made left/right judgments of the offset location relative to midline. The dependent variable was the point of subjective equality (PSE), calculated by a psychometric function (offset location x % right). Analysis examined factors of velocity (20°/s, 40°/s), duration (1s, 2s), and movement direction (leftward, rightward). Static sounds were also presented as a control. For each participant, PSEs for motion were corrected by subtracting the static PSE in the corresponding duration. Negative and Positive PSE values indicate RL and RM respectively.
Results: Results showed that sounds with shorter durations produced RL (M = -3.93°), while sounds with longer durations produced RM (M = +2.10°, p<.001). After controlling for duration, velocity still had an effect (p=.048) with 40°/s sounds producing RL (M = -1.57°), but not 20°/s sounds (M = -.26°). There was a significant effect of direction (p=.007) with RL in the rightward direction (M = -2.53°), but not in the leftward direction (M = +.70°. ns). There was an interaction between velocity and direction (p=.027). Both velocities that moved rightward show RL (20°/s: M = -2.43°; 40°/s: M = -2.62°), while leftward motion only had significant RM at the slower velocity (M = 1.92°).
Conclusions: These findings suggest that a minimum amount of time is needed for a stimulus to generate representational momentum; perhaps to predict where the sound is going or counteract attentional orienting effects of stimulus onset. An insufficient amount of time is associated with representational lag. Lastly, the directional asymmetry in spatial localization of moving sounds suggest that sounds moving across the left hemispace towards midline need more spatial information to generate representational momentum than sounds moving across the right hemispace.

TU48. A Model Framework for Simulating Spatial Hearing of Bilateral Cochlear Implants Users

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Category: Binaural Hearing and Sound Localization
Background: Bilateral cochlear implants (CIs) greatly improve the spatial hearing of CI users. However, substantial gaps in different aspects, such as localization, binaural unmasking, and speech understanding in challenging environments, still exist between bilateral CI users and normal hearing listeners. Multiple

aspects have been identified to limit binaural hearing with CIs in different ways, including various CI processing stages, the electrode-nerve interface, neural health, binaural interaction processes in brainstem neurons, as well as particularly complex and plastic cortical information decoding and decision making. However, it is often not possible to identify which of these aspects is causing which specific limitation, especially not in a quantitative manner. To help quantifying the role and interaction of these aspects, computer models can be employed. While models of isolated stages are often in good agreement with experimental data, their combination often does not necessary result in a quantitative and comprehensive simulation of perception with some kind of 'one-for-all' settings.
Methods: Here, we combine various CI processing strategies with physiological auditory model stages in a modular and open-source framework, resembling an artificial bilateral CI user. The stages include (a) a binaural signal generation stage with optional head-related impulse response filtering, (b) a CI processing stage not restricted to a specific manufacturer, (c) an electrode-to-auditory nerve model stage, (d) a binaural interaction model stage, and (e) a decision model stage. The framework allows for third party model extensions or substitutions. For demonstration and validation purposes, the framework was tested with three common psychoacoustic experiment setups: (1) detection of interaural cues in single- and multi electrode pulse trains, mimicking experiments with research platforms; (2) lateralization of pure tone, click, speech, and noise stimuli with different CI coding strategies, mimicking experiments with the CI processor's audio input; (3) the simulation of free-field localization with the CI processor's regular microphone input.
Results: In general, the bilateral CI model framework can simulate the average perception of bilateral CI users in all three exemplary experiment setups. This provides a starting point for more detailed investigations and other applications. Since the model framework deliberately was not tailored to one specific binaural research question, in order to reduce the gap between the model and an individual bilateral CI user, fine tunings or extensions with patient-specific and device-specific model parameters, and using other decision models are possible and necessary, but arguably not straight forward.
Conclusions: The framework offers a selection of coding strategies in combination with interchangeable model components of auditory pathway stages. Although the model was only demonstrated with lateralization and localization examples, it is possible to cover a wide range of binaural research topics. This work was supported by the European Research Council (ERC) Starting Grant No. 716800 to M.D.

TU49. Forward Masking of the Auditory Brainstem Response Differs Between Insectivorous and Frugivorous Bats

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Category: Brainstem: Structure and Function
Background: Echolocating bats rely on their auditory sense to support diverse behaviors, including navigation, obstacle avoidance, and foraging. This active sensing system requires precise temporal processing of sound, as echolocation calls may be emitted at rates as high as 150-200 sounds per second in the terminal "buzz" phase that precedes prey capture by insectivorous species. The high call repetition rate of insectivorous bats might therefore introduce forward masking effects that interfere with echo detection. However, bats may have evolved peripheral and brainstem specializations to prevent repetition suppression of the auditory response and facilitate detection of sounds separated by very brief time intervals. Here we assessed the time course of post-stimulus recovery of the auditory brainstem response (ABR) in two bat species: the frugivorous Carollia perspicillata which uses echolocation sequences to navigate, and the insectivorous Eptesicus fuscus, which uses echolocation to capture prey.
Methods: We recorded ABRs to broadband clicks and tones (4-84 kHz) in C. perspicillata (n=12) and E. fuscus (n=9) to generate audiograms. We additionally recorded forward masking ABRs to paired clicks presented at suprathrehold levels and at varying inter-stimulus intervals (ISI: 15, 12, 10, 8, 6, 5, 4, 3, 2 ms). We measured ABR wave amplitudes and latencies evoked by each click in the paired stimulus and compared response amplitudes to the first and second click of the pair as a measure of temporal masking. We evaluated response amplitude differences across ISIs within and among species using linear mixed-effects modeling.
Results: We observed significant species-specific effects of ISI on the amplitude of the ABR response to the second click in the stimulus pair. In E. fuscus, the ABR evoked by the second click in the pair was maintained at 80% of the original response amplitude, even when stimulated at ISIs less than 4 ms. E. fuscus response amplitudes to each click in the pair were significantly different only when stimulated at 2 and 3 ms

ISI. In contrast, C. perspicillata showed significantly reduced ABR amplitudes evoked by the second click when stimulated at ISIs less than 6 ms.
Conclusions: The two bat species demonstrated differential effects of forward masking in the ABR in which E. fuscus showed a shorter period of recovery from prior stimulation than C. perspicillata. These results indicate that reliance on high repetition rate echolocation sequences in insectivorous bats like E. fuscus may be supported by peripheral or brainstem specializations to reduce the time frame for post-stimulus recovery and enable responsiveness to sounds spaced by less than 6 ms, a critical time window for receiving incoming call echoes from the terminal buzz phase.

TU50. The Nuclei of the Lateral Lemniscus: Unexpected Players in the Descending Auditory Pathway
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Category: Brainstem: Structure and Function
Background: The processing of acoustic stimuli is modulated by feedback pathways that adjust feedforward signals in a context-dependent manner. All levels of the mammalian auditory pathway have been implicated in the top-down filtering of incoming information, with one notable exception: the nuclei of the lateral lemniscus (NLL). Contradicting this tenet, we have observed repeatedly that, following the injection of retrograde tracers into the superior olivary complex (SOC), numerous neurons are labeled in the NLL.
Methods: We have investigated systematically with retrograde tracers the projections from the NLL to the SOC of the rat. We made large injections of FluoroGold into the SOC to reveal to what extent NLL neurons contribute to descending projections, and focal injections of biotinylated dextran amine (BDA) to pinpoint the specific nuclei of the SOC innervated by each NLL.
Results: Our FluoroGold cases revealed that: a) The SOC is innervated by four nuclei or regions related to the lateral lemniscus (and collectively referred to as the NLL): 1. The ipsilateral ventral nucleus of the lateral lemniscus (VNLL). 2. The ipsilateral intermediate nucleus of the lateral lemniscus (INLL). 3. The medial paralemniscal region (PL) of both sides. And 4. The ipsilateral semilunar nucleus (SLN). The previously unrecognized SLN wraps around the INLL dorsally, medially, and caudally and consists of small, flat neurons. b) The dorsal nucleus of the lateral lemniscus and the sagulum are not significant sources of projections to the SOC. c) The nucleus with the most labeled neurons was the VNLL, followed by the PL. d) At least 30% of the neurons in the VNLL and INLL innervate the ipsilateral SOC.
Our BDA cases revealed that: e) The NLL innervate all SOC nuclei, except the lateral and medial superior olives. f) The ventral nucleus of the trapezoid body (VNTB), which receives projections from the auditory cortex and the inferior colliculus, is also the main recipient of the projections from the NLL. g) The VNLL targets preferentially the VNTB and the superior paraolivary nucleus (SPON). h) The main target of the INLL is the VNTB. i) The PL innervates bilaterally the dorsal ribbon of the SOC, which covers dorsally the SPON and the medial nucleus of the trapezoid body (MNTB). j) The main target of the SLN is the ipsilateral MNTB. SLN neurons are most likely excitatory. Therefore, this novel SLN-to-MNTB projection may represent the first known source of the non-calyceal inputs that activate principal MNTB neurons.
Our own preliminary data with mice and gerbils, along with data from the literature, suggest that the NLL-to-SOC projections are shared by other mammals.
Conclusions: These unexpected findings could have strong implications for the understanding of acoustic information processing in the initial stages of the auditory pathway.

TU51. Rapid FFR - Investigating a New Approach to Present and Analyse the Frequency Following Response
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Category: Brainstem: Structure and Function
Background: The FFR has many possible clinical implications, which is why its use in clinical settings (similar to the ABR) will likely prove advantageous. However, obtaining the FFR is time-consuming, with recordings to a single sound usually requiring around 6 minutes. We propose a technique that can drastically decrease acquisition time while still detecting a robust response.

Methods: To this end, we play a periodic steady-state sound continuously instead of interleaving shorter sounds with silent pauses. Additionally, rather than averaging across tens or hundreds of milliseconds of data as in the conventional FFR, we average across a single cycle of the stimulus. For example, the conventional FFR technique might average a 70ms stimulus 3000 times, with a 55ms break between each iteration, resulting in a 70ms ERP. In contrast, the rapid technique might present a steady-state vowel of 128Hz continuously for one minute, with the resulting ERP being only 7.8ms long. In this experiment, we use different steady-state vowels to illustrate the feasibility of the new technique in measuring the neural response to variations in spectral shape.
Results: Similar SNR values are obtained across the 7 lowest harmonics when comparing the rapid with the conventional technique. After investigating more deliberately if silences in between repetitions improve SNR values, we find no significant difference between stimulation with or without silences. Further, the measured F0 component benefits from 7.8ms averages (i.e. one-cycle averaging), but longer trial durations that lead to 62.4ms ERPs (i.e. 8-cycle averaging) yield higher or similar SNR values for higher harmonics compared to one-cycle averaging.
Conclusions: We are able to record similar response levels in two minutes with our rapid technique compared to six minutes in the conventional technique. This significant reduction in testing time is likely to make the FFR much more readily applicable in the clinic.

TU52. Differential Changes in Auditory Brainstem Responses After Cisplatin Treatment
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Category: Brainstem: Structure and Function
Background: The inferior colliculus plays an integral role in processing sound, including pitch discrimination, inhibition of auditory input, and sound localization. The long-term goal is to investigate if cisplatin induces anatomic changes in the inferior colliculus that can account for the audio-vestibular changes, such as hearing loss, vertigo, tinnitus and hyperacusis, seen in patients treated with cisplatin.
Methods: FVB mice were treated with cisplatin as in Fernandez et.al. (2019) and auditory function (ABRs and DPOAEs) tested before and 21 days after treatment. ABR thresholds, DPOAE amplitude, ABR wave I and V latency and amplitude were compared between saline and cisplatin treated group.
Results: Cisplatin-treated mice generally had normal ABR thresholds as saline-treated mice, except for a significant increase in thresholds at 32 kHz in cisplatin-treated mice compared to saline-treated mice. Cisplatin-treated mice with more weight loss tended to have higher ABR thresholds. There was no significant change in DPOAE amplitudes in cisplatin-treated mice compared to saline-treated mice. When cisplatin-treated mice were divided into either “normal” or higher ABR threshold groups, we compared the latency and amplitude of wave I and V between groups at 32 kHz. Cisplatin-treated mice with “normal” ABR thresholds had significantly greater wave V amplitudes, with reduced latencies, compared to saline-treated mice, while cisplatin-treated mice with higher ABR thresholds displayed no significant change in wave V amplitudes or latencies compared to saline-treated mice. There was a significant decrease in the interwave latency between waves I and V for cisplatin-treated mice with normal ABR thresholds compared to saline-treated mice, and no significant difference between cisplatin-treated mice with higher ABR thresholds and saline-treated mice.
Conclusions: We observed that cisplatin treatment can lead to changes in ABR waveforms in the auditory brainstem, as also reported following acoustic trauma. Intriguingly, we observed that a subgroup of cisplatin-treated mice with “normal” ABR thresholds had higher wave V amplitudes and reduced wave V latencies, indicative of hyperactivity, potentially due to reduced inhibition of the auditory input from the cochlea. This hyperactivity in the auditory brainstem prior to loss of ABR thresholds or DPOAEs could contribute to the tinnitus or hyperacusis reported in patients after cisplatin treatment. These changes in central auditory function may also be reflected in morphological changes in the auditory brainstem. If this hypothesis is supported in murine tissues, we will verify if these changes are also observed in human tissues.
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TU53. Activity-Dependent Synaptic Adaptation at the Developing Calyx of Held-MNTB Synapse
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Category: Brainstem: Structure and Function
Background: Early-life auditory input is an important driving force to shape the auditory neurocircuitry. It is known that loss of peripheral sound input at an early age causes both structural and functional deficits in the auditory pathway, even when peripheral sound sensitivity is reestablished, suggesting sound experiences during the critical period. However, the underlying mechanisms of how sound experiences modulate the auditory circuitry during this critical period remain largely unknown. The study aims to investigate how different sound environment modulates synaptic properties and plasticity of the auditory brainstem in juvenile mice.
Methods: Here, we performed ultrastructural analysis of the calyx of Held synapses using transmission electron microscopy (TEM) and whole-cell patch clamp recordings for examining excitatory synaptic transmission and plasticity in three groups of two-week old mice; normal hearing mice (WT), aged-matched mice that underwent extra sound stimulation (sound enrichment), and whirler mice with congenital deafness (Whrnwi/wi, sound deprivation).
Results: TEM image analyses show that the number of docked vesicles was increased in the sound enrichment group, but decreased in whirler mice, indicating the readily releasable pool size was changed by different sound inputs. We recorded excitatory postsynaptic currents (EPSCs) in MNTB neurons from three groups (P18-P21) and found an increase in paired-pulse ratio in the whirler mice and a decrease in sound stim mice when compared to WT.
Conclusions: This result indicates that the short-term synaptic plasticity at the calyx synapses dynamically changes in response to distinct sound experiences. Overall, the results show differential patterns of short-term synaptic adaptation among the three groups, providing some insights into activity-driven synaptic properties during the development that may be used to lay the groundwork in future therapeutic approaches for early-onset hearing deficits.

TU54. Neural Differentiation of Natural Speech Sounds in Humans
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Category: Brainstem: Structure and Function
Background: A distinguishing feature of the frequency following response (FFR), a predominantly subcortical auditory evoked response, is its ability to faithfully reflect stimulus features (fundamental frequency, harmonics, timing, etc.). To rigorously control these stimulus features, most previous studies have used synthesized speech to evoke the FFR. This synthesis enables focused comparisons between manipulated stimulus features, but it does not characterize real-world speech. In this study, we sought to determine whether FFRs to natural speech can also be dissociated by the features that differentiate each sound.
Methods: FFRs were recorded on 30 native English speakers (18 females) to 3000 repetitions (1500 per polarity) of six natural-speech syllables with varying spectra – four types of ‘da’ with different F0 trajectories (low-flat, high-flat, rising, and falling), ‘ba’, and ‘du’. FFRs to the opposing stimulus polarities were added and subtracted and averaged for each participant. Averaged responses were then fast-Fourier-transformed (FFT). FFTs to the added response reflect chiefly F0 encoding while subtracted FFTs reflect primarily harmonic encoding. FFTs were amplitude normalized (RMS) and run through a classification algorithm to determine whether responses could be differentiated by F0 and/or harmonic encoding.
Results: Classification analyses revealed that FFRs (both added and subtracted) to natural speech sounds are distinguishable from one another. Although both added and subtracted responses were effective in separating the neural responses to the different stimuli, the strength and biasing of the classification depended on the type of averaging (added vs subtracted). For the added FFRs, low F0 vs High F0 vs Rising/Falling F0 showed the greatest differences, while the subtracted best distinguished ‘a’ from ‘u’ responses which differed in their harmonic and formant information (but had the same F0).
Conclusions: Similar to FFRs evoked by synthesized speech, differences in natural speech features can be captured in the FFR and can be used to differentiate FFRs. These results highlight the feasibility of using natural speech in FFR studies. Natural speech tokens provide additional insights compared to synthesized speech because they more closely approximate the speech heard in everyday settings.

TU55. Ambient Sound Stimulation Tunes Axonal Conduction Speed by Regulating Radial Growth of Myelin on an Individual Axon-To-Axon Basis

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Category: Brainstem: Structure and Function

Background: Increased firing rates along axons following sensory maturation, skill learning or social engagement causes thicker myelination of active axons, while sensory or social deprivation results in thinner myelin sheaths. Current concepts about how axonal activity is communicated to myelinating oligodendrocytes involve vesicular or non-vesicular release of either glutamate or GABA from active axons, volume transmission and activation of oligodendrocytes to promote myelination, a well-suited scenario for fiber tracts where all axons myelinated by the same oligodendrocytes are equally active. Much less is known about adaptive myelination at the level of individual axons within a fiber tract. Neuronal firing in the auditory pathway is a combination of spontaneous activity and sound-evoked activity, with the latter starting at about P10-12 in mice. Mild and reversible sensory deprivation was achieved by raising mice with monaural earplugs from P10-P20. This does not only create an internal control to assess the physiological significance of adaptive myelination, but also allows the investigation of myelin regulation of individual axons in fiber bundles of mixed activity.

Methods: Changes in neuronal conduction speed were assessed by comparing auditory brainstem response (ABR) latency differences between both ears. In vivo single-unit recordings in the medial nucleus of the trapezoid body (MNTB) were performed and compared between control and deprived side. After sacrificing the mice, some brains were used to acquire electron microscopic (EM) images of the auditory nerve of the control and deprived side. Other brains received injections of different colored dextran dyes into the cochlear nuclei on both sides to track and analyze control and deprived auditory axons within the trapezoid body. Axon diameter and myelin thickness were measured in trapezoid body fibers using immunohistochemical labeling for neurofilament H and myelin basic protein.

Results: We found wave IV-I ABR latencies to be significantly longer when stimulating the deprived side, suggesting slower conduction speed through the auditory brainstem. This was corroborated by control fibers showing more radial growth of myelin compared to the deprived side. EM images revealed this radial growth being due to an increased number of myelin layers in control fibers. A general delay in development due to the earplugging was ruled out as MNTB neurons innervated by either the deprived or the control side within the same animals showed no differences in properties like synaptic delay, action potential half width and pattern of spontaneous activity, all known to normally change as a function of development.

Conclusions: In conclusion, our data suggest that radial growth of myelin in the auditory system depends on the level of sound-evoked activity and is not determined by the spontaneous activity, cellular identity of the neurons giving rise to the axons, nor the type of myelinating oligodendrocytes within the fiber tracts.

TU56. Sodium Leak Channel NALCN Underlies Noradrenergic Modulation of Cartwheel Interneurons of the Cochlear Nucleus

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Category: Brainstem: Structure and Function

Background: In the dorsal cochlear nucleus, feedforward inhibition from glycinergic cartwheel cells potently shapes the output of fusiform principal neurons. Previous studies (Kuo and Trussell, 2011) found that in cartwheel cells noradrenaline (NA) inhibited spontaneous spiking and the postsynaptic effects of that spiking, yet also enhanced evoked inhibitory currents in postsynaptic cells. These actions could be attributed to the effects of spontaneous firing on presynaptic depression. However, the identity of the effector channel underlying the inhibition of spontaneous firing by NA remained unknown

Methods: We developed a mouse line in which NALCN is deleted from glycinergic neurons by crossing NALCN flox/flox mice to GlyT2-Cre mice. Hemizygous offspring were crossed to NALCN flox/flox mice to obtain glycinergic neuron specific KO of NALCN. Patch-clamp recordings were made using K-gluconate or Cs based internal solution from cartwheel interneurons. Cell-attached (voltage-clamp) recordings were made using normal extracellular solution. For puff experiments, a small puffer pipette was placed near (100 µm) the soma of the recording cell.

Results: NALCN KO mice showed reduced spontaneous firing compared to WT mice. Puff application of NA to cartwheel cells resulting in outward currents (due to block of inward current) in WT mice but not in NALCN conditional knockout mice. GABA-B receptor activation by baclofen puff also resulted in an outward current. Such baclofen currents are usually attributed to GIRK K+ channels. Yet, in WT cartwheel cells, only 40% of outward baclofen current was blocked by the GIRK blocker Ba2+, suggesting an additional mediator of baclofen action. This mediator is likely NALCN, as 80% of outward baclofen current was blocked by Ba2+ in NALCN knockout mice. NA application hyperpolarized, and significantly increased firing threshold, in cells from wild-type mice but not from NALCN knockout mice. Finally, we isolated NALCN-mediated currents by blocking Ca2+, K+, and voltage-gated Na+ channels. NA or baclofen inhibited the isolated NALCN currents in WT mice. By contrast, in NALCN KO mice, there were no NALCN currents or effects of NA. Both modulators, NA and baclofen, appeared to act on the same population of NALCN, as application of baclofen completely occluded responses to subsequent application of NA

Conclusions: NALCN is a downstream effector of inhibitory G protein-coupled receptors in cartwheel cells. Specifically, the Na+ leak channel, NALCN, is the primary target of α2 NA receptor action and also mediates a large fraction of the effect of GABA-B receptors in these neurons. The sharing of NALCN by the two modulatory receptors suggests close apposition of both receptors with these channels.

TU57. Cochlear Contribution to Central Auditory Deficits in Animal Models of Fragile X Syndrome

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Category: Brainstem: Structure and Function

Background: Auditory processing deficits are common in neurodevelopmental disorders (NDs). Efforts to understand ND pathogenesis and develop treatment strategies primarily focus on the brain. The potential contribution of the peripheral nervous system has not been studied and considered for treating NDs. This knowledge gap is significant because the proper development and function of the brain depend on peripheral inputs from sensory organs during developmental critical periods.

Methods: Here we provide multiple lines of evidence in support of a cochlear contribution to Fragile X syndrome (FXS), a leading single-gene cause of intellectual disabilities with prominent sensory dysfunction. FXS results from gene silencing of FMR1 and loss of Fragile X mental retardation protein (FMRP), an RNA-binding protein.

Results: We first characterized the spatiotemporal distribution pattern of FMRP immunoreactivity in the inner ear of mice, rats, gerbils, and chickens. Across species, FMRP was expressed in hair/supporting cells and auditory ganglion (AG) neurons. While FMRP level in hair cells and auditory neurons in the brain was high at early postnatal ages and dramatically declined after hearing onset, AG neurons maintained strong FMRP expression into adulthood, implicating the unique importance of FMRP in AG development and function. To determine FMRP functions in AG neurons, we selectively knocked down Fmr1 expression in AG neurons of chicken embryos using in ovo electroporation of Fmr1-shRNA constructs. This autonomous FMRP deficiency led to compromised AG projection to the brain including aberrant axonal projection to the cochlear nucleus, defective synapse pruning, and delay in presynaptic machinery maturation. Consistently, Fmr1 knockout (KO) mice displayed altered signal transmission from the AG to the brainstem and reduced tonotopic precision in the ventral cochlear nucleus (VCN). In addition to alterations in circuit development, NDs are characterized by abnormal neuronal plasticity and shifted critical periods. We thus performed genetic and surgical deprivation of peripheral auditory inputs in wildtype and Fmr1 KO mice to investigate the effects of FMRP loss on deafferentation-induced neuronal loss and its critical period in the VCN. We found that the critical period was closed at postnatal 2 weeks in wildtype but not in Fmr1 KO mice, indicating a delayed closure without FMRP. Importantly, conditional Fmr1 KO (cKO) mice with selective deletion of FMRP in AG but not VCN neurons recapitulated this delay, confirming involvement of cochlear FMRP in shaping the temporal features of neuronal critical periods in the brain.

Conclusions: Together, these results identify evolutionally conserved, strong, and sustained FMRP expression in the auditory periphery which regulates the developmental pattern of the ear-brain projection and shapes afferent-dependent neuronal dynamics in the brain. Treating cochlear pathology might provide a potential avenue both necessary and promising for reducing the progress of auditory symptoms in FXS.

TU58. Cav2.1 Current Facilitation is Critical in Regulating Synaptic Transmission and Plasticity at the Calyx of Held/MNTB Synapse

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Category: Brainstem: Structure and Function

Background: Sound information encoding within the initial auditory processing stations requires reliable and precise synaptic transmission over a broad dynamic range of sound frequencies. In mammals, the calyx of Held/medial nucleus of the trapezoid body (MNTB) synapse is a critical synapse for encoding sound localization and temporal features of music and verbal communication. The calyx of Held, a glutamatergic presynaptic terminal that arises from the Globular Bushy Cell axon is the sole input driving action potential spiking in the MNTB. Cav2.1 is exclusively the calcium channel subtype in the mature calyx of Held terminal which controls the strength and dynamics of neurotransmission. The alternative splice variant Cav2.1 exon 37a in the calyx undergoes Calcium-dependent facilitation (CDF) while Cav2.1 exon 37b does not. CDF is hypothesized to be important for sustaining synaptic transmission to maintain the input firing rate needed for the encoding of auditory information.

Methods: Therefore, to test the role of Cav2.1 current modulation in the regulation of precise and reliable synaptic transmission, we expressed either Cav2.1 37a or Cav2.1 37b at the calyx of Cav2.1 knockout mice.

Results: we have observed no change in the amplitude of 1st EPSC and onset of EPSCs during high-frequency stimulation (300, 500 Hz), suggesting that neither the calcium current amplitude nor the coupling of Cav2.1 channels has changed when expressing either of the two splice isoforms. Importantly, calyces that express Cav2.1 37a showed facilitation of EPSCs which is comparable to that in wild-type calyces. However, calyces expressing Cav2.1 37b did not show synaptic facilitation, rather they increased the short-term synaptic depression.

Conclusions: We here identified distinct roles of calcium current facilitation in the auditory processing neurons, in which the Cav2.1 37a isoform is critical in supporting the natural facilitation of synaptic transmission thus maintaining firing rates over a wide range of sound frequencies. Results of this work and how the lack of CDF impacts the reliability and precision of naturalistic auditory stimulation patterns will be discussed.

TU59. Development of Precocious Avian Hearing: Frequency Specificity and Cross Species Comparison

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Category: Brainstem: Structure and Function

Background: Frequency specific processing is critical for survival, which is supported by the tonotopic organization of the auditory system. Development of the auditory system determines how spectral-temporal features of stimuli and vocalizations are encoded. Understanding the development of tonotopy in a variety of animal models can help explain the intricacies of frequency specificity and the factors necessary for successful development. Both the chicken embryo and chicken hatchling are excellent models for development, because of the chicken's precocious nature. At a molecular level, intrinsic properties of a neuron like resting membrane potential and ionic currents are near-mature at late embryonic stages. We previously have characterized the chicken hatchling auditory brainstem response (ABR) to click and tone burst stimuli. How this precocious development compares to other species is unknown.

Methods: We measured the click-evoked and tone-evoked ABR for 43 chicken hatchlings. Click-evoked ABR latency was compared to six other avian species and the mouse. Tone-evoked thresholds were compared to behavioral thresholds from adult chickens. To examine near-mature hearing function in embryos, we used whole-cell patch clamp electrophysiology to record from nucleus magnocellularis (NM), the avian analogue to the mammalian anterior ventral cochlear nucleus (AVCN). We sampled only from the caudal most area, termed NMc, to examine development at the lowest frequencies of the chicken hearing range.

Results: The chicken ABR was similar to other animal models. Despite a variety of recording parameters, the time of occurrence (latency) of Waves I, II, and III across six adult avian species and the mouse were all similar; the shortest latencies were seen in the mouse. However, tone-evoked thresholds for the chicken hatchling were higher compared to behavioral data in the adult. For the chicken embryo, NMc neuron development showed expected increases in ionic current for both depolarizing and hyperpolarizing stimulation. Surprisingly, high variability was seen at older embryonic ages, previously thought to be "near-

mature". This was true for both hyperpolarization activated current (Ih) and depolarization activated current (Ik).
Conclusions: These in-vivo and in-vitro results suggest that development extends beyond hatch for the chicken, perhaps in a frequency specific manner. Future studies should explore the development of intrinsic neuron properties in the chicken after hatch. These experiments would need to account for the acoustic and social environment of the chicken. Additionally, chicken ABR development hours or days before hatch could be compared to the hatchlings and juveniles of altricial avian species.

TU60. Case Studies Highlighting Use of Wideband Absorbance Measures in Pediatric Patients

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Background: Wideband absorbance (WA) measures in the adult population assist in characterizing mass- and stiffness-dominated pathologies, such as ossicular discontinuity and fixation. In the pediatric population, there are limited studies to characterize WA various congenital or acquired pathologies. Clinical measures performed to diagnose an otologic pathology include pure tone audiometry, tympanometry, acoustic reflexes, otomicroscopy, and imaging. For children, audiology results often yield an incomplete set of results due to limited patient participation, which affects the discussion about differential diagnosis and management options. WA, with its clinical feasibility and diagnostic sensitivity, may be an effective and desirable tool to aid in conductive hearing loss pathology differentiation. In cases of present CHL and tympanometry within normal limits, surgeons present options such as amplification, imaging, and surgery. Imaging, such as CT scans, has been found to have a range of sensitivities and specificities for confirming a diagnosis of a mass- or stiffness-dominated pathology (Chen, 2014). Without knowing the cause of a CHL middle ear exploration surgery might be recommended. Risks of anesthesia, surgical approach, co-morbidities, radiation, and untreatable hearing loss are considerations in this decision-making process. Moreover, surgical outcomes for individuals with ossicular fixation versus discontinuity are variable. Often, the most conservative pathway for effective management of CHL pathologies is preferred. As such, these authors sought to gather audiologic data with clinically available tools to explore suitability and applicability of WA on assisting in differential diagnosis of conductive pathology in the pediatric population.
Methods: Retrospective chart review was performed to gather patients who met inclusion criteria. Case studies highlight pure tone thresholds (audiogram), tympanometry, WA and CT scan written interpretation. Narrative of surgeon discussion for medical management and operative notes were also included for discussion. Ears were compared in the above areas for any differences in WA tracings. Case studies were selected from our clinical pediatric audiology population. The following clinical measurements were compared: pure tone audiometry (air and bone conduction), normal tympanometry, WA, acoustic reflexes, CT imaging, and intraoperative findings, when available. Patients were excluded if they had a history of prior ear surgery or significant history of ear infections.
Results: Chart review revealed multiple patients who meet the inclusion criteria for presentation. Patients had unilateral significant CHL, normal tympanometry, and variability in CT scan result interpretation. WA measures demonstrated significant differences when comparing ears. When comparing to the adult WA literature, measurements and morphology are consistent with pathologies such as ossicular fixation and discontinuity.
Conclusions: WA can show differences between individual ears as well as compared to normative data. WA is a clinically available measurement that can guide clinical decision-making and differential diagnosis in the pediatric population.

TU61. Open Board

TU62. Chatbot-Delivered Mobile Auditory Training Program in Experienced Hearing Aids Users: A Prospective Randomized Controlled Study

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Category: Clinical Otolaryngology and Pathology

Background: Auditory training (AT) is necessary to improve speech perception ability in patients with hearing loss. In this study, we evaluated the effectiveness of hearing rehabilitation with our novel chatbot-delivered mobile AT (CMAT) program on speech perception performance of experienced hearing aid (HA) users.
Methods: A total of 42 adult hearing loss patients who had worn bilateral hearing aids for more than 3 months were enrolled and randomly allocated to the AT or control group. In the AT group, CMAT was performed for 30 minutes a day for 2 months, and no intervention was provided in the control group.
Results: The AT group showed better improvement in word and sentence perception tests compared to control group ($p<0.05$). However, no significant difference was observed in phoneme and consonant perception tests. All participants were able to use CMAT without any difficulties and 85 % (17/20) of the AT group completed required training session.
Conclusions: Word and sentence perception performance were significantly improved in experienced hearing aid users after using the CMAT program. Also, CMAT showed high compliance and adherence over the two-month study period. Further investigations are needed to validate long-term efficacy in a larger population.

TU63. Impacts of the SARS-CoV-2 Virus on Chronic Otitis Media Complications

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Category: Clinical Otolaryngology and Pathology
Background: To compare the clinical features of Chronic Otitis Media (COM) complications of two-time cohorts of patients: "pre-COVID-19" and "COVID-19" pandemic period. The study aims to determine the effect of the SARS-CoV-2 Virus on the clinical course and complications of chronic otitis media.
Methods: Retrospective analysis of patients' documentation concerning procedures of COM complications at Izmir Ekol Hospital Otolaryngology Department. Analyzed database-pre-COVID-19 cohort (January-February 2019/2020): 1228 otological outpatient visits, 215 planned surgeries, and 15 urgent otological procedures; also COVID-19 cohort (March-April 2020/2021): 1042 otological outpatient visits, 345 planned surgeries, and 23 urgent otological procedures, respectively. Overall COM complications: 4 patients pre - COVID- 19 and 12 patients due Covid -19 pandemic period.
Analyzed outcome measures: sociodemographic and comorbidity anamnesis data, otological historical background, incidence proportion of otogenic complications with planned and urgent surgical procedures; incidence proportion of complications with the total number of emergency and planned outpatient consultations, and the total number of planned surgical procedures, general clinical and surgical evaluation, COVID 19 PCR testing background, prolongation of the recovery period
Results: There were 16 COM complications, 4 in the pre-COVID-19 and 12 in the COVID-19 cohort, including Subperiostal apse, Acute mastoid, Sever purulent otitides, dura defect with CSF fistula, and Facial paralysis. 90% (14) of these COM complicated patients were urgent and also planned operated. The cohort group patients also had Chronic otitis media anamnesis and COVİD-19 PCR positive background. There was no significant differentiation in gender, sociodemographic, and comorbidity anamnesis between the 2 groups. COM complications and urgent operation procedures were much more frequent in the COVID-19 period ($p < 0.02$), also prolongation the recovery period in the COVID-19 period ($p <0.01$).
Conclusions: It was observed that the complication of chronic otitis media was observed more frequently in the COVID-19 pandemic period than in the previous period. We think that there are many reasons for the progression of COM complications in patients infected with the Covid-19 virus during the pandemic period.
1. Patients cannot start the necessary treatment for COM in the early period, because of isolation
2. Since Covid 19 virus is a respiratory tract pathogen, it affects the Eustachian tube, causing further progression of ear diseases.
3. Covid-19 virus delays the clinical recovery of ear diseases by affecting the protection mechanisms of the general body.

4. We think that the anatomical and physiological properties of the middle ear structures are affected by the way of inflammation by means of hematogenous and direct contact (Eustachian tube reflux), which leads to the clinical worsening of COM disease and prolongs the recovery period in the post-intervention period.

The fact that all patients with complications had Covid PCR "+" infection proves that the SARS-CoV-2 virus negatively affects the clinic and recovery period

TU64. Long-Term Hearing Results Following Middle Fossa Surgery of Vestibular Schwannoma

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Category: Clinical Otolaryngology and Pathology

Background: Therapeutic options of small-sized vestibular schwannoma (VS) are watchful waiting, stereotactic radiosurgery and microsurgical resection. Small-sized VS confined mostly to the internal auditory canal might be considered for hearing preservation microsurgery using the middle fossa approach. The aim is to remove the tumor in full and preserve the hearing and facial nerve functions. This study presents the long-term hearing results in patients with sporadic VS after tumor resection performed with the microsurgical middle fossa approach at a single surgery unit.

Methods: 86 patients operated between 1998 and 2020, were retrospectively analyzed. Mean tumor size was 12.4 mm, including both extra- and intra-meatal parts. Pre- and postoperative data, including hearing results, were collected by medical chart review. Postoperative complications, including facial nerve function, were registered. Also, long term MRI follow up findings were documented and related to degree of hearing function.

Results: 55 (64%) of the patients had preserved hearing after surgery. The mean period between the surgical intervention and last available follow up audiogram were 8 years among these patients. 50 (58%) of the patients presented a tumor free status on postoperative follow up MRI. Only 3 patients (3%) required additional treatment and were referred to stereotactic radiosurgery. Facial nerve function was evaluated with the House-Brackman facial nerve grading system and 78 (91%) of the patients had preserved function postoperatively (HB I-II).

Conclusions: The middle fossa approach is an established treatment option for sporadic VS when the tumor is small, and the patient has serviceable hearing preoperatively. Stable long time hearing function is achieved to a high degree when the early postoperative audiogram shows serviceable hearing.

TU65. Longitudinal Deep Phenotyping of Hearing Instability Disorders – Clinical Protocol Structure and Initial Cohort Characterization

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Category: Clinical Otolaryngology and Pathology

Background: Clinical assessment of human diseases often starts with the identification of a phenotype, defined as an observable set of traits in a human disease. Diseases, including, but not limited to, Meniere's disease (MD), sudden sensorineural hearing loss (SSNHL), autoimmune/autoinflammatory inner ear disease (AIED), are characterized by either fluctuating and/or sudden changes in hearing, which can be termed hearing instability (HI) as a prominent phenotype. In some cases, HI disorders are characterized by endolymphatic hydrops, which is expansion of the endolymph-containing scala media, in the cochlea and vestibule. EH can now be visualized in vivo in patients utilizing contrast-enhanced delayed fluid-attenuated inversion recovery (CED-FLAIR) MR imaging. While treatments, both medical and surgical, exist for the vestibular symptoms that accompany some of these disorders, effective treatments for hearing loss in these diseases remain limited. The underlying pathophysiology of these diseases remain largely unknown with several theorized etiologies. Deep phenotyping of human diseases has been utilized to gain a better understanding of poorly understood diseases and to identify potential biomarkers and therapeutic targets in these settings. Recently, a deep phenotyping study has been initiated to study HI disorders utilizing a

combination of physiologic, immunologic, and magnetic resonance imaging-based investigations. In this presentation, we introduce this NIDCD-sponsored longitudinal deep phenotyping protocol at the NIH Clinical Center (NIH CC) and describe the phenotyping measures utilized to assess an initial cohort of HI patients. We characterize the baseline characteristics of an initial cohort of recruited patients.
Methods: Patients with presentations consistent with HI disorders were recruited for longitudinal deep phenotyping at the NIH CC. Recruited patients are assessed longitudinally during periods of both hearing instability and stability, respectively, over the course of 15 months. Deep phenotyping measures include audiometric and vestibular physiologic measures, CED-FLAIR MRI, and immunophenotyping of immune cells and peripheral plasma. Immunophenotyping measures include full spectrum flow cytometry (FSFC), single-cell RNA-sequencing (scRNA-Seq), and cytokine and proteome profiling of peripheral plasma.
Results: Longitudinal assessment protocol structure is presented with initial cohort of HI patients. An overview of phenotyping with audiometric and vestibular measures, as well as the use of CED-FLAIR MRI to both identify and quantify endolymphatic hydrops (EH) are detailed.
Conclusions: We present a structural overview of a deep phenotyping clinical protocol for HI disorders that is actively recruiting subjects. Deep phenotyping efforts may improve our understanding of the underlying pathophysiology of these poorly understood disorders.

TU66. Automated Classification of Middle- And Inner-Ear Mechanical Pathologies Based on Individual Acoustic Input Impedance and Audiogram

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Category: Clinical Otolaryngology and Pathology
Background: Machine-learning based automated classification method utilizing data from wideband acoustic immittance (WAI) and audiogram would lead to efficient initial diagnosis with higher accuracy and consistency than what has been available. WAI has been shown to have clinical value in the diagnosis of middle-ear mechanical pathology. Specifically, measured absorbance, which is derived from WAI, when combined with measured air-bone gap (ABG) has been shown to differentiate between stapes fixation (SF) and superior canal dehiscence (SCD) with a smaller classification error than ABG alone. The goal of this study was to determine whether an estimate of acoustic input impedance (Z), being potentially more informative than absorbance, could further reduce classification error.
Methods: WAI and ABG measurements in 70 pathological ears (SCD or SF) from a previous study were included in this study. |Z| was estimated for each ear by fitting parameters of an analog circuit model (that includes the impedances of the ear canal, middle- and inner-ear) for best agreement with individual WAI measurements. Cross-validation of logistic regressions was computed by randomly dividing the data in training (70%) and validation (30%) subsets. Overfitting was avoided by incorporating into the regression analysis a regularization that minimized the classification error across a 1000-fold cross-validation.
Results: Two-way classification of SCD and SF based on ABG+Z resulted in an error of 1.2% when averaged across the 1000 validation sets. For comparison, the corresponding error was 3.6% when the classification was based on ABG+Absorbance. When the classification was based on ABG alone the error was 7.1%, when based on absorbance alone was 23%, and when based on Z alone was 23%.
Conclusions: Generalization of these classification errors is limited by the specific characteristics of the data set used in this study, notably its size and definition of the observed pathologies. However, the very low error in classification within the context of the current data set suggests that the approach of our classification method using estimates of Z from WAI combined with ABG will aid towards a more accurate and efficient initial diagnosis.

TU67. Remodeling of Bowman's Glands in Hamsters Infected With SARS-CoV-2

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Category: Clinical Otolaryngology and Pathology
Background: The COVID-19 Pandemic has affected over 180 million people worldwide. One of its features with significant prevalence is anosmia. The current known mechanisms related to the anosmia

pathology mostly involve inflammation of olfactory clefts or Acute Respiratory Distress Syndrome (ARDS) which leads to damage to the olfactory epithelium or sensory innervations; however, little is known about how SARS-CoV-2 influences the olfactory epithelium's mucus secretions. Since our sense of smell is influenced by mucin production by the bowman's glands (BG), the purpose of this study was to analyze histological changes in BG of hamsters infected with SARS-Cov-2 which developed anosmia.
Methods: The histological changes in BG was characterized by quantifying the area of the BG. For this purpose, 32 Syrian Golden Hamsters were intranasally infected with SARS-CoV-2 alpha strain and 4 were administered phosphate-buffered saline as control. Paraffin-embedded nasal coronal sections were stained with Alcian Blue and Nuclear Fast Red. We verified the olfactory structures in the nasal cavity of the Syrian Golden hamsters, namely the main olfactory epithelium on the dorsal nasal conchae. The area of the BG was measured by tracing around the gland using NIH Image J software.
Results: Coronal Sections were analyzed at different days post-infection (DPI). The smallest BG area means were observed in the 2 DPI and 3 DPI groups. The largest area means of BG were observed in the 42 DPI group. ANOVA analysis showed a statistically significant decrease in BG size in the 2 DPI, 3 DPI groups compared to the 5, 8, 17, 21, 35, 42 DPI, and control groups (p-value < 0.05)
Conclusions: These findings suggest that SARS-CoV-2 infection significantly decreases mucin production at 2 DPI and 3 DPI. Additionally, the increase in BG area suggests that BG remodeling, and thus recovery, can be attained post-infection.

TU68. Clinical Features of Patients With Tinnitus in a Tertiary Institution in Colombia

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Category: Clinical Otolaryngology and Pathology
Background: Tinnitus is the perception of a ringing, buzzing, hissing or roaring sound in the absence of an external source. Several studies have been focused on the clinical characterization of tinnitus in Europe, Asia, and the United States, however, there is little data regarding the clinical features of tinnitus in Latin American patients. Herein, we performed a clinical and epidemiologic study in a tertiary institution in Medellín, Colombia using the European School for Interdisciplinary Tinnitus Research Screening Questionnaire (ESIT-SQ), which is a questionnaire widely used and translated to several languages that allow to identify onset, background, causes, critical situations that increase the tinnitus as well as the perception, quality, and location of the sound. This study is the first one in Latin America to perform a study using this clinical tool.
Methods: A cross-sectional study was conducted between October 2021 to April 2022 at Clinica Universitaria Bolivariana. The ESIT-SQ was applied to patients consulting to outpatient and in-patient settings who had or currently have tinnitus. Before the application of the questionnaire, all patients consented. This study was proved by the Institutional Review Board at Universidad Pontificia Bolivariana. A descriptive analysis was performed using STATA. Absolute and relative frequencies, mean and standard deviation were obtained.
Results: 146 patients participated in this study. Females (53.4%) were the most affected population. 101 patients presented tinnitus lasting more than 5 minutes over the last year. In this population, otitis media was the most common clinical antecedent (10%), followed by acoustic trauma (8.9%) and presbycusis (8.9%). Patients with tinnitus presented concomitantly headache (38.4%), followed by cervical pain (31.5%). Hypertension was mainly observed in the studied population (22.1%), followed by reflux (19.3%), dyslipidemia (14.5%) and anxiety (14.5%). When characterizing tinnitus, 44.9% of patients presented a progressive onset, while 33.7% indicated that presented sudden tinnitus. Intermittent (60.4%) and unilateral (39%) tinnitus were mostly reported, which are increased by silence, stress, anxiety, and high sound levels as coffee consumption. To date, 64.7% of patients do not receive any treatment. 27.7% of patients refer mil preoccupation about their tinnitus and 18.1% instead of their symptom, do not take care about it.
Conclusions: Unilateral intermittent tinnitus were mostly seen in patients at a Tertiary Institution. Some triggers may increase tinnitus such as beverages, stress, and anxiety. Even though, this symptom is common in this studied population, it is underdiagnosed and not commonly treated. Further follow-up should be done by healthcare providers to establish more appropriate diagnoses and treatment options.

TU69. Epidemiology of Gymnastics-Related Head and Face Injuries: Sex- and Age-Based Patterns Over the Past Two Decades

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Category: Clinical Otolaryngology and Pathology

Background: Gymnastics is a traditional, popular spectator sport that has been part of the Olympic Games since 1896. Since the Tokyo Olympics in 2020, the popularity of gymnastics in the US has been growing every day. However, there is minimal epidemiologic data on gymnastics-related injuries and the trends of these injuries across different age groups and between male and female performers.

Methods: Data were obtained from the National Electronic Injury Surveillance System (NEISS) 2001-2021 databases. Product variable was used to identify gymnastics-related cases, then head and face injuries were isolated using the Body Part variable. Data analysis and manipulation were performed using SPSS v27 (IBM, Armonk, NY) software, while GraphPad Prism 9 (GraphPad Software, San Diego, CA) was used for data visualization. Bivariate chi-squared analyses and binary logistic regressions were performed to describe the epidemiology of sports-related facial fractures.

Results: Analysis of gymnastics-related head and face injuries' frequencies in the US over the past two decades showed that 2019 had the highest number of injuries (n = 4000) while 2008 had the lowest frequency (n = 1512). The majority of gymnastics-related injuries are females (69.5%), while only 47.8% of admitted cases are females. Interestingly, the females' odds of being admitted was found to 61% lower than that of males (odds ratio = 0.39, 95% CI = 0.35 - 0.44, $p < 0.001$). In terms of age, high schoolers (13-18 yo) make up 28.6% of gymnastics-related injuries. According to the National Federation of State High School Associations surveys between 2001 and 2018, on average 9.9% of students participating in gymnastics are males. However, in our study, 25.1% of high schoolers' gymnastics-related injuries were males. Internal organ injury is the most prevalent type of injury (35.6%) followed by lacerations (20.1%). In term of location the majority of gymnastics-related injuries took place at a sport-related location (60.2%).

Conclusions: This study shows that even though the majority of gymnastics-related injuries are female, males with these injuries are more likely to be admitted. When the age group of high school students was analyzed, the results showed that males' representation in gymnastics-related injuries is higher than theirs in gymnastics participation. Further studies are warranted to identify the factors behind these trends and to analyze the outcome of these injuries.

TU70. Embryonic Inner Hair Cells Induce the Formation of Outer Pillar Vs Deiters' Cells

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Category: Development: Cellular/Systems

Background: The organ of Corti is divided into two compartments each with its own kind of hair and supporting cell (SC): IHCs with supporting IBCs, IPhCs and IPCs in the inner compartment (IC); OHCs with supporting DCs and OPCs in the outer compartment (OC). We have engineered mutants (Insm1 cKOs) in which nearly half of the embryonic OHCs transdifferentiate into IHCs, as well as mutants (Tbx2 cKOs) in which IHCs transdifferentiate into OHCs. By genetically switching the identity of IHCs and OHCs at various developmental stages, we can determine the effects of hair cell type in supporting cell identity.

Methods: We conditionally ablated Insm1 embryonically (with Atoh1-Cre), and Tbx2 at various embryonic to postnatal stages (with Atoh1-Cre, Gfi1-Cre, and Fgf8-CreER temporally induced by tamoxifen administration). We ascertained the conversion of OHCs to IHCs (Insm1 cKOs) and of IHCs to OHCs (Tbx2 cKOs) by immunohistochemistry. We examined the identity of the SCs in the various configurations by immunohistochemistry. We used explants to pharmacologically inhibit FGF signaling emanating from the supernumerary IHCs of Insm1 cKOs. We measured ABRs and DPOAEs in the various Tbx2 cKOs to functionally assess the IHC to OHC conversion (no ABRs) and their effect on the outer compartment structural integrity (reduced DPOAEs when affected).

Results: We find that OHCs in the inner compartment are not surrounded by outer compartment-like SCs (OPCs and DCs), whereas IHCs in the outer compartment are not surrounded by inner compartment-like SCs (IBCs, IPhCs and IPCs). However, we find that in the Insm1 cKOs, in which there are excess IHCs in the outer compartment, many DCs of rows 1 and 2 are replaced by OPCs. Inhibition of FGF signaling in explants of Insm1 cKOs prevents this DC to OPC conversion. Conversely, the embryonic conversion of

IHCs into OHCs by TBX2 removal resulted in a conversion of OPCs into DCs, a disorganization of the outer compartment and a reduction in DPOAEs. This effect is limited to the embryonic period, as the postnatal ablation of TBX2 in IHCs and their subsequent transdifferentiation into OHCs did no longer affect the ratio and disposition of OPCs and DCs. Consequently, these animals displayed normal DPOAEs (indicative of a normal outer compartment) despite absent ABRs (consistent with their lacking IHCs).
Conclusions: We conclude that the type of hair cell (inner or outer) does not induce the formation of the accompanying type of supporting cell (IBC, IPhC and IPCs vs DCs and OPCs). However, our results demonstrate an embryonic, non-cell autonomous role of IHCs in dictating the identity and distribution of outer compartment OPCs vs DCs. This role seems mediated by FGF signaling and is consistent with the pharmacological and genetic evidence that FGF8 secreted from IHCs induce POC vs DC differentiation.

TU71. The Role of Spontaneous Activity in Maturation of the Calyx of Held Nerve Terminal and Its Synaptic Target in the Medial Nucleus of the Trapezoid Body

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Category: Development: Cellular/Systems
Background: The formation of neural circuits in early development can occur independent of neural activity, but maturation and refinement is an activity-dependent mechanism. Intrinsic patterned spontaneous activity (SA) occurs in several brain regions during development, including the visual and auditory sensory systems. Interestingly, SA in these sensory systems occurs prior to the onset of external stimulation (in mice, ear canals and eyes open after P10), highlighting the importance of stimulus-independent activity during neural circuit formation. The calyx of Held (CH) is the primary terminus of globular bushy cells (GBCs), whose cell bodies are located in the ventral cochlear nucleus (VCN), and innervates principal cells (PCs) in the medial nucleus of the trapezoid body (MNTB). The CH:MNTB synaptic connection is utilized as a model system for studying the role of SA in neural circuit formation, in part because growth of the CH occurs rapidly (postnatal day (P)2-P6) resulting in mono-innervation, and key biophysical properties have been characterized. Previous manipulations to eliminate SA at the developing CH have involved genetic strategies that affect cochlear function, and may induce homeostatic compensatory mechanisms in GBCs.
Methods: Direct manipulation of synaptic transmission through viral vector mediated, rapid-onset expression of tetanus neurotoxin (TeNT) targeting GBCs was employed to silence activity at the CH:MNTB synaptic connection. Injections expressing TeNT were made in MORF3 reporter mice, which utilizes a stochastic translation switch for sparse labeling and a membrane-targeted GFP allowing for detailed structural analysis of the CH.
Results: Following unilateral high titer viral injections into the VCN at P0, mCherry fluorescence (co-expressed with TeNT) was detectable within 48 hours in CHs innervating the contralateral MNTB. Compared to non-transduced ipsilateral MNTB control recordings ($n = 9$), patch-clamp recordings from transduced P6 MNTB PCs ($n = 7$) showed a decrease in frequency (0.5 ± 0.2 Hz vs 3.5 ± 2.2 Hz; $p < 0.01$) and amplitude (60.1 ± 10.4 pA vs 65.2 ± 15.3 pA; $p = 0.047$) and increase in the decay rate (1.3 ± 0.2 ms vs 0.7 ± 0.1 ms; $p < 0.0001$) of spontaneous excitatory postsynaptic currents. MNTB PCs innervated by transduced calyces showed delayed transition from tonic to phasic firing (0% phasic/100% tonic vs 78% phasic/22% tonic), lower threshold current (65.7 ± 27.6 pA vs 186.7 ± 58.3 pA; $p < 0.001$), and increased input resistance (0.4 ± 0.2 GΩ vs 0.2 ± 0.1 GΩ; $p < 0.001$) compared to ipsilateral controls. Control mice at P9 have a CH collateral branch system that includes many short and long branches. Preliminary investigation of transduced calyces revealed reduced size of the CH and fewer collaterals.
Conclusions: This study highlights an important role for SA triggering rapid growth of the CH and the synchronous maturation of the MNTB PC physiological properties.

TU72. A ZnT3-HA Knockin Mouse to Study the Development and Plasticity of Zinc Signaling in the Auditory System

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Category: Development: Cellular/Systems

Background: Vesicular (synaptic) zinc is a vital neuromodulator for fine tuning synaptic transmission across many brain areas, including the hippocampus, amygdala, and the neocortex. Within the auditory system, synaptic zinc signaling plays a vital role in frequency discrimination, cell-specific gain modulation, and contrast gain control. Furthermore, synaptic zinc levels are modulated by sensory experience. This has been observed in the auditory system as synaptic zinc levels in the dorsal cochlear nucleus decrease after loud noise exposure. The vesicular zinc transporter, ZnT3, is responsible for moving zinc into vesicles for synaptic release, and the mRNA for ZnT3 is consistent with histochemical stains for synaptic zinc. Despite the importance of ZnT3 in the brain, conventional antibodies against ZnT3 have not been reliable in localizing and quantifying ZnT3 protein changes.
To overcome this limitation and to further understanding of mechanisms of auditory system signaling and plasticity, we recently designed and developed a mouse-line using CRISPR/Cas9, with the ZnT3 protein containing an epitope tag (ZnT3-HA). Using the more reliable anti-HA antibody, we performed immunohistochemical staining and confocal microscopy to localize and quantify changes in ZnT3 expression in the mouse brain across development and in response to noise exposure.
Methods: Using CRISPR/Cas9, a human influence hemagglutinin (HA) tag was knocked into the endogenous ZnT3 protein of C57Bl/6 mice. We used zinc autometallography (Timm stain) to confirm zinc and ZnT3-HA localization patterns.
To determine how ZnT3 expression changes throughout postnatal development, we measured ZnT3-HA immunofluorescence in the auditory cortex, auditory thalamus, inferior colliculus, and cochlear nucleus of mice aged postnatal day (P)0, P7, P14, P21, and P28.
To determine the dynamics of ZnT3 expression after noise exposure throughout the auditory pathway, we measured ZnT3-HA immunofluorescence in noise-exposed adult mice (P56).
Results: Critically, the ZnT3-HA immunofluorescence is consistent with Timm staining, which is known to preferentially label synaptic zinc, suggesting that this mouse-line is an effective tool for studying ZnT3 protein.
While ZnT3 is traditionally expected to be localized to synapses in adult mice, in brains from younger mice (P7), we observed strong ZnT3-HA staining in the cell bodies of these brain areas, suggesting a potential alternative role for ZnT3 in the still-developing brain. In noise exposed mice, we quantified the region- and layer- specific changes in ZnT3-HA expression.
Conclusions: Together, these data provide novel insights into the dynamics of ZnT3 protein expression both throughout development and after noise exposure. Such data will further our understanding of zinc signaling and plasticity mechanisms in both healthy auditory processing as well as pathology.

TU73. Investigating How Outside Signals Polarize the Morphogenesis of the Hair Bundle

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Category: Development: Cellular/Systems
Background: The hair bundle is a precise array of actin-based membrane protrusions (stereocilia) that transduce sound in hair cells. Graded height architecture across stereocilia rows is essential for hair bundle function, yet the underlying molecular machinery remains poorly understood. Inhibitory G proteins (GNAI) and their binding partner GPSM2 polarize hair bundle morphogenesis along both the planar and apico-basal axes. GPSM2-GNAI is first planar polarized at the lateral surface of nascent hair cells (the bare zone), and later enriched at the tip of immediately adjacent stereocilia only (row 1). This dynamic, dual localization is essential for proper stereocilia placement and for stereocilia elongation into the tallest row, respectively. Here we developed tools and a methodology to dynamically follow GPSM2 and GNAI3 outside and inside stereocilia. One of our goals is to ask whether protein accumulation at the bare zone serves as reservoir for specific trafficking to row 1, instructing its tallest identity.
Methods: We generated *Gpsm2*^{HaloTag} and *Gnai3*^{Egfp} knock-in mouse strains to track GPSM2 or GNAI3 proteins in neonate hair cells (P2) with subcellular and temporal resolution. HaloTag is a versatile hydrolase that permanently binds a variety of ligands. In this study, we use two cell-permeable ligands: 7-bromoheptanol (7Bro-Lig) as an unconjugated blocker and a conjugated fluorescent ligand (TMR-Lig). In addition to photobleaching, we established pulse-chase protocols to observe either the emergence of new signals (7Bro-Lig > TMR-Lig) or signal decay (TMR-Lig > 7Bro-Lig). Ex vivo, we explant *Gpsm2*^{HaloTag} or *Gnai3*^{Egfp} cochleae and either culture for various time intervals before fixation or perform time-lapse live

imaging. In vivo, we inject 7Bro-Lig in the posterior semicircular canal and fix the cochlea at various timepoints for TMR-Lig labeling.
Results: First, we verified that HaloTag-GPSM2 and GNAI3-Egfp localize normally at the bare zone and at stereocilia tips, supporting normal hair bundle morphogenesis and auditory function. Using *Gpsm2*^{HaloTag} explants, we titrated 7Bro-Lig (to block binding of TMR-Lig to HaloTag-GPSM2) and TMR-Lig (minimum concentration for robust signals). We assessed that competing 7Bro-Lig can prevent lingering intracellular TMR-Lig from binding to newly synthesized HaloTag-GPSM2. Having established conditions for pulse-chase, we quantified HaloTag-GPSM2 turnover at the bare zone and stereocilia tips. We obtained reasonably close values with fixed and live explanting approaches (t1/2 bare zone: 14.6h (fixed), 17.2h (live); t1/2 tips: 16.9h (fixed), 20.0h (live)), and in vivo via surgery (t1/2 bare zone: 19.1h). Currently, we are leveraging photobleaching using these tools to interrogate whether GPSM2-GNAI3 traffics directly from the bare zone, outside of the forming hair bundle, to stereocilia tips.
Conclusions: By generating tools and approaches to notably follow a discrete batch of GPSM2 protein in time and space, we are in a unique position to ask how outside signals dynamically influence hair bundle morphogenesis, and thus sensory function.

TU74. Chromatin Remodeling Protein CHD4 Regulates Axon Guidance of Spiral Ganglion Neurons in Developing Inner Ear

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Category: Development: Cellular/Systems
Background: Spiral ganglion neurons (SGN) are the primary afferent neurons in the cochlea that convey auditory signals from the sensory hair cells to cochlea nucleus in the brainstem. Improper function of SGNs results in sensorineural hearing loss. The chromodomain helicase binding protein 4 (CHD4) is an ATP dependent chromatin remodeler and a core component of the nucleosome remodeling and deacetylase complex that alters the epigenetic landscape. Missense mutations in CHD4 cause Sifrim-Hitz-Weiss syndrome (SIHIWES). Patients with SIHIWES show delayed development, intellectual disability, facial dysmorphism, and ear abnormalities including hearing loss. Although dynamic expression of CHD4 has been reported in many different inner ear cells, little is known about how loss of CHD4 function affects hearing loss.
Methods: To study the epigenetic function of CHD4 during SGN development, Neurog1(Ngn1) CreERT2 Chd4 conditional knockout (cKO) animals were generated. Cochlea tissues were collected and subjected to either immunostaining or single molecule fluorescence in situ hybridization (smFISH) to determine changes in protein or mRNA levels after Chd4 deletion. To clarify the molecular mechanism behind the phenotypes in Chd4 cKO animals, immortalized multipotent otic progenitors (iMOP)-derived neurons were used for CUT and Tag in order to identify genome-wide CHD4 binding sites. Cellular functions of CHD4 were inferred from CHD4 binding to nearby genes by performing gene ontology analysis.
Results: Cochleae from Chd4 cKO and control animals showed no macroscopic differences and Chd4 deletion did not dramatically affect SGN viability. However, SGNs from Chd4 cKO animals showed altered fasciculation of radial fiber bundles. In addition, the Chd4 cKO cochleae display improper turning of type II fibers. To understand the underlying molecular mechanism behind the observed phenotypes in Chd4 cKO animals, CHD4 specific binding sites from iMOP-derived neurons were used to infer SGN target genes. Gene ontology analysis unveiled significant CHD4 binding near genes involved in axogenesis, axon guidance, and ephrin receptor signaling pathway as well as ear development. We confirmed CHD4 binding at the promoter and enhancers of multiple ephrin and ephrin receptor genes. Finally, we employed primary SGNs and observed increased Epha4 mRNA expression after Chd4 deletion.
Conclusions: Taken together, the results of the study suggest that CHD4 is required for SGN axon guidance. Ablation of CHD4 chromatin remodeling activity alters gene expression of Epha4 and changes the stereotypic pattern of peripheral projections from spiral ganglion neurons.

TU75. Development of Efferent Innervation of the Cochlea – Diversity and Flexibility in the Murine Olivocochlear Circuit

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Category: Development: Cellular/Systems

Background: The cochlea receives direct efferent feedback from olivocochlear neurons (OCNs) in the superior olivary complex (SOC) in the brainstem. Medial olivocochlear neurons (MOCs) innervate outer hair cells (OHCs), but also synapse onto the peripheral fibers of the primary afferents in the cochlea, spiral ganglion neurons (SGNs). Lateral olivocochlear neurons (LOCs), housed in the lateral superior olive (LSO), innervate SGN peripheral fibers beneath the IHCs. OCNs have been implicated in protection from loud sounds and attentional modulation, and, importantly, their axons arrive in the cochlea when SGNs themselves are developing. Thus, OCNs are in a position to modulate auditory input at the earliest site of auditory activity and shape the developing cochlear circuit. However, lack of genetic access to this relatively small population of neurons has limited our understanding of their identity and development, which ultimately will help us understand their function.
Methods: We used single nucleus RNA sequencing of brainstems from Chat-Cre;Sun1-GFP mice to identify the transcriptional profiles of OCNs at two pre-hearing and one mature age: postnatal day (P) 1 (n=13); P5 (n=16), an important period for SGN synapse development and refinement; and P26-28 (n=32), when cochlear circuitry is grossly mature. Peptide expression was assessed in brainstems from hearing (TMC1+/-;TMC2-/-) and constitutively deaf mice (TMC1-/-;TMC2-/-) at pre- and post-hearing ages (P7 and P28). To analyze OCN wiring patterns in the cochlea, we sparsely labeled OCNs by administering low doses of tamoxifen to Ret-CreER;Igs7-GFP mice (n=46 axons, 9 mice of both sexes).
Results: Mature LOCs subcluster into two subtypes, distinguished by differential expression of a suite of neuropeptides. Peptidergic LOCs are restricted to the medial wing of the LSO, but their axons extend along the entire length of the cochlea, suggesting that despite their cell body position, their effects are unlikely to be strictly tonotopically restricted. LOC peptide expression is developmentally dynamic, increasing gradually across the LSO postnatally before becoming restricted to the medial LSO after hearing onset. Furthermore, peptide expression fails to restrict to the medial LSO in constitutively deaf mice, indicating that development of peptidergic expression in LOCs is activity-dependent. Using NPY levels to categorize peptidergic identity of sparsely labeled individual LOC axons in the cochlea, we found a diverse array of morphologies and connectivity patterns. However, there is little to no correlation between LOC axon wiring patterns and peptidergic identity.
Conclusions: Thus, despite having identified transcriptionally distinct subtypes, LOCs on the whole appear to innervate the cochlea opportunistically. Ongoing work is exploring the molecular logic and anatomical features of early wiring decisions of OCNs in the embryonic and neonatal cochlea to better understand how diverse efferent innervation patterns arise and how efferent and afferent wiring is coordinated in the developing cochlea.

TU76. A Nesprin-4/Kinesin-1 Cargo Model for Nuclear Positioning in Cochlear Outer Hair Cells
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Category: Development: Cellular/Systems
Background: Hearing loss is the most common sensory disorder, affecting approximately 6-8% of the world's population with a significant fraction due to genetic mutations. While most treatments today include amplifying hearing aids and cochlear implants, using an approach of viral vector gene therapy appears to be a promising biological strategy for rescuing hearing function. We previously reported rescue of hearing in the Syne4 model using AAV gene delivery. Nesprin-4, encoded by SYNE4, is a member of the linker of nucleoskeleton and cytoskeleton complex (LINC), and pathogenic variants in the gene lead to early onset and progressive hearing loss in humans. Syne4 deficiency in mice leads to nuclear mislocalization and cell death of the outer hair cells. Although exogenous delivery of Syne4 was sufficient to prevent nuclear mislocalization and deafness, it remained unknown how nesprin-4 mediates the position of the nucleus and which other components are involved in the process.

Methods: To explore and better understand the mechanism of nuclear positioning in hair cells, we validated the interaction domain of nesprin-4 and kinesin-1 using immunoprecipitation. We performed in vivo AAV gene delivery to test the function of nesprin-4 in which the kinesin-1 interaction domain has been mutated and its effect on nuclear positioning in hair cells. We further validated our results using gene editing on UNC-83, a functional homolog of nesprin-4, in C. elegans to examine its role in nuclear positioning.
Results: We found that the interaction between nesprin-4 and kinesin-1 is mediated by a motif of 4 conserved amino acids. Next, by using in-vivo AAV gene delivery, we showed that the nuclear position in outer hair cells entirely depends on this interaction, which is crucial for OHC survival and hearing. Likewise, UNC-83 interacts with Kinesin-1 via the same motif, disrupting it results in a nuclear migration defect.
Conclusions: Our work demonstrates the critical interaction between nesprin-4 and kinesin-1 for proper nuclear positioning in outer hair cells. In addition, our results show for the first time that outer hair cells rely on the microtubule cytoskeleton for nuclear positioning, emphasizing the utility of gene delivery in exploring fundamental aspects of hair cell biology.

TU77. Molecular Mechanisms of Type II Spiral Ganglion Neuron Development

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Category: Development: Cellular/Systems
Background: The molecular mechanisms dictating spiral ganglion (SGN) neuron growth and cochlear innervation must be determined, to understand normal auditory development. SGNs are bipolar neurons that relay auditory input to the cochlear nuclei after receiving glutamatergic input from mechanosensitive receptor hair cells. Type II SGNs represent a fascinating subdivision of neurons in the inner ear and have characteristics resembling nociceptors. Type II SGNs have a highly stereotyped projection pattern whereby they project past the IHCs, make a 90° turn toward the cochlear base synapsing with 10-15 outer hair cells (OHC). Core planar cell polarity (PCP) proteins have previously been shown to mediate type II SGN turning events, but whether additional axon guidance mechanisms are involved remains unknown. In this research, I am investigating axon guidance mechanisms that facilitate type II SGN guidance and OHC innervation.
Methods: I generated Efna3 and Vangl2 null mice carrying Neurog1CreERT2 and R26RtdTomato, permitting SGN sparse labeling. Conversely, Efna3; Vangl2 double knockouts (DKOs) are examined using anti-NF200. Using immunostaining and confocal imaging, I imaged and analyzed hundreds of type II SGNs in these models. In combination, 3D rendering in Imaris software was used to quantify type II SGN turning, branching and other growth and navigation characteristics.
Results: Immunostaining experiments using KO tissue to control for specificity, exhibited EPHRIN-A3 expression on the membranes of outer pillar and Deiters' cells of the cochlear epithelium. Compared to controls, Efna3 null mice showed a small, but significant increase in type II SGNs incorrectly turning toward the apex. Both Efna3 null and heterozygous mice showed increased proportions of type II SGNs with odd navigation behaviors (e.g., aberrant forking, misrouting). In addition, Efna3 nulls displayed decreased branch numbers, suggesting EPHRIN-A3 may normally serve as a positive growth cue. Once Efna3 mutant analysis was complete, Vangl2 mutants were examined in the same manner. As predicted, Vangl2 nulls displayed an immense rise in type II SGNs incorrectly turning to the apex. Vangl2 null and heterozygous cochleae both displayed an increased (but rare) number of type II SGNs possessing abnormal navigation behaviors, similar to Efna3 mutants. Vangl2 null cochleae also displayed a lower number of branches per fiber compared to control littermates, suggesting VANGL2 may also provide a positive growth cue. In ongoing experiments, Efna3; Vangl2 DKO mice will determine possible interactions between Eph/Ephrin and PCP signaling. Efna3; Vangl2 DKO type II SGN turning defects resemble Vangl2 nulls, suggesting the two signaling systems operate in a linear pathway. I am also using a cochlear culture preparation to examine the temporal aspects of EPHRIN-A3 on type II SGNs, with preliminary data showing growth cone collapse.
Conclusions: Taken together our findings suggest that Eph/Ephrin signaling may act downstream of PCP signaling to mediate type II SGN guidance during development.

TU78. Deletion of TgfBr1 Results in Extra Hair Cells in the Organ of Corti

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Category: Development: Cellular/Systems

Background: The organ of Corti (oC) is comprised of specialized mechanosensory hair cells and associated support cells arranged in a structurally complex mosaic that is necessary for auditory perception. In mammals, the oC is derived from the prosensory domain, a narrow patch of terminally differentiated precursor cells abutted by two regions of nonsensory cells, Kölliker's organ on the neural (medial) side, and the outer sulcus on the abneural (lateral) side. In the oC, the medial prosensory domain develops the single row of inner hair cells (IHCs) and the lateral prosensory domain separated by the tunnel of Corti develops the three rows of outer hair cells (OHCs). However, the signal pathways that mediate and maintain these boundaries and cellular specifications are largely unknown.
Recently, our lab showed that the transforming growth factor β Receptor 1(TgfβR1) is selectively expressed in cells within the lateral prosensory domain of the oC suggesting a possible role in the development of OHCs and/or Deiters' cells within this region. To examine in more detail the role of TGFβR1 in cochlear development, we sought to specifically delete the receptor during cochlear development.
Methods: To circumvent the embryonic lethality that occurs following germline deletion of TgfβR1, we generated two conditional knockout (cKO) mice using a floxed TgfβR1 allele in combination with two cre driver lines, Empty spiracles homeobox 2 (Emx2-cre/+) or Fibroblast growth factor 20 (Fgf20- Cre/+). Auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE) were used to assess auditory function in animals that reached adult age. In addition, standard immunohistochemical assays were used to examine morphology and cellular patterning of the oC structures.
Results: Emx2-cre mediated deletion of TgfβR1 resulted in embryonic lethality, while Fgf20-cre deletion generated viable animals. ABR and DPOAE assessments indicate normal hearing in Fgf20cre; TgfβR1-fl/fl mice. In addition, development of OHCs and Deiters' cells appeared grossly normal. However, a mild duplication of inner hair cells (IHCs) was observed in the apex of the cochlea. This duplication was evident as early as postnatal day (p)1.5 in Fgf20-cre; Tgfbr-fl/fl mice, suggesting a possible disruption in the medial boundary between Kölliker's organ and the prosensory domain.
Conclusions: The observed effects on the medial boundary of the oC are surprising given that the expression of TgfβR1 is restricted to the lateral portion of the prosensory domain. Possible explanations would be that the observed extra IHCs are a secondary effect of an outgrowth defect in the lateral domain as a result of the lack of TgfβR1, or that there is a genetic interaction between Fgf20 (which is heterozygous in Fgf20-cre/+) and TgfβR1. Future experiments will address these possibilities and provide further analysis of anatomical and physiological changes in the absence of TgfβR1.

TU79. Longitudinal Relationship Between Central Auditory Test Performance and Cognitive Function in Children Living With HIV

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Category: Development: Human Subjects
Background: Cognitive and literacy deficits may develop in children living with HIV (CLWH) even with modern antiretrovirals. Early markers of cognitive decline could be useful for targeting interventions. Central auditory tests provide simple, easy-to-perform measures of neurocognitive performance in CLWH. To use central auditory tests as a marker for cognitive and literacy function in CLWH, we need to determine the trajectory of developmental performance so that we can potentially assess predictive value. This study examines the trajectory of central auditory and cognitive test performance across age in a cohort of CLWH and HIV-negative children in Dar es Salaam, Tanzania. This will support central auditory model prediction of neurocognitive deficits in cross-sectional and longitudinal analyses.
Methods: 421 children (ages 3-10 years, 50% female) were administered central auditory and neurocognitive tests over the course of 5 years. Average number of observations (i.e. visits) for CLWH for HIV-negative children were 2.9 and 3.2, respectively. Central auditory tests included the Tiple Digit Test (TDT), the Hearing in Noise Test (HINT), and the Staggered Spondaic Word Test (SSW). Cognitive tests were from the Leiter International Performance Scale—3rd Edition (Leiter-3), which is a test of nonverbal intelligence and overall cognitive function. Non-linear functions were fit to the data (i.e. power, polynomial, and linear) to assess the best fit trajectory of performance across age. 3-D plots were also used visualize the tripartite auditory, cognitive, and age interaction. Linear mixed effect models were created using central

auditory function as predictors with Leiter-3 variables as outcomes with age as a factor. Differences in trajectory of CLWH and HIV-negative children were assessed across all central auditory tests.
Results: Non-linear and linear fits of central auditory function across age showed minimal differences in standard error and adjusted R2 between fits. Central auditory function and cognitive performance showed similar trajectories of improvement across age. The relationship between cognitive factors and specific central auditory tests varied; however, each was associated with at least one neurocognitive domain on the Leiter-3 across age. The TDT showed the strongest relationship to all Leiter-3 variables (all $p<.007$). The effect of increased age was also related to faster non-verbal processing speed and higher intelligence, but not for memory. Positive HIV status was strongly associated with slower processing speed and weakly associated with poorer nonverbal memory and intelligence.
Conclusions: Central auditory performance increased with age and non-linear fits did not offer significant advantages over linear ones. There were strong associations between central auditory function and cognitive performance across multiple neurocognitive domains independent of age. This longitudinal work is consistent central auditory tests tracking cognitive performance. Future work is focused on using central auditory function to predict neurocognitive deficits and potentially literacy in CLWH.

TU80. Structural Characterization of Mini-PCDH15 Engineered Proteins For Usher Syndrome Type 1F Therapy

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Category: Gene Therapy
Background: Hair cells, the sensory cells of the inner ear, carry actin-filled microvillus-like projections called stereocilia, which are interconnected by long protein filaments called “tip links.” Whether sound stimulation, change of head position, or linear acceleration, force applied to tip links enables hair cell mechanoelectrical transduction: the critical conversion of mechanical deflection into electrical signals our brain can understand. Tip links are formed by two pairs of extremely large Ca2+-binding proteins, cadherin-23 (CDH23) and protocadherin-15 (PCDH15), interacting tip-to-tip in a Ca2+-dependent manner. PCDH15 has 11 extracellular cadherin (EC) repeats interconnected by Ca2+-binding acidic residues that stabilize the protein. Mutations in the PCDH15 gene cause Usher Syndrome Type 1F, characterized by congenital hearing loss, balance deficit, and progressive blindness.
Adeno-associated virus (AAV) vectors have been shown to be efficient and effective for gene therapy. However, the PCDH15 coding sequence (>6 kb) is too large to fit in a single AAV vector. We have developed novel, shortened “mini-PCDH15” variants that retain key domains, but lack 3-5 EC repeats of the native protein, and thus fit in a single AAV. After deleting these EC repeats, we engineered new Ca2+-binding linkers (that are not found in nature) to connect the remaining EC domains. Some of the mini-PCDH15s are functional and rescued the balance deficit in newborn Pcdh15-ko mice and the hearing in Pcdh15fl/fl;Myo15-Cre mice. To be functional, we expect the mini-PCDH15s to recapitulate the PCDH15’s cis-dimeric architecture, which is yet to be demonstrated and iteratively optimized for further improving protein stability and durability of rescue.
Methods: We used site-directed mutagenesis and size exclusion chromatography coupled to multiangle light scattering, to evaluate cis-dimerization of mini-PCDH15s. In addition, we combined X-ray crystallography, negative stain electron microscopy, and molecular modelling to study their structures. Furthermore, we built structural models for steered molecular dynamics simulations to evaluate their elastic properties. Finally, we used differential scanning fluorimetry of purified mini-PCDH15s to evaluate their stabilities.
Results: Here, we present a structural and computational study of mini-PCDH15s — including the first X-ray crystal structure of an artificial EC3-EC7 linker that partially rescues hearing function and shows decreased Ca2+-binding occupancy and a bent structure. Our results show that mini-PCDH15s behave as dimers in solution, showing diverse conformational arrangement and stabilities, which might modulate binding to CDH23. In addition, we report low-resolution negative stain images, cryo-EM results, and molecular dynamics simulations that reveal the flexibility, rigidity, and Ca2+-binding occupancies of mini-PCDH15s as compared to the native PCDH15.
Conclusions: Our study provides the first structural design, characterization, and atomistic view of mini-PCDH15 variants that rescue hair-cell mechanotransduction in mice. This work promotes the use of a

rational, iterative, structure-based mini-gene approach to develop gene therapies for large cadherins and possibly other proteins as well.

TU81. An Integrated Adeno-Associated Vector Development Platform for Inner Ear Disorders

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[1]Sensorion SA

Category: Gene Therapy

Background: The inner ear is a diverse mosaic of highly specialized cells including sensory hair cells, supporting cells and spiral ganglion neurons, all of which play an important role in the process of hearing. More than half of the congenital non-syndromic deafness cases have a genetic cause that affect specific cell populations in the cochlea. Gene therapy is a promising avenue for those patients, but requires precise spatiotemporal transgene expression. AAV-based gene transfer shows great potential for the treatment of hearing loss, as they provide stable gene expression over long period of time in post-mitotic cells and are relatively safe to use. Furthermore, AAV cell-specific tropism and transgene expression patterns can be fine-tuned through AAV capsid engineering and the addition of regulatory sequences in the expression cassette. Development of new AAV gene therapy programs for hearing loss is dependent on the efficient screening of new capsids and vectors of interest targeting specific cell population within the cochlea, and on the subsequent production of the candidates to be evaluated.
Methods: Transcriptomic datasets, bioinformatic and computational tools were used to isolate regulatory elements present in genes that are expressed in unique cochlear cell populations and implemented to generate new cell-specific AAV expression cassettes. Targeted capsids modifications approaches were implemented to develop novel capsids for various inner ear indications by altering particular epitopes. We optimized an in-house research grade AAV production protocol yielding high viral titers compatible with in vivo transduction of mouse cochlear cells, with no need for specialized equipment such as bioreactors. The platform not only opens the possibility to produce multiple AAV vectors in parallel for the screening of novel capsids or new regulatory motifs for transgene expression, but also to validate them both in vitro, ex vivo and in vivo.
Results: Through this process, we generated two independent AAV libraries of 1/ new synthetics capsids encapsulating an eGFP expression cassette or 2/ AAV serotypes with reporter transgenes containing a variety of regulatory sequences. An initial screen was performed ex vivo by transducing rodent cochlear explants. Selected candidates were further validated by in vivo injection into the inner ear of rodents. With this integrated approach, we could screen for up to 20 expression cassettes or synthetic capsids per week, enabling us to quickly expand the molecular toolbox available to precisely target cells of interest in the cochlea. Tropism and transduction efficacy studies were completed by local tolerability studies, audiometry (includes auditory brainstem responses (ABRs),distortion product otoacoustic emissions (DPOAEs) recordings) and startle response tests.
Conclusions: In summary, we have developed an integrated AAV discovery platform that allow us to design new AAV vectors tailored for addressing a variety of inner ear pathologies and to test for their specificity and efficacy in vivo.

TU82. Efficient Study of Human Inner Ear Organoids by an Editing Reporter in Combination With Novel Lipid Nanoparticle (LNP) and AAV Delivery

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Category: Gene Therapy

Background: The lack of human inner ear tissue presents a major challenge to the development of treatment for inner ear diseases, including the diseases that lack animal models. The emergence of human induced pluripotent stem cells (hiPSCs) derived human inner ear organoids (IEOs), in which diverse inner ear cell types are produced, serve a valuable tool to overcome the obstacles. However, it remains a major challenge to evaluate IEOs primarily due to a lack of efficient delivery systems. Further, the rapid progress in editing technology has made it possible to edit organoids efficiently. However, it is difficult to analyze edited cells in the organoids due to the lack of a reporter system to monitor editing events in situ. Our goal is to

construct a reporter human iPS to monitor editing and test the use of lipid nanoparticles (LNPs) and AAV for the delivery of editing complex into the IEOs for efficient editing.
Methods: PiggyBac donor plasmid containing "CAG-STOP-sfGFP" cassette was delivered into a hiPSCs cell line along using PiggyBac system. Stable hiPSC-CAG-STOP-sfGFP cell lines were acquired by puromycin selection. Human IEOs were established by sequential modulation of signaling pathways to derive hair cells from the hiPSC-CAG-STOP-sfGFP cells. LNP complexed with Cas9 mRNA and sgRNAs and dual AAV carrying Cas9 and sgRNAs were transfected into differentiated human organoids, respectively. Editing was studied by GFP expression and NGS, together with inner ear cell markers.
Results: 1. We inserted "CAG-STOP-sfGFP" cassette into hiPSCs genome and demonstrated that spCas9/sgSTOP disables the STOP signal that leads to sfGFP expression in the iPSCs by editing.
2. We direct hiPSCs (hiPSC-CAG-STOP-sfGFP) into IEOs by mimicking embryonic development. The inner ear organoids expressed key markers of otic differentiation and developed hair cells. ESPN labeled stereocilia were detected in the organoid-derived hair cells. The IEOs further developed likely auditory neurons that were connected with the hair cells by potential synapses labeled with CtBP2.
3. We evaluated the delivery of GFP reporter gene by different AAV serotypes into the organoids. We observed AAV2 facilitated superior delivery efficiency compared to other serotypes. We studied the delivery by LNPs with the Cas9 mRNA and sgRNAs and by dual AAVs to edit the reporter IEOs. We showed that LNP and dual AAV mediated efficient delivery and editing in the inner ear organoid cell types including hair cells.
Conclusions: We have established a fluorescent reporter system in hiPSC-derived IEOs. We screened and identified AAV2 and LNPs as ideal vehicles to deliver Cas9/sgRNA complex into organoid cells including hair cells, resulting efficient editing. Our study is an important step towards the development of genome editing therapy, with a platform to screen novel delivery vehicles for diverse genome editing complexes.

TU83. Development of a Utricle Culture System for Exploring Dual AAV-Mediated Expression Kinetics of Human Otoferlin

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Category: Gene Therapy
Background: Otoferlin is a calcium sensor involved in neurotransmitter release from inner hair cells (IHCs) in the cochlea that is essential for proper communication with the auditory nerve. To address deficiencies in otoferlin, DB-OTO (AAV1Myo15-hOTOFv5) is a gene therapy designed to restore hearing function to patients with biallelic mutations of otoferlin. Because the coding sequence for the complete human OTOF (hOTOF) gene exceeds the packaging capacity of an AAV1 vector, DB-OTO is composed of two AAV1 vectors, DB-OTO-5 and DB-OTO-3, which encode the 5' and 3' components of hOTOF. When present together, the two vectors reconstitute a functional hOTOF gene cassette for expressing full-length OTOF protein isoform 5. While this technique has shown promise for restoring hearing in mice, little is known about the kinetics of dual vector recombination in target cell types.
Methods: To determine the kinetics of dual vector recombination in the context of human Otoferlin expression in hair cells, we developed an ex vivo culture system using utricles of adult Otof-Q828X mice, which contain an hOTOF nonsense mutation. We evaluate the utility of the utricle explant as an ex vivo assay for observing expression driven by dual vector recombination using dual vectors of AAV with a split GFP transgene driven by the Myo15 promoter. Furthermore, we establish methods to evaluate transcript levels of hOTOF in single utricles by reverse transcription quantitative polymerase chain reaction (RT-qPCR), as well as protein content by immunohistochemistry (IHC), and determine the length of time to detect full length, recombined hOTOF transcripts and protein.
Results: We observe specificity of the Myo15 promoter for expression of human Otoferlin in utricular hair cells. Furthermore, we observe that hOTOF RNA and protein can be detected in individual mouse utricles and expression of recombined hOTOF RNA and protein is dose-dependent. Expression of hOTOF RNA can be detected as little as 3 days post-administration and plateaus by 14 days. Expression of hOTOF protein is not detectable shortly after administration, but is detectable by 7 days post-administration and plateaus by 14 days.
Conclusions: Dual vector recombination provides a method to deliver large genes, but the timing and efficiency of transduction, generation of full-length mRNA, and protein expression with such a method have

not previously been described. We established the length of time to detect full-length hOTOF transcripts, the timing for transcripts to plateau, the timing to produce hOTOF protein, and the timing for protein quantity to plateau using an ex vivo culture system. An understanding of these kinetics would enable a better estimate for the timing of recovery of hearing in a human patient treated with dual vector AAV-based gene therapy and for the kinetics of dual vector AAV therapy in general.

TU84. Effects of Molecular Nano-Motor (MNM) -NOX3-DsiRNA- Delivered by Intracochlear Infusion in a Cisplatin-Induced Hearing Loss Model in Hartley Guinea Pigs

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Category: Gene Therapy

Background: Cisplatin, an antineoplastic drug widely used in the treatment of many cancers, causes permanent hearing loss. Cisplatin treatment is associated with an increase in reactive oxygen species (ROS) in the cochlea, leading to hair cell damage and apoptosis. NOX3, an isoform of NADPH oxidase linked to ROS generation, highly expressed in cochlea, is dramatically upregulated by cisplatin. Its knockdown by local administration of siRNA prevents cisplatin ototoxicity. However, due to their size and negative charge, the diffusion of the siRNAs into the cells is limited. Aposense's innovative and unique technology is comprised of novel Molecular Nano-Motors (MNMs), small molecules which are conjugated to dsiRNA and interact with the electric field inherent to all cell membranes to achieve transmembrane delivery of their nucleic acid cargo. Our study aims to demonstrate the innovative potential of using MNM-dsiRNA in hearing disorder therapies. Using intracochlear (IC) infusion, we studied (1) a comparative biodistribution profile between Cy3-dsiRNA and MNM-Cy3-dsiRNA in the cochlea; (2) the protective effects of dsiRNA and MNM-dsiRNA against NOX3 on cisplatin induced hearing loss (CIHL).

Methods: In the biodistribution experiment, Cy3-dsiRNA and MNM-Cy3-dsiRNA were intracochlearly infused for 24 hours in male Hartley Guinea pigs. The dsiRNA compounds were intracochlearly delivered using an Alzet® osmotic pump. Six hours after infusion, cochleae were sampled for flat surface or cross section preparations. The biodistribution of Cy3-dsiRNA (Group 2; n=6) and MNM-Cy3-dsiRNA (Group 3; n=6) was observed at the apex, mid and base of the cochlea, and targeted cells identified in comparison to naïve cochleae (Group 1; n=3).

For CIHL in male Hartley Guinea pigs, for 24 hours, an iPRECIO® pump intracochlearly delivered vehicle (Group 1; n=8), dsiRNA^{NOX3} (Group 2; n=6) and MNM-dsiRNA^{NOX3} (Group 3; n=4). Infusion started 24 hours before slow IP administration of cisplatin (10 mg/kg). ABR and DPOAE were measured prior to pump implantation and 3 days after cisplatin infusion (T_{+3DAYS}).

Results: In the biodistribution study, both flat surface and cross section preparations demonstrated greater biodistribution of MNM-Cy3-dsiRNA from base to apex and a better penetration in all cochlear cell types than Cy3-dsiRNA.

At T_{+3DAYS}, the CIHL demonstrated by lower DPOAE amplitudes and greater ABR thresholds was significantly prevented by MNM-dsiRNA^{NOX3}. No effect was observed after dsiRNA^{NOX3} treatment.

Conclusions: The MNM technology resulted in a greater base to apex biodistribution of dsiRNA and a substantially wider penetration of dsiRNA among cochlear cell types compared to naked dsiRNA alone. In addition, despite the lack of efficiency of naked dsiRNA^{NOX3} in our CIHL model, MNM-dsiRNA^{NOX3} demonstrated significant protective effects against cisplatin ototoxicity.

To conclude, MNM-dsiRNA constructs represent a promising and innovative technology to be used in hearing indications. Formulation development is needed to allow delivery into the middle ear.

TU85. Assessment of AAV-Mediated Innate and Adaptive Immunity in the Mammalian Inner Ear

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Category: Gene Therapy
Background: Adeno-associated virus (AAV) is a safe and effective viral vector that has been widely used in gene therapy studies. However, it has been shown that the host immune response to AAV may affect its potency, efficacy, and persistence. Following the recent FDA approval for AAV-based gene therapies for Leber's congenital amaurosis and spinal muscular atrophy, as well as the increasing number of proof-of-concept studies showing that gene therapy is effective at improving the auditory function in various mouse models of hereditary hearing loss, inner ear gene therapy is now closer to clinical application than ever before. Therefore, it is critical to evaluate the immune responses triggered by AAV in the mammalian inner ear in order to ensure the safety of AAV-mediated gene therapy applications. In this study, we examine the innate and adaptive immune responses triggered by AAV-mediated gene delivery in the mouse inner ear.
Methods: The B6.129P2(Cg)-Cx3cr1tm1Litt/J (CX3CR1GFP) mouse model is used for evaluating the innate immune responses. CX3CR1 is a fractalkine receptor expressed in immune cells. In cochlea, CX3CR1GFP/GFP mouse expresses eGFP signals in resident macrophages. We injected AAV2.7m8-CAG-TdTomato or vehicle into the inner ears of CX3CR1GFP/GFP mice using the posterior semicircular canal (PSC) approach between 8 to 12 weeks old. The activation of resident macrophages was assessed immunohistochemically at various time point and compared to non-surgery control by evaluating the GFP-expressing macrophages in cochlea. For evaluating the adaptive immune responses, we injected AAV7m8-CAG -GFP and vehicle into C57BL/6J mice at 8 to 10 weeks old. Neutralization assay was performed to assess the presence and quantity of neutralizing antibodies against AAVs produced by B cells. ELISpot assays were performed to assess IFNɣ secretion mediated by antigen-specific T cells against AAVs at 4 weeks after the injections.
Results: Macrophage activation was observed in Rosenthal's canal, spiral limbus, and Reissner's membrane of CX3CR1GFP/GFP mice at 14 days after AAV2.7m8-tdTomato injection. However, the number of resident macrophages did not change in the cochlear lateral wall. Neutralizing antibodies against AAV2.7m8-CAG-GFP were detected at 4 weeks after AAV2.7m8-CAG-GFP injection. Cellular immune responses against capsid peptide of AAV2.7m8 was not detected in 6 of 7 mice. Only 1 mouse showed a low positive response.
Conclusions: Inner ear gene delivery with AAV2.7m8 can trigger both innate and adaptive immune responses. Careful examination of the immune responses triggered by AAV is critical for the successful implementation of AAV-mediated inner ear gene therapy as a treatment for hearing loss and dizziness.

TU86. The Gear Portal - New Tools and Updates

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Category: Genetics A: Genomics and Gene Regulation
Background: The gene Expression Analysis Resource (gEAR) Portal continues to grow as a familiar resource within the hearing community as a tool to display, analyze and share multi-omics data. Now with over 1000 datasets and 1600 users, we continue to add features based on user feedback.
New tools and updates in the last year include a transfer learning implementation, cross-organism gene mapping, and a complete refactor of the data upload system. There have also been improvements in usability, documentation and increased resources to handle additional users and larger datasets.
Methods: The portal runs on Google Compute Engine resources, implemented as a web application via Python3, Javascript, HTML5, CSS3, the Scanpy module, and visualizations using D3.js, Plotly and Dash Bio. Data are stored in a combination of MariaDB and H5AD binary files.
Results: Transfer-learning techniques, such as ProjectR, enable feature mapping between datasets including cell classification and biological process annotation without a priori knowledge. Doing this with two datasets of the same species is relatively straight-forward, but orthology mapping techniques are required to support datasets from different organisms.
We have created bidirectional orthology mappings for five organisms used within the portal and in HDF5 format for rapid accession. The projectR tool uses this when projecting between datasets with different

organism sources so that genes can be included between them even if they have different gene symbols. This mapping functionality is also used in front page search capabilities for similar purposes. Finally, we have completely refactored how users upload their own datasets into the portal, with a focus on making this process easier while adding far more rich annotations and metadata to the uploaded expression matrices. Previously the upload steps involved filling out a spreadsheet and uploading a data package separately. Now, an interactive upload form which guides the users in describing their data properly. We have employed controlled vocabularies for fields which were previously free-form, and users will be able to decorate their data matrix columns with metadata following principles of the FAIR/TRUST initiatives. Better curation of incoming data will allow gEAR users to ask "show me all datasets containing inner hair cell data", for example, which isn't currently possible.
Conclusions: The authors wish to thank the entire hearing community for their support of the gEAR Portal and making it the primary repository for data exploration in the hearing field.

TU87. Leveraging Published Multi-Omic Data for Discovery and Catalysis of Inner Ear Research via projectR - No Coding Needed

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Category: Genetics A: Genomics and Gene Regulation
Background: The wealth of published multi-omic data identifies molecular signatures of cell types, cell states, and tissue types. These signatures are often derived from principal component analyses, marker genes, or other bioinformatic analyses, and stored as gene lists. However, is there a way for biologists to easily find these lists and then query their own and others data, quickly and efficiently, without requiring programming skills?
The projectR package (Sharma et al. 2020, Stein-O'Brien et al. 2019) enables in silico transfer learning experiments. Transfer learning is a technique used to apply patterns or features learned from one dataset to another. Here we describe our implementation of projectR in gEAR, to enable inner ear researchers to capitalize on the wealth of inner ear derived datasets and discover intersecting molecular dynamics across the various studies, samples, and cells - without requiring programming skills.
Methods: In order to use the projectR package for transfer learning in gEAR the input is a set of genes (genecart) representing the pattern signatures, or learned features, and a target dataset with overlapping genes. Existing gEAR tools, such as the comparison tool, the single-cell analysis workbench, and multigene viewer can be used to generate genecarts with weighted patterns.Passing the genecart of patterns returns a projection of pattern weights across the new target dataset samples. These patterns can be plotted against sample conditions from the target dataset to assess the learned dynamics from the original dataset to the new target data, even across species.
Results: As an example for the successful implementation of the projectR package, we performed a principal component analysis on a cochlea-specific scRNAseq mouse dataset. Here PC1 (see presentation/poster) exhibited the highest levels within the Crabp1+, Hair Cells, Medial Interdental, and Supporting cell types. To see if there are shared transcriptional elements in utricle cells we projected utricle data into the transcriptional space of principal component weights belonging to the genes associated with the cochlea dataset. Within the utricle target dataset, PC1 exhibited higher levels in the Non-Sensory Epithelial and Supporting cell types as well as the hair cells. The projection of these cochlear principal components show greater levels within the same specific cell types in the utricle, indicating that gene expression dynamics captured in the cochlear PCA is preserved across inner ear structures.
Conclusions: Integration of projectR into the gEAR platform will greatly aid researchers in the discovery of novel biological phenomena such as latent cell types, cell states across different sample types and across organisms.

TU88. TMC1 p.D569N Mutation Induces Tip-Link Aberrant Morphology and Outer Hair Cell Loss

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Category: Genetics A: Genomics and Gene Regulation
Background: TMC1 encodes transmembrane channel-like protein isoform 1 (TMC1), a major component of the hair cell mechano-transduction channel. Both dominant and recessive TMC1 mutations (associated

with DFNA36 and DFNB7/11, respectively), have been reported and in aggregate represent ~2% of genetic hearing loss. In this project, we focused on two dominant mutations – p.D572H and p.D572N – associated with postlingual and progressive sensorineural hearing loss. To decipher the mechanisms involved in this TMC1-associated hearing loss, we studied a Tmc1 mouse model with an orthologous mutation (D569N).
Methods: Auditory function was assessed at postnatal day p15, p21, p30 and p60 in Tmc1D569N/+ and wild-type (WT) mice by measuring auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE). Cochlear cell morphology was analyzed using scanning electron microscopy (SEM) and hair cell loss was quantified by immunolabeling methods.
Results: Juvenile Tmc1 D569N/+ mice (p15) displayed significant sensorineural hearing loss as evidenced by high-to-low frequency hearing threshold shifts and reduced amplitudes of DPOAEs. By p30, the hearing loss was progressed to profound across all frequencies. Morphologically, alteration of outer hair cell (OHC) tip-link morphology was seen resulting in elongated tips. Tmc1 D569N/+ mice also showed substantial OHC loss, greatest in the basal turn of the cochlea.
Conclusions: The early and rapid of progression of OHC loss suggests that for the Tmc1D569N/+ mice, gene therapy in the post-natal period may be challenging.

TU89. Integration of Single-Cell RNA-Seq Datasets for Hair Cell organs: Exploring Methods for Batching Experiments and Interspecies Comparisons

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Category: Genetics A: Genomics and Gene Regulation
Background: Hair cells are special mechanotransductory cells that are conserved in many species and importantly are the sensory receptors for the auditory and vestibular systems as well as the lateral line in fishes. While other non-mammalian hair cells such as in zebrafish have shown remarkable ability to regenerate from surrounding supporting cells, most mammalian hair cells cannot regenerate, and their irreversible damage is a main cause of sensorineural hearing loss.
Utilizing single-cell RNA sequencing, which provides an unprecedented opportunity to explore high-resolution gene expression profiles, we hope to compare gene expression of hair and supporting cells between mammals and non-mammalian counterparts. Although there has been an explosion of single-cell data that explore zebrafish and mouse hair cells, few studies have focused on integrating datasets, especially for cross-species comparison. In general, meta-analysis of single-cell RNA sequencing data has been limited due to computational limits although various methods have been proposed. Our goal is to explore the ability to combine and analyze multiple single-cell based datasets of hair cells and supporting cells of multiple sensory organs and model organisms including mouse and zebrafish.
Methods: We curated zebrafish single-cell RNA sequencing datasets from published studies that comprise the early developmental timeline from 4–7 dpf and adult from both the inner ear and lateral line. Similarly for mouse, datasets from E12.5-P2 and multiple adult timepoints (7 and 22 weeks) that include the cochlea and vestibular organs were included. We benchmarked existing methods for integration such as Harmony, BKNN, ComBAT, and scVI, and curated a homology table in order to conduct mouse and zebrafish interspecies comparison. Further data processing and manipulation was done via python through the anndata and scanpy packages
Results: Of the available methods, integration with the scVI method provided meaningful comparisons between different datasets from varying origins. A single map database of zebrafish hair and supporting cells was generated with both inner ear and lateral line neuromast data which contains over 47,000 cells from 15 independent experiments across development. These zebrafish data were compared by integrating with mouse single cell data. In this process, we provide a workflow and discuss common pitfalls in appropriately integrating multiple datasets, especially from different experiments and samples.
Conclusions: We demonstrate the ability to integrate and compare hair cell single-cell datasets from zebrafish and mouse. We benchmark tools and propose a workflow for appropriately conducting meta-analyses for single-cell data.

TU90. Chromosome 3q Amplification Promotes Tumorigenesis in Head and Neck Cancer by Suppressing Immune Programs

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Category: Genetics A: Genomics and Gene Regulation
Background: Tumor microenvironment is known to inhibit the host cell immune response to aid tumor cell survival by evading the immune activity. The use of immunotherapy in HNSCC patients as last resource after surgery, radiation, and chemotherapy is a possible treatment approach. However, reports suggest that approximately only 20% of patients respond adequately to immune checkpoints inhibitors as immune therapy.
Investigations on HNSCC patients have highlighted amplification of multiple genes in Chromosome 3q namely, ZNF639, GNB4, ACTL6A and PIK3CA as drivers of oncogenic events in both HPV-ive and +ive HNSCC. The present study evaluated the effects of mutations in amplified 3q region in HNSCC patients undergoing immune therapy and the effects of pharmacologically targeting genes present in 3q region to elicit an immune response in HNSCC cells.
Methods: The present study is a retrospective analysis of HNSCC patients who underwent immunotherapy and had molecular evaluation performed via next generation sequencing at Massachusetts General Hospital (MGH). All study protocols and procedures were approved from the Institutional ethics committee (IEC). Patients were divided based on response to immune checkpoints blockers (ICB) into four groups: Complete Response (CR), Partial Response (PR), Stable Disease (SD) and Progression Disease (PD). In our patient cohort, the most altered genes were identified. The drug/inhibitor treatment was used against the identified 3q locus targets in HNSCC cells. To evaluate the impact of the drug treatment on the immune response, we tested the gene expression of immune gene using qPCR by SyBr green method. The data was analyzed using Graphpad Prism software at a statistical significance threshold of 0.05.
Results: The present study cohort comprised of 91 patients who underwent therapy with ICB. Among them, 3 patients had a complete response, 22 patients were partial responders, 14 had stable disease, and 55 patients had a disease progression (non-responders to immunotherapy). The 4 most mutated genes observed in our cohort were TP53, PIK3CA, CDKN2A and TERT. PIK3CA is present on chromosome 3q locus along side ZNF639, GNB4, ACTL6A. All these genes have been previously reported as altered in HNSCC patients. Furthermore, in our analysis, we observed 27.2% of patients with PIK3CA alterations in responders' groups versus 32.7% of patients with altered PIK3CA in non-responders' group. A pan-PIK3CA inhibitor and alpha-specific subunit PIK3CA inhibitor were used against PIK3CA mutated and wild type HNSCC cells. The treatment with selected inhibitors showed increase in the expression of immune genes in HNSCC cells, suggesting a beneficial effect overcoming tumorigenic immune evasion.
Conclusions: The present study highlights that targeting mutations in 3q locus may have impact on the expression of immune response genes that may have further implications in the management of HNSCC patients undergoing immunotherapy.

TU91. Generation of Humanized Knock-In Mouse Model and Patient Ipsc Line for the TMEM43 p.(Arg372Ter) Variant Causing a Progressive Hearing Loss

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Category: Genetics B: General
Background: Hearing loss is the most common sensory disorders. Recently, mutations in the TMEM43 gene have been associated with hearing loss (HL). TMEM43 encodes a transmembrane protein that is associated with connexin-linked function in cochlear glia-like supporting cells (GLSs). However, the role of TMEM43 in the inner ear remains largely unknown and no therapeutic strategies have been developed for the treatment of, the TMEM43-assoacited HL. Animal models, such as mice, have provided invaluable information on inner ear development and HL. However, there are differences between animal and human gene sequences, that can influence HL susceptibility and complicate the development of therapeutic strategies, such as gene therapy approaches. In order to further investigate the mechanism underlying TMEM43-assocaited HL and develop therapeutic strategies, it is necessary to establish more accurate in vivo or in vitro models that more accurately represent, human genetics. To investigate the role of the TMEM43 p.Arg372Ter variant on the development of hearing loss and facilitate the testing of gene therapy-

based approaches, we are developing two complementary approaches – humanized mice and human induced pluripotent stem cell – based models.
Methods: A lymphoblastoid cell line (LCL) derived from a patient with the late-onset progressive hearing loss carrying the TMEM43 c.1114C>T (p.Arg372Ter) variant was used to generate iPSC line (UMi040-A). This line was, characterized for pluripotency, genome stability, and differentiation potency. In addition, a humanized knock-in (KI) mouse model of the TMEM43 p.(arg372Ter) was generated. Heterozygous mice bearing the human TMEM43 p.(Arg372Ter) variant were identified and confirmed by Sanger sequencing. Auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) tests will be performed on these mice over time to establish the impact of this variant on hearing.
Results: Successful reprogramming of the human lymphoblastoid-derived iPSC line was validated by immunocytochemical staining and qRT-PCR for several pluripotency markers. Genomic stability was assessed by q-band karyotyping showing no discernable genomic alterations during the reprogramming process. Loss of both the OriP/EBNA-based reprogramming construct and the EBV used to transform the original lymphoblastoid line was confirmed by PCR analysis. In addition, the differentiation potency of the iPSC line was validated using the Trilineage differentiation approach followed by ICC screening for markers of the three primary germ layers. Establishment of the humanized mouse model was confirmed by successfully identify heterozygous mice that had human TMEM43 bearing the p.(arg372Ter) gene integrated into the mouse genome by Sanger sequencing.
Conclusions: The establishment of both a patient-derived iPSC line and a humanized mouse model of the TMEM43 p.(Arg372Ter) variant will allow us to study the role of this variant on inner ear function and hearing loss, as well as provide complementary platforms for the development of novel gene therapy-based approaches to correct TMEM43-associated HL.

TU92. Open Board

TU93. Accelerated Cognitive Decline Associated With Hearing Loss and Bilateral Vestibulopathy: Insights From a Prospective Cross-Sectional Study Using the Repeatable Battery for the Assessment of Neuropsychological Status Adjusted for the Hearing Impaired in the DFNA9 Population
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Category: Genetics B: General
Background: DeaFNess Autosomal dominant 9 (DFNA9) is a hereditary disorder known to affect both hearing and vestibular function in its carriers. Its phenotype is characterized by a progressive Sensorineural Hearing Loss (SNHL) and vestibular dysfunction evolving towards Bilateral Vestibulopathy (BV) by the 3rd to 5th life decade. Recent studies have identified the impact of hearing loss and vestibular dysfunction on cognitive functioning.
The main objective of this study was to investigate how cognitive functioning of carriers of the p.Pro51Ser variant in the COCH gene is affected by the disease and compare these results with a matched healthy control group.
Methods: Forty-six carriers of the pathogenic p.Pro51Ser variant in the COCH gene were included in this study, of which 38 met the Bárány Society criteria and were thus diagnosed with BV. All subjects were between the age of 22 and 72 years old. Each control was individually matched based on age, gender and education level. A cognitive, vestibular and hearing assessment was performed in all subjects. All participants completed the Repeatable Battery for the Assessment of Neuropsychological Status, adjusted for the Hearing Impaired (RBANS-H), a cognitive test battery which includes subtests probing Immediate and Delayed Memory, Visuospatial/Constructional, Language and Attention.
Results: Overall, the DFNA9 patients demonstrated significantly lower scores on the Immediate Memory subscale and lower Total Scale scores than their healthy matched controls. The total sample was divided into two groups: age < 55 years old and age ≥ 55 years old. The DFNA9 group aged ≥ 55 years old obtained

significantly lower scores on the Attention subscale and lower Total Scale scores than their matched controls. Cognition of DFNA9 patients aged < 55 years old did no longer differ significantly from their matched controls.
Conclusions: This cross-sectional study found that DFNA9 patients demonstrated cognitive deficits in comparison with their healthy matched controls, especially in the Total Scale and Immediate Memory subdomain. The DFNA9 group aged ≥ 55 years old obtained significantly lower scores on the Total Scale and Attention subscale. This finding, however, was not observed for the age group younger than 55 years old. Further research is needed on the individual trajectory of SNHL and vestibular function, and how hearing rehabilitation affects cognitive functioning.

TU94. A Common FUT2 Variant is Associated With Differentially Expressed Genes and Shifts in the Nasopharyngeal Microbiotas in Patients With Otitis Media

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Category: Genetics B: General
Background: Otitis media (OM) is a leading cause of childhood hearing loss. Variants in FUT2, which encodes alpha-(1,2)-fucosyltransferase, have been previously identified in association with increased susceptibility to OM though the downstream effects of this variant on mucosal gene expression and microbiotas within the context of OM is unknown.
Methods: We examined gene expression in relation to carriage of a common pathogenic FUT2 c.461G>A (p.Trp154*) variant using RNA-sequence data from saliva samples from 28 patients with OM. Resulting differentially expressed genes were then used as input for network and pathway enrichment analyses. Expression of the genes identified by differential expression analysis was also examined in bulk mRNA and single-cell RNA-sequence data from wildtype mouse ME mucosa after inoculation with non-typeable Haemophilus influenzae (NTHi). Furthermore, microbiotas were profiled from ME and NP samples of 65 OM patients using 16S rRNA gene sequencing.
Results: In human carriers of the FUT2 variant, FN1, KMT2D, MUC16 and NBPF20 were downregulated while MTAP was upregulated. FN1, KMT2D and MTAP are connected within a single protein-protein interaction network of which pathways enrichment analyses revealed 27 significant pathways in KEGG and 21 significant processes in PATHER GO-slim BP. Post-infectious expression in the mouse ME recapitulated these transcriptional differences, with the exception of Fn1 upregulation after NTHi-inoculation. In the ME, Propionibacterium (nominal p=0.04) and Anoxybacillus (nominal p=0.02) were associated with variant carriage whereas Haemophilus (nominal p=0.03) and Moraxella (nominal p=0.02) were associated with wildtype FUT2 genotype. In the NP, Propionibacterium is also enriched (nominal p=0.01) in FUT2 variant carriers whereas Actinobacillus (nominal p=0.03), Selenomonas (nominal p=0.03) and Candidate Division TM7 was associated with wildtype genotype (FDR-adj-p=0.009). There were no significant changes in alpha- or beta-diversities in either the ME or NP.
Conclusions: Overall, the FUT2 c.461G>A variant was associated with transcriptional changes in processes related to response to infection and with increased load of potential otopathogens in the ME and decreased commensals in the NP. These findings provide increased understanding of how FUT2 variants influence gene transcription and the mucosal microbiota, and thus contribute to the pathology of OM.

TU95. Genotype-Phenotype of Usher Syndrome in a Diverse Patient Cohort

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Category: Genetics B: General
Background: Usher syndrome (USH) is a multi-sensory disorder that impacts patients' visual and auditory systems. Typical USH presentation includes retinitis pigmentosa, sensorineural hearing loss, and may include vestibular dysfunction. USH is divided into three subtypes, each with unique genotypic and phenotypic presentations. Onset of hearing and visual symptoms varies by USH subtype, making accurate

diagnosis critical for appropriate treatment. In this study, we review the genotype and phenotype presentations of USH patients in a diverse, multidisciplinary setting.
Methods: Patients of Bascom Palmer Eye Institute who were evaluated for USH were included in this review. A total of 198 USH patients were evaluated by ophthalmology for retinitis pigmentosa. Patients received genetic testing to confirm USH diagnosis. Test results were collected for genetics, ophthalmology, and audiology. Molecular Vision Laboratory, Invitae, GeneDX and multi-gene panels were used for the detection of nucleotide variations in coding exons and flanking introns in the USH genes by target enrichment (capture) and Next Generation Sequencing (NGS).
Results: Of the 198 patients who were reviewed, a total of 190 met the clinical diagnostic criteria for USH. All patients had a family history consistent with autosomal recessive inheritance. 58 of these cases have a conclusive molecular diagnosis by genetic testing. Of the 58 patients, 20.1% were diagnosed with USH Type 1 (n=12), 75.9% were USH Type 2 (n=44) and 3.4% were USH Type 3 (n=2). Among the USH1 patients, 9 (75%) patients had pathogenic variants in MYO7A. The most common variant in the MYO7A gene was c.1903 T>C (p.Cys635Arg) (4/18). We identified 2 USH1C cases (16%) and one USH1D case, who was found to have two presumably pathogenic variants in the CDH23 gene. Of the patients who were USH2, 88% (39/44) were found to have a pathogenic variant in the USH2A gene. The most common variant among USH2A patients was c.2299delG (p.Glu767Serfs*21) comprising 14/88 alleles. Two patients who were diagnosed with USH3 in our group were found to carry the c.144T>G (p.Asn48Lys) variant in a homozygous state. Of the patients with a molecular diagnosis, a majority (81%) self-reported hearing loss during the ophthalmology visit and had routine ophthalmology follow-up. However, only 19% of patients were evaluated at the University of Miami Ear Institute. As such, audiologic phenotypes could not be easily established.
Conclusions: Usher syndrome is a multi-sensory, heterogenous disorder that requires comprehensive, multi-disciplinary care. This study highlights the need for structured multi-disciplinary collaboration to ensure that both aspects of the sensory impairment are well managed. Further genotype-phenotype analysis of sensory systems in USH patients is necessary to define its natural history.

TU96. RFX2 Compensates for the Loss of RFX1 and RFX3 in the Vestibular System

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Category: Genetics B: General
Background: The group 1 RFX transcription factors (TFs), RFX1, RFX2, and RFX3, are master regulators of ciliogenesis. Our laboratory has previously shown that conditional deletion of both Rfx1 and Rfx3 (Rfx1/3;Gfi1-Cre) from hair cells (HCs) of mice results in profound hearing loss, an abrupt loss of all outer hair cells shortly after the onset of hearing, and a mild, late onset vestibular phenotype. However, Rfx1/3;Gfi1-Cre mutant HCs do not have kinocilia or planar cell polarity defects. Due to significant homology in functional domains, a similar role in ciliogenesis, and expression in both cochlear and vestibular HCs, we hypothesized that RFX2 functions to compensate for the loss of RFX 1/3 in inner ear HCs. Here, we investigated the compensatory role of RFX2 for RFX1/3 in kinocilia development and maintenance, as well as its function in the vestibular system. Additionally, we explored the signaling cascade downstream of the group 1 RFX transcription factors in the vestibular system.
Methods: We generated a triple conditional knockout (cKO) mouse of the group 1 RFX TFs (Rfx1/2/3 cKO), by crossing an Rfx2 knock out mouse model (Rfx2Gt) with our already existing Rfx1/3;Gfi1-Cre mouse. Vestibular function of Rfx1/2/3 cKO and littermate controls was measured using vestibular sensory evoked potentials (VsEPs) at 1, 3, and 6-months of age. Vestibular tissues of Rfx1/2/3 cKO and littermate controls were harvested from early postnatal ages until 6-months of age for assessment by immunohistochemistry and scanning electron microscopy. To unravel the transcriptional cascade downstream of the group 1 RFX TFs, we performed single cell RNA-sequencing (scRNA-seq) on the

sensory epithelia of pooled saccules and utricles of postnatal day (P)5 Rfx1/2/3 cKO and littermate controls using the 10X Genomics platform. Analysis of these datasets was conducted using Seurat v4.
Results: A loss of Rfx1, Rfx2, and Rfx3 together leads to a robust vestibular phenotype, with significantly elevated VsEP thresholds as early as 1-month of age that appear to progressively worsen as mice age. Additionally, vestibular kinocilia of Rfx1/2/3 cKO mice are normal during development (P10) but have an abnormal morphology with shortened height at 6-months of age, suggesting a possible role of Rfx1/2/3 in the maintenance of vestibular kinocilia. scRNA-seq analysis of P5 Rfx1/2/3 cKO and littermate controls show differentially expressed genes in vestibular HCs.
Conclusions: Our data show that RFX2 has a compensatory role for RFX1/3 within the vestibular system and that the group 1 RFX TFs have an essential role in vestibular function, possibly through vestibular kinocilia maintenance. Finally, we reveal part of the group 1 RFX signaling pathway in vestibular HCs, identifying candidate genes for maintaining vestibular function.

TU97. Dual Diagnoses of Genetic Hearing Loss Identified on Multigene Panels: Considerations for Clinical Care and Genetic Counseling

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Category: Genetics B: General
Background: Advances in genetic testing technology and increased awareness of its benefits in the diagnosis of hearing loss have made genetic testing a vital component of care for deaf and hard-of-hearing persons. Given the prevalence of genetic hearing loss and high carrier frequency of pathogenic variants in hearing loss-associated genes, comprehensive genetic testing may identify dual genetic diagnoses in deaf and hard of hearing persons. This presents a challenge for genetic counseling, and medical and audiological management. However, there is little data about the frequency and subsequent challenges in genetic counseling for dual diagnoses of hearing loss. Here, we present a series of cases with dual genetic diagnoses for hearing loss.
Methods: We used targeted genomic enrichment and massively parallel sequencing to screen all known genes associated with non-syndromic hearing loss and multiple syndromes in a large multiethnic cohort of approximately 6,000 probands. Following bioinformatics analysis to screen for single nucleotide and copy number variations, identified genetic variants were discussed in the context of the individual's medical and family history in a multidisciplinary meeting including clinicians, geneticists, scientists, bioinformaticians and a genetic counselor.
Results: We identified a series of cases of dual genetic diagnoses of hearing loss including probands with dual non-syndromic hearing loss diagnoses and probands with dual syndromic hearing loss diagnoses. For example, a proband with congenital profound sensorineural hearing loss was homozygous for the c.35delG in GJB2 (DFNB1) but also carried two in trans pathogenic variants in USH2A (Usher Syndrome type 2A). The DFNB1 diagnosis correlates with their profound hearing loss, masking milder hearing loss phenotype associated with pathogenic USH2A variants.
Conclusions: Our data highlights the complexity of genetic counseling and how the specialized training and knowledge of genetic counselors can: facilitate appropriate evaluation and pre-test counseling, provide prognosis, allow for education about inheritance and recurrence risk in a family, initiate familial variant testing for at-risk relatives, and ensure appropriate follow-up care for individuals who are deaf or hard-of-hearing and their families. Future studies can explore the psychosocial aspects of dual diagnoses on patients and families and their impact on medical and audiological management.

TU98. Audiologic and Genetic Findings in a Diverse Patient Cohort

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Category: Genetics B: General
Background: Hearing loss is a common heterogeneous sensory disorder that impacts patients across the lifespan. Non-syndromic hearing loss has been associated with more than 8,000 causative genetic variants, and the specific variant can impact the success with hearing loss treatment options. Yet, genetic testing is

not routinely administered when hearing loss is diagnosed, particularly in adults. In this study, genetic testing was completed in patients with known hearing loss to better understand genotype and phenotype presentations in genetic variants associated with hearing loss.
Methods: A total of 104 patients who were evaluated at the University of Miami Ear Institute were enrolled and received genetic testing. Information including genetic test results, audiologic evaluation, and hearing loss treatment were collected. The samples were first tested for the GJB2 gene and two recurrent GJB6 deletions (GJB6-D13S1830 and GJB6-D13S1854) given prevalence of GJB2 mutations. Simultaneously, the mitochondrial genome (mtDNA) was amplified using long-range polymerase chain reaction (PCR) and sequenced for 6 prevalent pathogenic mitochondrial variants. Samples negative for common deafness mutations were enriched for the complete coding regions and splice site junctions of the genes using a proprietary targeted capture system developed by GeneDx for NGS with copy number variations (CNV) calling (NGS-CNV).
Results: Of those 104 patients enrolled, 41 had a solved causative variant, 18 had one missing allele, and 45 yielded no genetic diagnosis. For patients with a solved causative variant, 26 were simplex cases and 15 were multiplex cases. Most patients presented with an autosomal recessive (AR) inheritance pattern (n=36), 27 of which presented with congenital hearing loss. Of those with AR inheritance pattern, variants on nine different genes were detected. 39% of solved cases were positive for GJB2 mutation (n=16) with c.35delG being the most common pathogenic variant. Five out the six c.35delG homozygous cases identified as Hispanic white and 1 identified as non-Hispanic white. 93% of patients with a solved causative variant were using hearing aids, cochlear implants, or a combination of both devices.
Conclusions: Known genotype-phenotype correlations are useful in understanding hearing loss presentation in certain homogenous groups, but clinical patient groups are often more heterogenous. In this diverse patient group, we explored genotypes and phenotypes in a multidisciplinary setting. In patients with solved cases (n=41), presentation was similar amongst Hispanic and Non-Hispanic groups, as well as males and females. This patient cohort had a disproportionate number of White patients, but several Asian and African American patients had solved variants as well. Despite the variety in race and ethnicity in this patient group, GJB2 remains the predominant solved cause for non-syndromic hearing loss. Other genetic variants accounted for only one or two cases per variant.

TU99. The Natural History of TMPRSS3-Related Hearing Loss: A Multi-Center, International Study
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Category: Genetics B: General
Background: There are over 120 genes known to be related to non-syndromic hearing loss (NSHL). TMPRSS3 is a high confidence NSHL gene that codes for a serine protease expressed by multiple cell of the inner ear, including supporting cells, hair cells, and SGNs. TMPRSS3 mutations are an important cause of NSHL, accounting for ~2% of cases. It is necessary to have well-powered, genotype-to-phenotype correlations to best understand the natural history of TMPRSS3 variants and their implications for care, decision making, and future clinical trials of gene therapy. Here we present the largest TMPRSS3 natural history study to date, involving 141 individuals from 10 institutions across 6 countries.
Methods: A request for data was sent to 14 institutions in six countries. This data included demographics, such as sex, race, and age at hearing loss onset. TMPRSS3 mutations were provided by cDNA, protein change, and locus identifier. Serial audiograms were collected for each participant, both before and after cochlear implantation. Individuals with variants in other known hearing loss genes were excluded from analysis. Individuals were assigned to three groups by the combination of missense (M) and loss of function (LoF, premature stop and splice variations) alleles: M/M, M/LoF, LoF/LoF. Individuals were also grouped by the protein domain in which specific variants occurred. We stratified by sex and race. Audiograms were averaged for each grouping by participant age to correlate specific genotypes and protein domains with phenotypes over time.

Results: We analyzed data from 10 institutions in 6 countries on 141 individuals with confirmed TMPRSS3 variants. 9 individuals were excluded due to variants in other known hearing loss genes. Grouping by genotype showed 66% M/M, 28% M/LoF, and 6% LoF/LoF. The SRCR domain carried the most variants of any of the TMPRSS3 protein domains. The population was 57% female. Audiologic phenotype analysis by genotype group, demographics and age, pre and post CI, and protein domain is ongoing and will be completed by the time of presentation.
Conclusions: All patients with TMPRSS3 mutation display severe to profound hearing loss. Understanding the natural history of the TMPRSS3 mutations is the first step towards developing interventions. To our knowledge, we have assembled the largest genotypic and phenotypic data set to date on TMPRSS3. M/M genotypes are the most common variations in TMPRSS3. Completion of the audiologic phenotype analysis will determine the severity and progression of each genotype, and analysis after cochlear implantation will determine the efficacy of CI for specific TMPRSS3 variants. This analysis will facilitate robust clinical care and decision making. The most commonly mutated protein domain is the SRCR domain. We will explore the molecular effects of mutations in this domain in the lab to better understand the function of the TMPRSS3 serine protease in hearing and HL.

TU100. Identification of a Novel Missense Mutation in the LCCL Domain of Cochlin Which Causes DFNA9

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Category: Genetics B: General
Background: Non-syndromic autosomal dominant hereditary hearing loss DFNA9 is associated with variants of COCH (coagulation factor C homology) gene. COCH encodes cochlin, which is expressed at high levels in the cochlea. Cochlin is composed of an N-terminal signal peptide, an LCCL (Limulus factor C, Cochlin and Lgl1) domain and two von Willebrand factor A-like (cWFA) domains. Almost half of reported COCH mutations that cause DFNA9 occur in the LCCL domain, which is proteolytically cleaved and may be involved with the innate immune response in the cochlea. In this study, we report a novel pathogenic variant of COCH segregating in a DFNA9 family.
Methods: We ascertained a 54-year-old woman with a history of progressive sensorineural hearing loss and a family history of hearing loss which follows an autosomal dominant inheritance pattern. Audiologic testing was performed on the patient and unaffected family members. Exome sequencing was performed to identify the genetic cause of hearing loss. We relied on in silico pathogenicity prediction software scoring, gene mutation databases, and molecular modeling of the protein structure to select for likely pathogenic variants in known hearing loss genes. To study the pathophysiology of the COCH variant, we used in vitro cellular systems and super-resolution microscopy to examine its effects on COCH protein trafficking.
Results: We identified a novel p.Val90Glu variant in COCH which is predicted to be pathogenic as the cause of DFNA9 in an American family. Molecular modeling showed a significant disruption of the local interacting network and folding of the LCCL domain because of the substitution of the hydrophobic and neutral valine residue at position 90 with a negatively charged glutamate residue. Additionally, immunocytochemistry in COS7 cells and super-resolution fluorescence microscopy of the trafficking pathways of wild type and mutant cochlin showed that the latter heavily accumulates in the endoplasmic reticulum (ER). Moreover, secretory vesicles carrying mutant cochlin were almost absent at the periphery of the cell, at the vicinity of the plasma membrane (PM), indicating that the mutant cochlin is likely sequestered in the ER compartment due to misfolding.
Conclusions: Our study uncovered a novel p.Val90Glu pathogenic missense variant of the cochlin LCCL domain which causes DFNA9 in an American family. This mutation is predicted to disrupt the structure of

cochlin, which leads to its sequestration in the ER, and thereby hinders its exit from the ER compartment towards the secretory pathway.

TU101. Novel SCN3A(NaV1.3) Mutant Found in One Family With Non-Syndromic Hearing Loss Showed Defective Channel Activity

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Category: Genetics B: General
Background: SCN3A(NaV1.3) is a candidate gene for deafness at the DFNA16 locus which maps to Chromosome 2q23-24.3. In inner ear, sodium channels are important for inner ear fluid regulation and cochlear response to electric stimulation. In recently performed whole-exome sequencing for 1 family with hearing loss, we found a novel mutation in SCN3A: a missense mutation (c.3614A>G; p.His1205A).
Methods: To examine whether the missense mutation have functional impact, we performed whole-cell patch clamp in HEK293T cells which were transiently expressed wild-type (WT) and mutant (p.His1-205Arg) SCN3A channel proteins. We also used western blot and immunohistochemistry(IHC) for detecting SCN3A channel proteins expressed in cochlear.
Results: Patch-clamp analysis demonstrated that p.His1205Arg mutant channels showed defective channel activity, such as decreased whole-cell currents, depolarized voltage dependence of activation, slowed down activation and inactivation at -30 mV. In addition, we detected SCN3A channel proteins expressed in cochlear by western blot. And from the results of IHC, these channel proteins were seemed mainly located to the hair cells and spiral ganglion.
Conclusions: SCN3A channel proteins are seemed to be located to the inner ear hair cells and spiral ganglion. And the novel SCN3A mutant found in family with hearing loss showed defective channel activity. These findings indicate that SCN3A channel dysfunction may contribute to hearing loss, and screening for this gene is important for prevention and treatment of this condition.

TU102. Big Data to Therapy: Precision Medicine for the Deaf

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Category: Genetics B: General
Background: Precision medicine is transforming our ability to treat human disease, as well as promoting health for individuals of all ages. The field of deafness is optimal for implementing precision medicine, as over 200 deafness genes are known worldwide, with correlations between phenotype, genotype and specific populations. These correlations are also reflected among the diverse Israeli Jewish ethnic groups. While next-generation sequencing (NGS) has rapidly advanced gene discovery, about half of inherited deafness still remains unsolved. Additionally, with the availability of electronic medical records (EMR) for deaf individuals, large numbers of hearing impaired patients are available to be used for improving personal treatment based on large-scale studies.
Methods: Utilizing the Tipa Biobank of 60,635 samples, including 3,807 hearing impaired, NGS is being performed on 1189 deaf from the Biobank, with audiograms documenting their phenotype, and the hearing loss diagnosed before the age of 60. For bioinformatics meta-analysis, we designed a unique pipeline for variant detection in a hearing-impaired population with very little background information, including structural variant analysis. Following variant detection by bioinformatics mega-analysis, pathogenic variants are being evaluated. New variants will be applied in audiology and genetics clinics, setting a guideline for precision genetic counseling and personalized audiological treatment and rehabilitation for deafness in Israel. In parallel, novel variants are being functionally investigated by CRISPR/Cas9 gene editing and gene therapy.
Results: Our designed unique pipeline for bioinformatics meta-analysis used for our initial NGS results of 506 samples yielded over 10% solved cases and additional 20% cases with inconclusive variants. All variants detected are known and novel variants in known deafness genes. Our next step is to expand the ability of our pipeline to detect variants in non-deafness genes on a big-data scale.
Conclusions: Solving the etiology of deafness in the diverse Israeli Jewish population in a large-scale study and finding genotype-phenotype correlations are the key for precision medicine for hearing loss, including diagnosis, prevention and treatment. This work can also be applied world-wide as a model study.

The research is supported by the Israel Precision Medicine Partnership Program.

TU103. Evidence of Tip-Link Breakage During the Mechanotransduction-Driven Stereocilia Remodeling in Inner Ear Hair Cells

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Category: Hair Cells: Anatomy and Physiology

Background: Inner ear hair cells detect sound and head movements by the deflection of stereocilia, the microvilli-like projections at their apical surface. These actin-filled sensory organelles are precisely organized in a staircase-like bundle, in which shorter row stereocilia are connected to their taller neighbors via tip-links. In mammalian auditory hair cells, the stereocilia cytoskeleton is maintained by a constant influx of calcium through the mechano-electrical transduction (MET) channels, which are located at stereocilia tips and partially open at rest by tip-link tension. We previously showed that blockage of MET channels in mouse inner and outer hair cells for 24 hours causes significant retraction (~200-500nm) in transducing (second and third row) stereocilia, yet tip-links and MET channel activity are still present (Velez-Ortega, et al., Elife, 2017). However, it is unknown whether tip-links are sliding down together with retracting stereocilia, or whether they constantly break and re-form during stereocilia retraction. Here we evaluated the presence of tip-links at several time points after the blockage of MET channels in mammalian and non-mammalian inner ear hair cells.

Methods: We cultured mouse organ of Corti explants as well as frog amphibian papillae and sacculi in the presence of MET channel blockers or vehicle controls. Then, we evaluated stereocilia morphology and tip link counts via scanning electron microscopy (SEM). We also recorded MET currents via whole-cell patch-clamp recordings, and imaged spontaneous bundle oscillations with a high-speed video camera system. We are currently evaluating tip-link composition via SEM and immunogold labeling against protocadherin-15.

Results: We have found that, similar to mouse cochlear hair cells, frog stereocilia can also retract after MET channel blockage. Interestingly, in the frog sacculus, tip links and MET currents were present 24 hours after MET channel blockage but significant tip link loss was evident at earlier time points (5-6 hours). Therefore, we examined the tip link integrity in mouse inner hair cells after 2 and 5 hours of exposure to MET channel blockers and again found a significant decrease in tip link counts. Furthermore, existing tip-links at these timepoints often appeared abnormally short but recovered their normal appearance after 24 hours of MET channel blockage, which is consistent with a two-stage mechanism of tip link regeneration that may involve a temporary tip-link made of protocadherin-15 at both ends (Indzhykulian et al., PLoS Biology, 2013).

Conclusions: Our data suggests that the MET-dependent stereocilia remodeling is a common phenomenon for hair cells of at least two different vertebrate classes. In addition, we found evidence that tip-links cannot easily slide down the stereocilia shaft and are likely to break and re-form during MET-dependent stereocilia remodeling.

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TU104. Transmembrane Channel-Like Proteins Regulate Membrane Viscosity in Mammalian Cochlear Hair Cells

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Category: Hair Cells: Anatomy and Physiology

Background: Auditory mechanotransduction occurs in the hair bundle, an organelle composed of rows of linked stereocilia that increase in height in a staircase-like manner. Hair bundle deflection exerts force onto the tip link that is translated to mechanically gated (MET) ion channels located at the tops of the shorter stereocilia. There is a limited but growing body of data suggesting that stereocilia membrane properties modulate MET channels. Stereocilia membrane are more fluidic than the cell body membrane and are selectively sensitive to calcium and voltage. Transmembrane Channel Like proteins (TMCs) are considered part of the MET channel machinery, but also have membrane scramblase activity that regulates membrane homeostasis in hair cells.

Methods: To further investigate the role of the membrane in modulating MET channel behavior, we used a novel viscosity sensor BODIPY 1c whose changes in fluorescence lifetime allowed precise spatial and dynamic monitoring of membrane properties within live hair cells. BODIPY1c can also enter hair cells through MET channels and fluorescently label the cytoplasmic membranes allowing us to identify hair cells with functional MET channels.
Results: We show that the membrane viscosity of stereocilia and soma of mammalian cochlear hair cells vary during development. Membrane viscosity decreases and strongly correlates with the onset of MET. TMIE and TMC1 mutant mice, both of which lack TMC1 in stereocilia have significantly higher membrane viscosity compared to litter mate controls at P10. In TMC1 mutants, the stereocilia membrane viscosity strongly correlates to the level at which the bundles are transducing. Inhibition of MET channel current in P10 rats for 30 mins with 1 mM curare, however, did not alter the membrane viscosity.
Conclusions: Together this data suggests that the membrane viscosity of the stereocilia and hair cell soma undergoes developmental changes that correlated strongly with the onset of MET even though MET current is not driving the decrease in membrane viscosity with development. Lack of TMC1 inhibits these developmental changes to the membrane. Further studies are ongoing to determine the functional relevance between MET channel gating and membrane mechanics.

TU105. Elevator-Like Movements of Prestin Associated With Electromotility

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Category: Hair Cells: Anatomy and Physiology
Background: The outstanding acuity of the mammalian ear relies on cochlear amplification, an active mechanism based on the electromotility (eM) of outer hair cells. eM is a piezoelectric mechanism generated by little-understood, voltage-induced conformational changes of the anion transporter homolog prestin (SLC26A5). Recent structural studies used cryo-EM methods to reveal distinct states of prestin that might be visited during eM.
However, the occurrence of different states relied on the presence of inhibitory anion substrates, raising the concern that these structures represent inhibited conformations rather than intermediates of the electromotile cycle. Also, the proposed voltage dependence of transitions between these states cannot be shown directly by cryo-EM approaches.
Methods: To resolve the voltage dependence of prestin's conformational landscape and thereby identify structural transitions underlying eM, we probed the dynamics of prestin under physiological ionic conditions and with control over the membrane potential by applying a combination of MD simulations, cysteine modifications, and voltage-clamp fluorometry (VCF).
Results: Equilibrium MD simulations of prestin revealed a conformational landscape that goes beyond but includes previous experimental structures, and identified putative extended and compact states. These conformations resembled inward-facing and occluded states of an anion transport cycle and transition predominantly involved a translational-rotational movement of the transport ('core') domain along the scaffold ('gate') domain. Probing with cysteine-reactive compounds confirmed dynamic transition between these states in electromotile mammalian prestin under physiological ionic conditions and identified an additional outward-facing state in transporting non-mammalian prestin. Finally, VCF with fluorophores attached to the transport domain allowed to directly monitor its translational dynamics. Importantly, VCF established that core-domain movement is voltage dependent, precisely matching the voltage dependence of electromotility, thus tightly linking the elevator-like reorientation with eM.
Conclusions: These findings not only provide direct functional evidence for an elevator mechanism for SLC26 transporters for the first time, but indicate that electromotility results from an elevator-like core domain reorientation in prestin.

TU106. Nanometric Localization of Stereociliary Proteins With Sub-Immunogold Scanning Electron Microscopy

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Category: Hair Cells: Anatomy and Physiology

Background: Many proteins crucial for detecting mechanical forces are located at or below the membrane of hair cell hair bundles. Determining the localization of these proteins at high-resolution within the hair bundle is instrumental for understanding their functions. Although immunofluorescence can confirm a protein's presence in the hair bundle, determining a more precise localization (for example: at tip-link insertion points, in shorter stereocilia, or along stereociliary heights) remains challenging. The most resolutive spatial protein localization is achieved by pairing electronic microscopy (EM) with the detection of gold bead-conjugated antibodies, known as Immunogold-EM. Immunogold Transmission Electronic Microscopy (TEM) requires tissue sectioning. Because the ideal tissue sectioning plane cannot be controlled at the nanoscale level, Immunogold-TEM is laborious and difficult to use for quantification purposes. Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) subverts the sectioning issue by sequentially milling and imaging an embedded sample. Unfortunately, the number of cells that can be imaged by FIB-SEM is constrained, limiting the significance of quantifiable results. Thus, there is a need for an imaging technique that provides EM spatial resolution and quantifiable protein detection from large sample numbers. Scanning Electronic Microscopy (SEM) paired with gold-labeled proteins could theoretically allow large numbers of cells to be directly imaged and quantified. However, while Immunogold-SEM has been used previously to localize epitopes external to the membrane, it has failed to detect intracellular ones. We have now developed a protocol enabling consistent detection of submembranous proteins with nanoscale resolution by SEM: SUB-Immunogold-SEM.
Methods: We identified roadblocks in SEM sample preparation protocols preventing submembranous protein detection. Then, we evaluated and optimized different alternatives addressing the roadblocked steps. We quantified and compared the hair-bundle distribution of several submembranous proteins with different distribution patterns and expression levels.
Results: We first evaluate our protocol's efficiency by quantifying actin-binding protein EPS8 labeling at the tips of the tallest stereocilia. Next, we resolve the non-homogenous distribution of calcium pump PMCA2 along the height of outer hair cell tall row stereocilia – low expression at their tapers, enrichment along their central heights, and low expression at their tops. Moreover, the low PMCA2 expression found in inner hair cells was also preserved, demonstrating the technique's sensitivity. We further emphasize SUB-Immunogold-SEM's sensitivity by detecting the few TMC1 molecules of the mechanotransduction channel complexes at the tips of transducing stereocilia. Finally, we exhibit additional applications of SUB-Immunogold-SEM, including the detection of CRE-recombined cells within an epithelium, the ability to perform dual-protein labeling, and its performance in multiple tissue types.
Conclusions: SUB-Immunogold-SEM represents a new alternative for the community to obtain the distribution of submembranous proteins in cells with exposed surfaces. The main advantages of this technique are nanometric localization, preservation of the cell surface, and ease of investigating large sample numbers.

TU107. Rictor/mTORC2 Regulates Inner and Outer Hair Cell Function via the Actin Cytoskeleton

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Category: Hair Cells: Anatomy and Physiology
Background: The mammalian target of rapamycin (mTOR) kinase is part of a key signaling pathway regulating many cellular processes, such as cell growth, metabolism and aging. Central components of the pathway are two multiprotein complexes, mTOR complex 1 (mTORC1) and mTORC2. Recent studies indicate an important role for mTORC1 in regulating cochlear hair cell death and regeneration. In contrast, the role of mTORC2 is less well known in the inner ear. Rapamycin-insensitive companion of mTOR (RICTOR) is the unique, defining and essential subunit of mTORC2. Rictor deletion disrupts mTORC2 function, among which is the regulation of the actin cytoskeleton. The aim of our study was to investigate the role of Rictor/mTORC2 specifically in the sensory hair cells of the inner ear.
Methods: We generated postnatal conditional Rictor knockout mice (Ric-cKO) by crossing Myosin15-Cre mice with Rictorfl/fl mice. Hearing function was assessed using auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) measurements. We performed in depth morphological analysis of the cochlear sensory epithelium using confocal microscopy, scanning (SEM) and transmission electron microscopy (TEM) as well as histologic analyses. Furthermore, we assessed vestibular function by behavioral tests.

Results: Ric-cKO mice suffer from an early-onset and profound hearing loss. Already at hearing onset, Ric-cKO have elevated ABR and DPOAE hearing thresholds. At 4 weeks of age, Ric-cKO show profound hearing loss and at 12 weeks of age they are completely deaf. While reduced DPOAEs indicate an outer hair cell dysfunction, there is no significant inner or outer hair cell loss at 4 weeks of age. SEM analyses show a degeneration of stereocilia at 12 weeks of age; however, with no difference on mechanoelectrical transduction channel function as shown by FM1-43 labelling. Synapse numbers were significantly reduced in inner hair cells at 4 and 12 weeks of age. The actin cytoskeleton has been shown to be important for synapse function in inner hair cells. Accordingly, using high magnification confocal microscopy we found a reduction in the actin cytoskeleton surrounding the inner hair cell synapses of Ric-cKO mice. In contrast to auditory function, vestibular function was not affected in Ric-cKO mice.
Conclusions: We show that the ablation of Rictor expression impairs the actin cytoskeleton, whose dynamic structural organization is crucial for stereocilia and synapse maintenance and function. Thus, the mTORC2 signaling pathway plays a central role in regulating inner and outer hair cell function. These results give us novel molecular insights on a central regulator of cochlear hair cell function.

TU108. In Vitro Reconstitution of Myosin 15 and Elongation Complex Proteins Required for Hearing
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Category: Hair Cells: Anatomy and Physiology
Background: In the cochlea, afferent neurotransmission in response to sound is mediated by the deflection of actin-rich protrusions known as stereocilia. Myosin 15 (MYO15A) is a molecular motor protein critical for establishing the size and shape of these mechanosensory organelles and its mutation causes human hereditary hearing loss, DFNB3. Myo15a is spliced to produce at least two protein isoforms, MYO15A-1 (aka MYO15A-L) that maintains the size of shorter transducing stereocilia, and MYO15A-2 (aka MYO15A-S) that is required for stereocilia elongation and assembly of the hair bundle “staircase” architecture. MYO15A-2 is hypothesized to stimulate actin polymerization at the growing stereocilia tips by delivering the elongation complex (EC) of proteins, WHRN, EPS8, GPSM2 and GNAI3. How EC proteins stimulate actin polymerization is unknown, however we recently reported that MYO15A itself can directly stimulate actin polymerization via its actin-binding ATPase domain. Here, we explore the hypothesis that the EC forms a regulatory complex to control MYO15A-2’s activity within the growing hair bundle.
Methods: We set out to isolate the active holoenzyme, consisting of the MYO15A-2 heavy chain (262 kDa), in complex with its accessory light chains. To attempt this complex expression, we used the biGBac system to package multiple gene expression cassettes encoding: a) FLAG-EGFP-MYO15A-2, b) the myosin chaperone UNC45A, along with light chains c) MYL6, d) MYL12B and e) CETN2 into a single baculoviral transfer vector using Gibson assembly. Full-length WHRN-FLAG and EPS8-FLAG were individually cloned into conventional baculoviral transfer vectors. Baculovirus was packaged using Sf9 insect cells, tittered, and used to express recombinant protein in Sf9 cells. Proteins were captured from cell lysates using FLAG affinity chromatography followed by size exclusion chromatography (SEC). Enzymatic activity was measured using ATPase assays and protein binding assessed using single-molecule mass photometry (MP).
Results: We successfully purified MYO15A-2 to homogeneity in equimolar stoichiometry with MYL6 (1:1), MYL12B (1:1) and CETN2 (1:1) light chains. MYO15A-2 was soluble, 99% pure by SDS-PAGE, and monomeric when assessed using label-free mass photometry. EPS8 and WHRN were also soluble when extracted from Sf9 cells and separately purified to > 99% homogeneity. Purified WHRN eluted in the void volume when analyzed by SEC, and appeared turbid, consistent with previous reports that WHRN undergoes liquid-liquid phase separation at high concentrations. Our results lay the foundation for future biophysical and structural characterization of a key complex required for stereocilia elongation. Experiments are ongoing to directly test if WHRN and / or EPS8 can regulate MYO15A-2 enzymatic activity and its ability to regulate actin polymerization.
Conclusions: Funded by R01 DC 011827.

TU109. Volume Regulation Drives Atypical Endocytosis During Damage Repair in Cochlear Hair Cells
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Category: Hair Cells: Anatomy and Physiology
Background: Exposure to aminoglycoside antibiotics causes damage and death of sensory hair cells in the inner ear, resulting in permanent sensorineural hearing loss. When cochlear cultures from early postnatal mice are exposed to a high dose of neomycin, rapid externalization of phosphatidylserine (PS) and extensive membrane blebbing occurs at the apical pole of hair cells (Goodyear 2008). Blebbing/ectosome formation and PS externalisation in hair cells have also been reported in response to benzamil and curare (Ballesteros 2022). Blebs as large as 5 µm in diameter occur as a result of neomycin treatment. Remarkably, following aminoglycoside washout hair cells rapidly repair this damage; blebs are internalized, and membrane lipid asymmetry is restored. Here, we aim to better understand the mechanisms by which hair cells retrieve these large areas of membrane during recovery.
Methods: A combination of live imaging, SEM, and TEM tomography was used to characterize the process of membrane internalization during this repair process. The fate of bleb membrane was tracked in the cell by confocal microscopy of the outer leaflet of the neomycin-induced membrane blebs, labelled with fluorescently conjugated annexin V; or by TEM, utilising electron-dense cationized ferritin to decorate bleb membrane prior to internalisation. Determinants of membrane retrieval were assayed by the presence of canonical endocytosis inhibitors, as well as channel blockers, during the recovery process. The presence and properties of volume regulated anion channels (VRACs) were determined by whole-cell patch clamp recordings of OHCs in cochlear cultures, in response to superfusion with hypotonic extracellular solution.
Results: Live DIC imaging reveals that large blebs located in the vicinity of the kinocilium rapidly decrease in size and are resorbed into the cell within the first 15 minutes of recovery. Initial membrane internalisation is not blocked by canonical endocytic pathway inhibitors, therefore membrane endocytosis is determined to be atypical. The internalization process was characterized at the ultrastructural level using serial section TEM tomography, indicating that this bulk endocytic process involves the formation of large multi-laminated structures consisting of multiple concentric membrane folds. The dispersal of membrane within the cell is actin dependent as it is prevented by the presence of jasplakinolide. Bleb retrieval was blocked by inhibiting VRACs with niflumic acid or DCPIB during recovery. Exposure to hypotonic solutions caused an outwardly rectifying current in OHCs that showed partial inactivation at positive membrane potentials, indicative of VRAC currents.
Conclusions: Sensory hair cells have an astonishing capacity for membrane internalisation and maintenance of the integrity of their apical surface. Neomycin-induced bleb retrieval is dependent on the activation of hair cell VRACs. Blebs are removed from the apical surface of sensory hair cells by rapid internalization of large areas of membrane by a previously undescribed macro-endocytic process.
Research funded by Sussex Neuroscience and RNID grant G101.

TU110. The Role of Human TMC Proteins in Phospholipid Distribution in Cochlear Hair Cells

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Category: Hair Cells: Anatomy and Physiology
Background: Phospholipids in the plasma membrane of hair cell stereocilia regulate sensory transduction current, adaptation and gating. Interestingly, pharmacological inhibition of the mechanosensory transduction complex affects phospholipid distribution across the inner and outer membrane leaflets, causing the loss of normal phospholipid distribution (Goodyear et al., 2008; Ballesteros and Swartz., 2022). TMC1 and TMC2 form the ion channel at the mechanosensory transduction complex from inner ear hair cells (Pan et al., 2013; 2018). The structure of TMC proteins is reminiscent of TMEM16 proteins, which are Ca2+-activated ion channels and lipid scramblases (Hahn et al., 2009; Ballesteros et al., 2018; Pan et al., 2018). Therefore, it was recently proposed that mouse TMC1 could function as a lipid scramblase at decreased intracellular Ca2+ concentrations and provide a pathway for lipid diffusion along their concentration gradient (Ballesteros and Swartz, 2022).
Methods: We investigated the role of human TMC1 and TMC2 in the externalization of phosphatidylserine, a lipid typically localized in the inner leaflet of the plasma membrane, in cochlear hair cells from Tmc1Δ/Δ; Tmc2Δ/Δ mice using viral delivery of hTMC coding sequences. We also studied phosphatidylserine localization in murine models harboring dominant and recessive Tmc1 mutations that cause hearing loss in humans and mice.
Results: We determined that expression of exogenous human TMCs can induce phosphatidylserine externalization in the apical membrane of live cochlear hair cells upon ion channel blockage. Additionally,

our results suggest that aberrant (but not absent) mechanosensory transduction can cause phosphatidylserine externalization without pharmacological channel blockage.
Conclusions: Consistent with prior data (Ballesteros and Swartz, 2022), we conclude that human TMCs can contribute to phospholipid homeostasis during mechanosensitive transduction dysregulation.

TU111. Phospholipid PIP2 and TMIE Regulate Slow Adaptation in Mammalian Hair Cells

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Category: Hair Cells: Anatomy and Physiology
Background: The mechano-electrical transduction (MET) process allows the transduction of mechanical information from sound and head movements into electrical signals. MET occurs at the hair bundle level and is triggered by stereocilia deflection. During a sustained displacement, the receptor current peaks and then decays, indicating a gradual decrease in MET channel open probability, a process that is called "adaptation". Adaptation shifts the operating range of the channel and might be necessary for preserving the system's sensitivity and filtering (Crawford et al., 1989; Eatock et al., 1987, Ricci et al., 2005). The slow adaptation process operates with a time constant on the order of 10-100 ms and requires Ca2+ entry through the channel and the activity of myosin motors, in particular Myosin1c (Myo1c) in the vestibular system (Holt et al., 2002; Yamoah and Gillespie, 1996; Caprara et al., 2020). Recently, we demonstrated that the mechanism of slow adaptation doesn't involve the upper tip-link insertion movement as hypothesized by the motor model (Caprara et al., 2020), questioning its molecular mechanism.
Methods: Using electrophysiological recording in mouse vestibular and cochlear hair cells, we tested an alternative hypothesis involving the activity of myosin, PIP2, and TMIE to regulate slow adaptation. PIP2 inhibition affects slow adaptation in bullfrog saccular hair cells (Hirono et al., 2004), and TMIE is an essential subunit of the MET channel and contains charged amino acids that mediate binding to phospholipids, including PIP2 (Cunningham et al., 2020). In particular, we hypothesized that PIP2 is the major player in the slow adaptation process, and it can directly bind the MET complex by binding TMIE. Instead, myosins at the tip of the shorter stereocilia have a secondary role by transporting and concentrating PIP2 to the MET channel proximity.
First, using a pharmacological approach, we tested if PIP2 is required for slow adaptation, and then we determined its interplay with Myo1c. Lastly, we tested if the slow adaptation was affected in the TMIER82C mice, where the binding of TMIE with PIP2 is disrupted.
Results: Our results showed that PIP2 is necessary to regulate slow adaptation. The addition of exogenous PIP2 rescues slow adaptation when Myo1c is inhibited, indicating that PIP2 is the key mediator of slow adaptation and Myo1c has a supportive role. Preliminary data in TMIER82C mice also showed a significant reduction in slow adaptation.
Conclusions: These results support a hypothesis that PIP2 is a direct mediator of slow adaptation and functions downstream of the Myo1c, which is likely responsible for transporting and concentrating PIP2 near MET channels. These data provide the first view of a revised molecular mechanism of slow adaptation in mammals, the key process that preserves the system's sensitivity and allows us to detect a wide range of sound intensities with extremely high precision.

TU112. Pou4f3-/- Utricular Hair Cells Take up FM1-43 and Show Decreased Sensitivity to Aminoglycosides

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Category: Hair Cells: Anatomy and Physiology
Background: The transcription factor POU4F3 turns on in hair cells at the onset of differentiation, and its expression is maintained into maturity. Mice lacking functional Pou4f3 lose all cochlear hair cells by early postnatal ages, are deaf, and exhibit clear signs of vestibular dysfunction. However, recent studies have shown that, in contrast to earlier reports, hair cells are present in the vestibular epithelia of both Pou4f3-/- and Pou4f3DTR/DTR adult mice. While these Pou4f3mutant hair cells do have rudimentary hair bundles, their functionality has not been tested directly. We therefore sought to assess their functionality using in vitro assays for the presence of mechanotransductive (MET) channels and to determine their susceptibility to neomycin, an ototoxic aminoglycoside that enters hair cells through MET channels.

Methods: To assess the presence of MET channels, control and Pou4f3-/- utricles from adult mice were exposed utricles to the fluorescent dye FM1-43 or neomycin-conjugated Texas Red (neo-TR). As a negative control, BAPTA was used to disrupt tip links prior to exposure to either FM1-43 or neo-TR. The caspase-detecting assay CellEvent was used to assess the extent of caspase-mediated cell death in response to neomycin exposure.
Results: Pou4f3-/- utricular hair cells take up both FM1-43 and neo-TR, although the overall level of loading for both appeared lower by comparison with heterozygous littermates, suggesting that the bundles retain some degree of function. Interestingly, the extent of cell death in response to neo-TR exposure was significantly reduced by comparison with littermates. Whether this is a result of the decreased uptake of neo-TR or a novel resistance to neomycin remains to be determined.
Conclusions: Our work shows that, despite having significantly shortened stereocilia, Pou4f3-/- utricular hair cells retain some mechanotransduction activity. In addition, these hair cells appear to be less susceptible to neomycin-induced cell death. Future studies will more fully characterize the physiological state of Pou4f3-/- hair cells through electrophysiological recordings and transcriptional profiling.

TU113. The Conductance and Organization of the TMC1-Containing Mechanotransduction Complex in Auditory Hair Cells

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Category: Hair Cells: Anatomy and Physiology
Background: Hair cells of the inner ear convert acoustic stimuli into electrical responses through activation of mechanoelectrical transducer (MET) channel. Each MET channel is activated by tension in a tip-link extending from the side wall of one stereocilium to the tip of the adjacent shorter stereocilium, where the channel is located. Transmembrane channel-like protein 1 (TMC1) is thought to form the ion-conducting pore of the MET channel, assisted by accessory subunits TMIE, LHFPL5 and CIB2. Based on cryo-EM studies, LHFPL5 is most likely connected to the C-terminus of PCDH15 at the lower end of the tip link. Knowledge of the organization of the channel complex is still incomplete, but a structure based on homology with TMEM16A indicates 10 transmembrane (TM) domains and is confirmed by cryo-EM of the nematode TMC-1.
Methods: Using single channel analysis and ionic permeability measurements, we characterized six missense mutations in the purported pore region of mouse TMC1.
Results: MET currents could be recorded and characterized in cochlear hair cells of mouse mutants at postnatal day 6. All mutations reduced the Ca2+ permeability of the MET channel and resulted in deafness and hair cell apoptosis by postnatal day 30. In addition, Tmc1 p.E520Q and Tmc1 p.D528N reduced channel conductance whereas Tmc1 p.W554L and Tmc1 p.D569N lowered channel expression without affecting the conductance. Tmc1 p.M412K and Tmc1 p.T416K reduced only the Ca2+ permeability. The accessory subunits, LHFPL5 and TMIE, are required for targeting TMC1 to the tips of the stereocilia. We found sufficient expression of TMC1 in outer hair cells of Lhfpl5 and Tmie knockout mice to determine the properties of the residual MET channels, which could still be gated by hair bundle displacement. Single-channel conductance was unaffected in Lhfpl5-/- but was reduced in Tmie /-, implying TMIE very likely contributes to the pore. Both the working range and half-saturation point of the residual MET current in Lhfpl5-/- were substantially increased. Based on counts of numbers of stereocilia per bundle from scanning electron micrographs, we estimated there are three to four MET channels per tip link, implying that each PCDH15 and LHFPL5 monomer contacts two channels.
Conclusions: The consequences of the missense mutations studied endorse TMC1 as the pore of the MET channel, the mutations occurring in purported transmembrane domains 4-7. We suggest that TMIE very likely contributes to the pore and LHFPL5 is part of the mechanical coupling between the tip-link and the MET channel. Based on counts of numbers of stereocilia per bundle from scanning electron micrographs, we estimated there are three to four MET channels per tip link, implying that each PCDH15 and LHFPL5 monomer contacts two channels.

TU114. Changes in Clinical Auditory Physiology in Macaque Monkeys at 2 and 8 Months After Noise-Induced Temporary Threshold Shifts

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Category: Hearing Loss: Consequences and Adaptation
Background: Cochlear synaptopathy (CS) in rodents results in reduction of the amplitude of Wave I of the auditory brainstem response (ABR) to suprathreshold stimuli. In macaques and humans, ABR Wave I responses are small and variable. Excessive variability could limit the use of the ABR as a sensitive non-invasive indicator of auditory system integrity, including cochlear synaptic function, in clinical populations. Here we test the utility of the ABR as a sensitive measure of pathology by describing measurements in macaques following noise exposures designed to cause CS that caused temporary threshold shifts (TTS) and perceptual deficits.
Methods: Testing was conducted in anesthetized young adult macaque monkeys (Macaca mulatta, 6-10 years old) before and after noise exposure (120 dB SPL octave band noise from 2-4kHz for 4 hours) at two time points: 2-months (n= 9 male, n= 4 female) and 8-months post-exposure (n= 6 male, n= 4 female). Noise exposures induced TTS that recovered within three weeks, and caused perceptual deficits in masked detection tasks. ABRs (vertex-to-mastoid) were obtained to clicks (100μs) and chirps (1.6ms) presented at many sound levels (30-90 dB SPL in 5-10 dB steps). ABRs were also obtained to click pairs at varying interclick intervals (ICI: 10 to 1ms), to clicks at many presentation rates (200/s to 27.7/s), and chirps presented in a masker with varying high-pass cut-off frequencies (0.4 - 32 kHz). All ABR Waves (I, II, and IV) were analyzed for amplitudes and latencies. Distortion product otoacoustic emissions (DPOAEs) were measured from 1 – 10 kHz.
Results: The variability in ABR amplitudes found in normal hearing monkeys were also observed post-noise-exposure. Amplitudes of response to clicks and chirps increased on average post-exposure. The increases tended to be larger in female monkeys compared to male monkeys. ABR amplitudes to clicks at higher presentation rates decreased post-exposure compared to pre-exposure values when they were normalized (re: amplitudes at 27.7 clicks/s) to reduce within-subject variability. ABR amplitudes to click pairs and to masked chirps did not change post-exposure. ABR latencies did not change post-exposure in any stimulus condition. DPOAEs were unchanged at 2- and 8-months post exposure relative to pre-exposure.
Conclusions: Normalized ABR amplitudes in response to clicks at high rates appeared to highlight trends at the group level that may indicate decreased synaptic function. The high variability rendered the ABR largely uninformative for detecting small changes that may be present after TTS. Analysis at the individual subject level will need to correlate physiological, behavioral, and histological findings, to better assess the utility of the ABR as a sensitive indicator of auditory function.

TU115. Insight in the Conditions to Participate in Daily Life for Young Children With Moderate Hearing Loss

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Category: Hearing Loss: Consequences and Adaptation
Background: Children with moderate hearing loss (MHL) have a loss in sound sensitivity between 40 and 70 decibel (dB HL) and often use hearing aids (HAs) to increase their access to sounds and speech. Despite the use of a HA, however, these children still encounter challenges while growing up in a sound-dominated society. Few recent studies indicated that children with MHL are at risk for language difficulties. Yet, we do not have a complete picture of the daily acoustic environments and linguistic input for this specific group of children. Based on the model of "inconsistent access," we distinguish three key factors that are essential for promoting linguistic development in young children with MHL: 1) hearing technology, 2) the auditory environment, and 3) caregivers' linguistic input. Using this model the current study aims to provide a comprehensive insight in the daily acoustic and linguistic environments in which young children (0-4 years) with MHL learn language.
Methods: We use technology combined with caregivers' reports to gain a clear and comprehensive overview of the amount of time young children with MHL spend in optimal and suboptimal situations for language learning. We assessed this not only in the home environment but also in other environments such as day care and early intervention groups (groups for young children with HL, meant to stimulate speech and broad development for young children with HL). In this project, we used HA software allowing for daily datalogging, 2) LENA (Language Environment Analysis), a small device attached to a child's clothes

providing all-day recordings of children's acoustic and linguistic environments, 3) Room acoustic measures, such as Speech Transmission Index (STI), reverberation time, and background noise in daily environments, 4) questionnaires on child- and parent characteristics.
Results: To date, visiting six intervention groups, we have collected data on the acoustic environments of 22 children with MHL. This acoustic data will be compared to the available acoustic norms for schools and other environments. Furthermore, preliminary data on parent- and child characteristics and their relation to HA use (N=8) are presented, as well as preliminary data on the acoustic home environment.
Conclusions: In order to enable children with MHL optimal access to spoken speech signals, it is important to have a thorough understanding of their daily acoustic environments and HA use. Using the data on acoustic environments, we will provide an estimate of the amount of time that children spend in environments sufficient for spoken language learning. Based on these findings recommendations for acoustics at intervention groups for children with MHL will be developed.

TU116. High Dose Psilocybin and Cannabidiol Accelerates Recovery of Blast-Induced Hearing Loss
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Category: Hearing Loss: Consequences and Adaptation
Background: Blast-induced hearing impairment has become an increasingly common casualty in military and civilian situations. Temporary and permanent hearing loss, tympanic membrane perforation, tinnitus, and hyperacusis are common signs of blast exposure. No treatment exists for the permanent sensorineural hearing loss that may result from blast injury. It has been hypothesized that the accumulation of reactive oxygen species in hair cells causes subsequent apoptosis contributing to hearing loss. We focused on two drugs with the potential to benefit multiple pathways implemented in blast-induced hearing impairment. Cannabidiol (CBD) is an abundant non-psychoactive phytocannabinoid that is hypothesized to reduce inflammation and to inhibit gliosis via its action on the CB-2 receptor. Psilocybin (PS) is speculated to stimulate neural plasticity through microglia regulation and possess antioxidant effects as a result of 5-HT1A receptor activation. Neither drug has been studied for the prevention and treatment of blast-induced hearing loss. Our primary aim was to test the therapeutic effects of CBD and PS on blast-induced hearing loss with a secondary aim to assess the proper dosage for efficacy. **Methods:** Hearing loss was produced with controlled blast exposure. Exposure groups (n=5-11) consisted of blast injury and 6 treatment arms: placebo and 5 active substance groups: 1) Combined low dose CBD 5mg and PS .03mg, 2) Combined medium dose CBD 15mg and PS .10mg 3) High dose CBD 25mg, 4) High dose PS .17mg, 5) Combined high dose CBD 25mg and PS .17mg. Treatment was given to rats orally by gavage once daily for seven days beginning within one hour of blast exposure and continuing for six more daily doses. Auditory brainstem response (ABR) was measured at baseline, 24 hours, 72 hours, 7 days, 14 days, and 21 days post-blast. ABR thresholds were obtained at frequencies of 12, 16, 22, and 32 kHz. Group mean differences of ABR thresholds were assessed using one-way ANOVA statistical analysis with Tukey's test for post-hoc analysis. **Results:** Starting at 7 days through 21 days post-blast, combined high dose CBD and PS treated rats had significantly lower hearing thresholds than vehicle at all frequencies tested. In addition, on days 14 and 21, high dose PS alone demonstrated significantly lower hearing thresholds compared to vehicle at 16, 22, and 32 kHz.

Conclusions: This data suggests that high dose PS and CBD can significantly expedite the recovery of blast-induced hearing loss. Although the therapeutic effects of PS and CBD have not been fully elicited, these findings support their potential as a promising treatment worthy of further studies, especially since pharmaceutical treatments for hearing loss have yet to come to fruition.

TU117. In Vivo MRI in Uni- And Bilaterally Deafened Rats Identifies Reduced Volume Development of the Cochlear Nucleus
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Category: Hearing Loss: Consequences and Adaptation
Background: The consequences of early uni- or bilateral deafness on the anatomical development of the central auditory system are not yet fully understood but are of great importance for the optimal treatment recommendation. Patients suffering from early deafness would strongly benefit from strong indicators pointing towards best treatment options. Our previous histological study shows that the volume of the cochlear nucleus (CN) of neonatally single-sided deafened (SSD) rats is reduced on the deafened side, while the volume of the hearing side is comparable to that of normal hearing (NH) animals. Aim of this study was to investigate the effect of uni- and bilateral neonatal deafness on the development of the CN in vivo using magnetic resonance imaging (MRI).
Methods: Twenty Wistar rats with different hearing experience (NH, SSD, or bilaterally deafened (BD)) were studied. The animals of the SSD and BD groups were neonatally deafened by treatment with ototoxic aminoglycosides. Normal hearing or hearing loss were determined by measuring acoustically evoked auditory brainstem responses. On postnatal day (P) 30, 60, 90, and 120, T2- and diffusion weighted MRI measurements were performed on each animal under anaesthesia. Following the last imaging, one animal of each group was used for high-resolution ex vivo MRI, followed by histological assessment. The volume determination from in vivo data was done by extracting structures from diffusion-based maps and using them as boundaries.
Results: Using in vivo MRI, we verified a strong effect of unilateral and bilateral neonatal deafness on the volume development of the CN. By directly comparing the three cohorts NH, SSD, and BD, we identified significant differences in the development of the CN volume depending on its auditory input (acoustic input vs. no input). Already on P30, a CN connected to a hearing ear was significantly larger than that connected to a deaf ear. The mean volume difference of 15.5 % at P30 increased to 32.8 % at P90 and then stabilized. Comparison with ex vivo MRI and histology data was consistent with the in vivo MRI results, although there was a mean shrinkage of the volume compared with in vivo of about 7.8 % and 4.8 %, respectively.
Conclusions: To our knowledge, we are the first to investigate the effect of deafness on the developing CN in vivo using MRI. Our MRI data identified that both, unilateral and bilateral neonatal deafness results in early severe anatomical changes in the CN. Reduced volume development compared to a normal hearing system was observed as early as P30 and increased by P90. This confirms the importance of diagnosing deafness in patients as soon as possible in order to prevent reduced or impaired development of the central auditory system with the help of hearing prostheses.

TU118. Wide Dynamic Range Compression Reinstates Adaptation to Noise for Hearing-Impaired Listeners

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Category: Hearing Loss: Consequences and Adaptation
Background: ‘Adaptation to noise’ refers to an improvement in word-in-noise recognition when isolated words are delayed a few hundred milliseconds from the noise onset. This adaptation is thought to occur because auditory neurons shift (adapt) their dynamic range toward the prevailing noise level preceding the word, which can improve the neural encoding of speech spectral and/or temporal (envelope) cues. If this were the case, adaptation should be less for hearing-impaired (HI) than for normal-hearing (NH) listeners because HI listeners often have reduced cochlear compression, which would make the internal noise representation more fluctuating than normal and result in neurons not having a prevailing level to adapt to. Furthermore, it should be possible to restore adaptation for HI listeners by providing them with a fast-acting hearing aid (HA) to reduce the noise level fluctuations. The aims of the present study were to investigate if adaptation to noise is indeed reduced for HI listeners, and if it can be restored by providing them with a fast-acting but not with a slow-acting HA.
Methods: 25 HI listeners participated (mean age = 61.5 years; SD = 9.3 years). HI listeners were fitted with a software-based, multichannel HA that provided linear amplification, or slow acting (2 ms attack time; 1000 ms release time), or a fast-acting (2 ms attack and release times) wide dynamic-range compression. Speech reception thresholds (SRTs) (i.e., signal-to-noise ratios at 50% recognition) were measured for noise-masked natural (unprocessed) and tone-vocoded disyllabic words preceded or not by a 1-second noise

precursor, that could be steady or fluctuating in level. Adaptation was estimated as the difference in the SRTs without and with precursor.
Results: In steady noise and with linear amplification, HI listeners did not show adaptation for natural words, and they showed less adaptation than NH listeners for vocoded words (0.7 for HI vs 1.7 dB for NH). With wide dynamic-range compression, either fast or slow acting, HI and NH listeners showed statistically similar adaptation ($p \geq 0.309$), both for natural (0.5 dB for NH vs 0.7 dB for HI) and vocoded (1.7 dB for NH vs 1.2 dB for HI) words. In fluctuating noise, adaptation occurred for vocoded words only (0.7, 0.8, and 1.3 dB for the linear, slow, and fast acting HA, respectively), and it was correlated with the variance in level fluctuations at the output of the HA (Spearman $rs=0.220$, $p=0.064$), thus suggesting that adaptation to noise increases when the HA reduces the noise level fluctuations.
Conclusions: HI listeners show less adaptation to noise than NH listeners. Wide dynamic range compression can reinstate adaptation to noise for HI listeners. [Work supported by the Spanish Ministry of Science and Innovation (grant PID2019-108985GB-I00), and the European Regional Development Fund].

TU119. Exploring Listening-Related Fatigue in Children With and Without Hearing Loss Using Self-Report and Parent-Proxy Measures

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Category: Hearing Loss: Consequences and Adaptation
Background: Children with hearing loss appear to experience greater fatigue than children with normal hearing (CNH). Listening-related fatigue is a type of fatigue often associated with an increase in effortful listening or difficult listening situations. This has been observed in children with bilateral hearing loss (CBHL) and, more recently, in children with unilateral hearing loss (CUHL). Available tools for measuring fatigue in children include general fatigue questionnaires such as the child self-report and parent-proxy versions of the PedsQLTM Multidimensional Fatigue Scale (MFS; consisting of general, sleep/rest, cognitive and total domains) and the PROMIS Fatigue Scale. Recently, the Vanderbilt Fatigue Scale (VFS-C: child self-report; VFS-P: parent-proxy report) was introduced with a specific focus on listening-related fatigue. The aims of this study were to explore fatigue levels experienced by CNH, CUHL and CBHL using both generic and listening-specific fatigue measures and compare outcomes from the child self-report and parent proxy reports.
Methods: Seventy-seven children aged 6-16 years (32 CNH; 19 CUHL; 29 CBHL), and their parents/guardians, completed the above fatigue questionnaires online. Kruskal-Wallis H tests were performed to compare fatigue levels between the CNH, CUHL and CBHL. To determine the agreement between parent-proxy and child self-report measures, Bland-Altman 95% limits of agreement were performed.
Results: All child self-report fatigue measures indicated that CBHL experience greater fatigue than CNH. Only the listening-specific tool (VFS-C) was sufficiently sensitive to show greater fatigue in CUHL than in CNH. Similarly, all parent-proxy measures of fatigue indicated that CBHL experience significantly greater fatigue than CNH. The VFS-P and the PROMIS Fatigue Parent-Proxy also showed greater fatigue in CUHL than in CNH. Agreement between the parent-proxy and child self-report measures were found within the total and general domains of the PedsQL-MFS and the PROMIS Fatigue Scale.
Conclusions: Our results suggest that CBHL experience greater levels of daily-life fatigue compared to CNH. CUHL also appear to experience more fatigue than CNH, and listening-specific measures of fatigue may be better able to detect this effect. Further research is needed to understand the bases of fatigue in these populations and to clarify whether fatigue experienced by CBHL and CUHL is comparable in nature and degree.

TU120. Quantitative Aspects of Vestibular Mitochondria

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Category: Inner Ear: Anatomy and Physiology
Background: In this study, we quantified multiple aspects of mitochondrial structure in the vestibular periphery. Our main hypothesis in this research is that mitochondria are structurally heterogeneous,

depending on which hair cell type is under consideration and the specific locations of mitochondria in relation to other hair cell organelles. One such structural relationship that we have reported on previously is the polarization of mitochondrial crista junctions (CJs), openings of the internal mitochondrial membrane into the intermembrane space that is likely responsible for transport of molecules from the mitochondrial matrix out to the cytoplasm.
Methods: Inner ear vestibular tissue obtained from Long-Evans rats under an approved UIC IACUC procedure was used for electron microscope tomography. Data were processed with the IMOD (v. 3.13.6) software package (Kremer et al., 1996) and serial tomograms were joined using the etomo subroutine (IMOD). Manual segmentation was done with the 3dmod subroutine (IMOD) to obtain final models. Reconstructions were visualized using 3dmod (IMOD), including its SLICER option, to track relevant features in three dimensions. Several IMOD drawing tools (Sculpt, Join, Warp, Interpolator) and a proximity analysis subroutine (Mtk) with its randomization feature, were also used.
Results: Quantitative analyses were performed to test our hypothesis, including counts of total mitochondria within both types of hair cells (with a comparison to cochlear hair cells), efferent boutons, and afferent calyces, counts of CJs on either side of a mitochondrion in relation to other cellular organelles, analyzing polarization ratios (side toward vs side away) vs distances, bioenergetic calculations of ATP production, and the Mtk proximity analysis test was performed using CJ randomization as a control. Results support our hypothesis of structural heterogeneity of mitochondria, as CJs are polarized toward specific organelles within the cell and appear to be non-randomly distributed. Finally, vestibular hair cells have less than half the number of mitochondria compared to cochlear hair cells, although certain of these are quite a bit larger than cochlear mitochondria.
Conclusions: These results have functional implications for the various structural differences. CJ polarization toward organelles that use energy can transport ATP more efficiently. For example, the cuticular plate is an organelle that functions to return stereocilia rootlets back to their original position. Our results show a non-random orientation of mitochondrial crista junctions toward the cuticular plate. These structural differences may provide key information to better understand their function and to address mitochondrial deafness and dizziness disorders.
Supported by NIDCD R21-DC013181 (AL) and University of Illinois at Chicago Chancellor's Undergraduate Research Awards (CURAs, to several members of Team Mito, DR, ZA, PS, JL).

TU121. Instance Segmentation of Hair Cell Mitochondria in Fib-Sem Datasets for Morphological Analysis

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Category: Inner Ear: Anatomy and Physiology
Background: Cochlear hair cell histology has been extensively studied with light and electron microscopy, but interest in capturing subcellular structural morphology has led to the rise in adoption of newer 3D electron microscopy techniques, such as focused ion beam scanning electron microscopy (FIB-SEM). FIB-SEM can generate nanometer scale, isotropic imaging datasets enabling visualization of subcellular structures in great detail. A single outer hair cell may have over a thousand mitochondria and their morphology cannot be reasonably analyzed by hand without great effort, presenting a need for automated solutions. Due to highly variable mitochondria morphology and their dense packing, current approaches for biomedical instance segmentation are suboptimal. Here we present a new FIB-SEM hair cell mitochondria segmentation dataset which we use to train a novel, deep learning approach for automatic 3D mitochondria instance segmentation.
Methods: A FIB-SEM stack of a single outer hair cell (voxel size of 3.84×3.84×20nm) containing over 700 mitochondria was annotated in 3D using Dragonfly. We utilized this dataset, supplemented by other publicly available datasets, to train a 3D U-Net architecture to perform instance segmentation using a novel skeleton embedding paradigm. The trained U-Net predicts a morphological skeleton of mitochondria along with a set of 3D pixel embeddings onto said skeleton, by which individual instances may be inferred. We compare this approach to a semi-automated, deep learning enhanced, watershed segmentation pipeline we developed in Dragonfly.
Results: Our fully automated mitochondria instance segmentation solution can accurately segment densely packed mitochondria which are often too large to fit in the neural network's receptive field and is comparable in accuracy to our semi-automated, watershed segmentation approach. The automated approach

is more time-efficient and calculates similar distributions for mitochondria volume and surface area in a fully unsupervised fashion.
Conclusions: The development of this approach increases accessibility for analyzing mitochondria within large 3D electron microscopy stacks, enabling in-depth investigations of mitochondria morphology in normal and pathologic conditions.

TU122. TMPRSS3 Expression in Mouse Cochlea

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Category: Inner Ear: Anatomy and Physiology
Background: Cochlear implantation, which is the standard treatment for inherited sensorineural hearing loss, has shown variable outcomes in patients with DFNB8/10 carrying mutations in TMPRSS3 (Elbracht et al, 2007; Weegerink et al, 2011; Eppsteiner et al, 2012; Shearer et al, 2018; Tropitzsch et al, 2018; Holder et al, 2021). Both, CI performance and speech perception test results have been reported to be poorer for individuals carrying deleterious mutations in genes that discriminately affect the function of spiral ganglion neurons (SGNs) than for individuals with mutations affecting the sensory organ in the cochlea. Therefore, heterogeneity in CI performance in these individuals could plausibly be explained by an associated dysfunction of the SGNs. Here, we hypothesize that Tmprss3 is expressed in all or in specific subtypes of type I SGNs in the murine cochlea.
Methods: To determine its expression pattern, we performed immunostaining for Tmprss3 on fixed frozen decalcified mouse cochlear sections. Using the high specificity and sensitivity of RNAscope probe design strategy for in situ hybridizations, we also tested the spatial expression pattern of Tmprss3 mRNA in the mouse cochlea. For both assays, we co-stained with cellular markers for organ of Corti and SGN cell types. As a third line of evidence, we also estimated the relative expression of Tmprss3 using an RT-qPCR assay with TaqMan probes on RNA isolated from the organ of Corti, spiral ganglion neurons, and the brainstem of two different age groups of mice.
Results: In our immunostainings, Tmprss3 protein was detected in the organ of Corti, where it labelled hair cells and supporting cells lining the scala media. In contrast, hardly any specific immunolabelling was detected in cells of Rosenthal's canal. Evident from our RNAscope assays, Tmprss3 transcripts were also strongly detected in the organ of Corti, and moderately in the spiral limbus as well. However, with very few punctuate dots observed in the Rosenthal canal, its expression in the SGNs seems to be very limited. Data from RT-qPCR experiments confirmed its stronger expression in the organ of Corti compared to SGN tissue. Hardly any Tmprss3 transcripts were detected by RT-qPCR in the brainstem.
Conclusions: Conclusively, our results indicate a rather sparse expression of Tmprss3 in the spiral ganglion neurons, which is in agreement with a recently published meta-analysis of transcriptome data (Chen et al 2022). Following the spiral ganglion hypothesis, pathogenic mutations of TMPRSS3 and their effect on SGN health have been proposed as one possible explanation for the variable CI outcomes. However, with its rather minimal expression in the SGNs, this falls into question and suggests an indirect role of TMPRSS3 for the health of the SGNs and its relevance in CI performance.

TU123. Cannabinoid Receptor Signaling in the Hearing Mammalian Cochlea

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Category: Inner Ear: Anatomy and Physiology
Background: The endocannabinoid system provides important homeostatic regulation of CNS synapses. The wide expression of cannabinoid receptor 1 (CB1) throughout the brain demonstrates the breadth of its influence on neuronal functions. Multiple RNAseq datasets demonstrate the presence of CB1 receptors in the auditory periphery (Milon et al., 2021, Shrestha et al., 2018, Petitpré et al., 2018, Sun et al., 2018, Li et al., 2020). However, the molecular mechanisms and the physiological effects are still elusive (Ghosh et al., 2021). The study here characterizes the localization of CB1 receptors in the hearing mammalian cochlea and explores its effects on hearing function.
Methods: First, the expression pattern of the enzyme involved in the synthesis of the endocannabinoid 2-AG, DGL-alpha was examined with immunofluorescence microscopy in 1-3 months old mice. DGL-alpha is highly expressed in type II auditory nerve fibers (ANFs) and supporting cells such as outer pillar cells.

DGL-alpha labeling is present, albeit at a lower level of intensity, in IHCs as well. The expression pattern of genes encoding the endocannabinoid receptors, Cnr1 and Cnr2, were examined in reporter mice. Cnr1 is highly expressed in type II ANFs. Cnr1 is expressed at a low level in all ANFs as confirmed with smFISH. Cnr2 is expressed in the resident immune cells of the cochlea.
Results: Second, to assess the effect of the CB1 receptor on auditory function, we measured auditory brainstem responses (ABRs) at 2 and 3 months in CB1 receptor-knockout mice (CB1KO) and wildtype littermates (WT), all on a C57BL/6N background. Clicks and pure tones at 8, 12, 16, 24 and 32 kHz were tested. While ABR thresholds were similar at two months, at 3-months of age CB1KO mice showed significantly elevated ABR thresholds compared to WT at 24 kHz. When comparing WT and CB1KO at 2 month of age, wave 1 amplitudes were elevated in the CB1KO animals. This elevation was significant across all levels tested for the 12 and 16 kHz stimuli. The elevation of the wave 1 amplitude in the CB1KO group is lost at 3 months of age.
Conclusions: Finally, we recorded from type I ANF dendrites in P16-31 rats and showed that the cannabinoid receptor agonist WIN22512-2 (WIN, 2-5 µM) irreversibly reduced ANF spontaneous firing rates in extracellular loose-patch recordings. The CB1 receptor specific antagonist (AM251, 5 µM) could partially block the effect of WIN, suggesting that CB1 receptors modulate ANF activity.
In summary, the loss of CB1 receptors could contribute to accelerated hearing loss through affecting type I ANF activity. Possible CB1 receptor mechanisms on type II ANFs still need further assessment to allow for formulating a hypothesis regarding function.

TU124. Single Cell Transcriptomic Atlas of the Murine Cochlea

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Category: Inner Ear: Anatomy and Physiology
Background: The functional molecular data available to date in the cochlea has been obtained through the identification of the causal genes for deafness, a total of 140 genes responsible for isolated deafness and 300-400 genes for syndromic deafness. These advances open the way for curing deafness by gene therapy approaches, a clinical domain in which only prostheses can be presently proposed to patients. However, progress in the development of gene therapy requires a complete inventory of the cochlear cell types and an exhaustive characterisation of the genes expressed by each cochlear cell type with their developmental dynamics. To this purpose, we constructed a transcriptomic atlas including more than 120,000 cochlear cells and nuclei.
Methods: The cochleae were extracted from C57bl6J mice before and upon hearing onset,
and on P20 when the cochlea is mature. After tissue dissociation, the cochlear cells and nuclei were sorted by flow cytometry and underwent RNA-sequencing.
Results: Our dataset provides a comprehensive map of deafness genes and transcription
factors expressed in all cochlear cell types, the identification of new markers for poorly characterized cell types, and evidence for a yet unidentified cell type based on transcriptomic signatures and hybridization assays. In addition, overlooked candidate diseased cochlear cell types for several monogenic forms of deafness were also revealed.
Conclusions: Our study provides an exhaustive and highly detailed map of gene expression in the mouse cochlea in a timeframe corresponding to the postnatal development of the human ear compatible with gene therapy.

TU125. GluA3 Subunit of Ampa Receptors Are Required to Prevent Synaptopathy at Inner Hair Cell Ribbon Synapses in Female Mice

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Category: Inner Ear: Anatomy and Physiology
Background: AMPA-type ionotropic glutamate receptors (AMPARs) mediate fast synaptic transmission at inner hair cell (IHC) ribbon synapses. Antagonism of Ca2+-permeable AMPARs (CP-AMPARs) at IHC synapses may be sufficient to prevent synaptopathy and loss of cochlear sensitivity (Sebe et al., 2017; Hu et

al., 2020). Post-synaptic AMPARs at IHC-ribbon synapses are comprised of the dimers of dimers of GluA2, 3, and 4 subunits. However, the role of each AMPAR subunit in IHC synaptic transmission is still unresolved. Our recent studies in male mice (ARO 2022) showed that lack of GluA3 reduced hearing sensitivity and wave-1 amplitude, was not observed until 8-weeks of age (García-Hernández et al., 2017). Since there are sex differences in hearing and hearing loss (Shuster et al., 2019; Villavisanis et al., 2020; Milon et al., 2018), and knowing that the GluA3 gene is in the X chromosome (Mahadevaiah et al., 2009; Trivisano et al., 2020), we hypothesized that in the absence of GluA3, and when compared to male mice, females may have an early onset of hearing loss.
Methods: We used female C57BL/6 wild type (WT) and Gria3KO mice from postnatal weeks 3-13, reared in animal rooms at normal 'ambient' sound levels (55-70 dB SPL) or in 'quiet' sound levels (40-55 dB SPL). Using ABRs, we examined the role of GluA3 in auditory processing. With immunofluorescence and confocal microscopy, we studied the molecular anatomy of IHC-ribbon synapses with antibodies to GluA2, GluA3, GluA4, and the presynaptic ribbon protein CtBP2/Ribeye. With transmission electron microscopy and serial section reconstruction we studied pre- and post-synaptic ultrastructure of IHC-ribbon synapses.
Results: ABR thresholds and wave-1 amplitude in 3-week-old female WT and Gria3KO mice were similar. However, in mice raised in ambient sound, the ABR thresholds increased, and wave-1 amplitude decreased significantly at 5-weeks and 13-weeks in the Gria3KO, compared to WT mice. In contrast if the mice were raised in quiet, ABR thresholds and wave-1 amplitude were similar between Gria3KO and WT mice at 5-weeks. Confocal imaging analysis showed a similar number of paired synapses, although the numbers of lone ribbons and ribbonless synapses were increased in Gria3KO mice in ambient sound compared to WTs. As well, the ratio of GluA4 to GluA2 subunits per synapse was increased in the KO relative to WT, suggesting an increase in CP-AMPARs in the KO. Ultrastructurally, dendritic swellings were observed in afferent endings of cochlear nerve fibers on IHCs by 5-weeks of age in Gria3KO, but not in WT, and only in those KO mice reared in ambient sound levels.
Conclusions: Our data show that lack of the GluA3 AMPAR subunit results in synapses with increased vulnerability to AMPAR-mediated excitotoxicity at ambient sound levels. This AMPAR dysregulation apparently leads to presbycusis, particularly in female mice.

TU126. Reversible Contrast Enhancement for Visualization of Human Temporal Bones Using Micro Computed Tomography

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Category: Inner Ear: Anatomy and Physiology
Background: The gold standard for study of auditory pathology has long been post-mortem histology of temporal bones. Histological processing is time intensive and limits utility of sectioned temporal bones for applications which require their three-dimensional structure. Micro computed tomography (microCT) is an alternative imaging modality that can be improved with the use of contrast agents, of which iodine solutions, osmium tetroxide (OsO4), and phosphotungstic acid (PTA) are most commonly used. Iodine staining can be removed via leaching or chemical destaining with sodium thiosulfate (STS). Combining microCT with improved resolution from iodine staining could provide a reversible and non-destructive imaging method for the study of temporal bones. We compare microCT images obtained with three different contrast agents and demonstrate the feasibility of reversible iodine staining in human temporal bones.
Methods: Human temporal bones were drilled to expose the round and oval windows. Three contrast agents - Lugol's iodine solution (I2KI), PTA, and OsO4 – were compared for their ability to stain soft tissue structures of the inner ear. All stains were applied in the form of concentrated aqueous solutions in which the temporal bone samples were immersed. Additionally, contrast was injected directly into the cochlea via the round window membrane. Each specimen was stained for 48 hours followed by a 24-hour washing period in either distilled water (I2KI, OsO4, STS) or 70% ethanol (PTA). Specimens were then imaged in respective washing solutions and returned to staining solution for additional 48-hour intervals up through 240 hours.
Results: I2KI staining effectiveness peaked between 48 and 96 hours. In I2KI stained bone, the basilar membrane (BM), Reissner's membrane (RM), and spiral ligament (SL) were visualized in the basal, middle, and apical turns of the cochlea. Clear distinctions between the scala tympani (ST) and scala vestibuli (SV)

were appreciated. Furthermore, the AN and its branching structure was seen. PTA staining showed poorer resolution of intracochlear structures and the AN than I2KI staining at all time points. OsO4 stained bone was imaged at a single 48-hour timepoint. In OsO4 stained bone, the BM was visualized in all cochlear turns, but the RM was only weakly observed in the basal turn. The stria vascularis (StV) and SL were well defined in the basal and middle turns, and the AN trunk was clearly visualized. Chemical destaining with STS was effective in removing I2KI from temporal bone specimens. The structural details of the cochlear interior and AN were no longer appreciated in the whole mount and mid-modiolar virtual two-dimensional sections after 48 hours of treatment with STS.
Conclusions: Reversible I2KI staining with microCT can be used as an alternative approach for the study of the inner ear which preserves three-dimensional structure.

TU127. The Shape of Water – Adaptations in Cochlear Morphology of Seals and Otters

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Category: Inner Ear: Anatomy and Physiology
Background: Pinnipedia (including true seals, eared seals, and walrus) and Lutrinae (otters) are two closely related taxa in the group of Carnivora that returned to an aquatic lifestyle. Despite being the focus of numerous studies investigating their aquatic adaptations and ecology, how impedance matching in water and directional hearing work in those taxa is not completely understood.
Methods: Using µCT-scans, we analysed morphological traits of the inner ear and other auditory structures involved in sound perception in 52 skulls of 38 taxa of the canine subgroup of Carnivora, Caniformia. Additionally, to investigate the relationship between form and function, we used available audiogram data for 20 (in air) or 9 (underwater) taxa respectively and correlate hearing abilities with cochlea morphology.
Results: The examination of cochlea shape space through principal component analysis showed that 82% of shape variation in our samples is explained by the first two principal components. Significant correlations for aquatic shape adaptations were found for numerous factors like: the number of cochlear turns, cochlea spiral height and width, and different shape types. From disc-shaped cochleae in seals to tower-shaped and pyramid-shaped cochleae in Mustelinae (weasels) and otters. Important anatomical traits not directly related to the cochlea include the area size of the fenestrae and tympanum, as well as the distance between tympanum and oval window. Where we identified changes in the proportions of the fenestrae and the tympanic sulcus and characteristic ratio differences for aquatic and terrestrial species. Additionally, we recorded enlarged openings of a bony canal from the round window to the bulla-mastoid junction. It is in close range to an opening in the skull base in all Pinnipedia, which conceivably relates to pressure compensation. This adaptation, the “external cochlear foramen”, has formerly only been known from Phocidae (true seals).
Conclusions: Our data suggest that numerous morphological changes in the ears of aquatic taxa (reduced cochlear spiral height and number of turns, centroid size etc.) significantly affect hearing ability, notably the characteristic frequency.

TU128. Statistical Shape Modelling of the Human Inner Ear

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Category: Inner Ear: Anatomy and Physiology
Background: The structures that make up the human inner ear (IE), including the cochlea, vestibule and semicircular canals, are highly variable in shape. Recently, there has been increased interest in IE anatomy because of advances in auditory implants, robotic surgery, and virtual reality simulation. Statistical shape models (SSMs) are useful tools to analyze variation in morphology. While traditional clinical imaging modalities lack adequate resolution, micro-computed tomography (micro-CT) provides sufficient resolution for optimal visualization and morphological analysis of the IE structures. The objective of current study is to develop a SSM of the human IE using micro-CT images and validate it using surgically relevant morphological measurements.

Methods: Fifty-four temporal bones were obtained. All samples were scanned using a micro-CT scanner and the reconstructed images had a voxel size of 50 µm. The IE was semi-automatically segmented for all samples and exported as surface mesh models. The models were aligned using fiducial and rigid registrations. Three SSMs were developed using aligned 3D mesh surfaces: a model of the cochlea; a model of the vestibule and semicircular canals; and a model of the entire IE. Using principal component analysis, the dominant modes demonstrating the greatest sources of variation for each model were investigated and visualized. Additionally, manual morphological measurements were made on all samples to validate the SSMs. Specifically, the following were quantified: volume variation for 3D meshes used in SSMs, manual measurements of A- and B-values for the cochleae, and internal diameters and thicknesses of the semicircular canals.
Results: The SSMs developed in this study revealed that IE structures varied significantly in size. The SSM of the entire IE complex revealed that the major source of variation is in the semicircular canals. The SSM of the cochlea revealed noticeable variation of the lateral-wall diameter and curvature, especially along the basal turn. Higher modes of variation revealed morphological changes in the apical turn and overall angular length of the cochlea. The SSM of the vestibule and semicircular canals revealed high variation in the diameter of the anterior and posterior semicircular canals compared to the lateral one. All SSM-based findings were cross validated against the morphological measurements.
Conclusions: Three SSMs were developed and validated for the human IE. The results revealed the major sources of variation among the cochlea and additional IE structures. The outcomes of this study may be used for future development of cochlear and vestibular implants.

TU129. Spect Molecular Imaging of the Inner Ear in CBA/CaJ Mice

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Category: Inner Ear: Anatomy and Physiology
Background: Molecular imaging, also known as nuclear imaging, utilizes a suitable radiotracer to quantify biological functions inside the body. Here, we used a state-of-the-art imaging technique – single-photon emission computerized tomography (SPECT) to image the inner ear of young adult CBA/CaJ mice. Thallium-201 (201Tl) – a radiotracer analogous to potassium physiologically, was used as the radiotracer, as the cochlear scala media is unusually rich in potassium (~150 mM), relative to normal extracellular fluids.
Methods: Phantom Experiments:
Phantom experiments were performed to find the detectable radiation levels in volumes equivalent to cochlear volumes of different mammals. Instant thin layer chromatography (iTLC) paper in an Eppendorf tube was used as the phantom and imaged using a Siemens Inveon® multimodality microPET/SPECT/CT platform.
In-Vivo Imaging:
Anesthetized, young adult CBA/CaJ mice (2-3 months old) were intravenously injected into tail veins with a 201Tl radioactive solution (upto 400 µCi/animal). Brain, cochlea as well as the whole body were imaged using our microPET/SPECT/CT system.
Ex vivo Experiments:
At the end of the imaging session, mice were sacrificed, and radiation signals were measured from various body regions, including the brain and cochlea, using our gamma spectroscopy – ion chamber (BioDex-500) system. Ex vivo measurements (SPECT-CT image and gamma spectroscopy) from dissected cochleae were carried out to confirm the in vivo findings further.
Results: The detectable radiation signals (SPECT imaging as well as gamma spectroscopy) were observed from four phantom volumes used in the experiments: 0.8µL ~ mouse cochlea, 1.2 µL ~ guinea pig scala media, 3.0 µL ~ rat cochlear and 10.0 µL ~ guinea pig cochlea. Both SPECT signals and radioactivity levels increased as the phantom volume increases and there was a linear relation between these two quantities. SPECT 201TI signals were observed from the mouse cochlea in-vivo, indicating that 201TI can enter the bony cochlea structure and can be measured with a potassium ion analogue radio tracer. Regions of Interest (ROI) were drawn with the help of the CT structural images. Average cochlear signals were some of the strongest of any body region, and about two times that of the brain signal. Maximum 201TI accumulation was observed in the kidney, consistent with previous literature. Ex-vivo experiments further confirmed the in-vivo findings. Radioactivity signals (measured using gamma spectroscopy) were measured from various body tissues; and the cochlear signals were 2.2 times that of the brain. A pair of cochleae from an animal

were dissected and SPECT-CT imaging was performed. No difference was observed between the two cochleae, indicating the equal absorption of 201TI on both sides of the peripheral auditory system.
Conclusions: Our study shows that molecular/nuclear imaging can be used to effectively measure key physiological properties of the cochlea related to the magnitude of the endocochlear potential.

TU130. Imaging GABAergic Activity in the Cochlea

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Category: Inner Ear: Anatomy and Physiology
Background: Medial Olivocochlear (MOC) efferent neurons are predominantly known for their role in the inhibition of outer hair cell (OHC) electromotility. It is however, reported that MOCs also express markers of GABA synthesis, suggesting that they could engage in GABAergic signaling. Indeed, previous work has shown that in the mouse cochlea, incubating with a GABA B receptor antagonist leads to enhanced acetylcholine release from MOCs, suggesting that GABA acts on the pre-synaptic MOC terminal. Previous work using electron microscopy has also shown that MOCs form connections with cell types other than OHCs, though it is not clear whether these represent functional synapses. One target is afferent type II spiral ganglion neurons (SGN). Type II SGNs respond to sound at loud but non-damaging levels, and are also thought to be involved in responses to cochlear trauma. This work investigates whether a functional GABAergic synapse exists directly between MOCs and type II SGNs.
Methods: To explore whether there is a functional GABAergic synapse between MOCs and type II SGNs we used immunohistochemistry to localize indicators of GABAergic signaling in the mouse cochlea. We used whole-cell patch-clamp electrophysiology in acutely dissected mouse cochleae to investigate post-synaptic responses of type II SGNs to exogenous GABA application. To assess endogenous release of GABA in the outer spiral bundle (OSB) we performed posterior semi-circular canal injections of a genetically encoded GABA-sensing fluorescent reporter (iGABASnFR.F102G). We then performed simultaneous MOC electrical stimulation and iGABASnFR.F102G imaging of fibers in the OSB.
Results: Immunohistochemical results confirm that genetically labelled MOC terminals from ChAT-Cre; tdTomato mice contain the GABA synthesis enzyme glutamate decarboxylase (GAD). Type II SGNs are labeled with an antibody against GABA A β3 receptor subunits, apposed to MOC terminals. Furthermore, whole-cell voltage-clamp recordings from the dendrites of type II SGNs show evoked currents when treated with 1 mM exogenously applied GABA. By imaging iGABASnFR.F102G while performing electrical stimulation of MOCs to evoke neurotransmitter release, we visualized GABA-evoked increases in fluorescence of iGABASnFR.F102G in type II SGNs.
Conclusions: The close apposition of GAD-positive MOC terminals with GABA A β3 receptor subunit positive type II SGNs suggests that these cells are equipped to engage in GABAergic signaling. Electrophysiological evidence confirms that type II SGNs respond to GABA. Using iGABASnFR.F102G we have found that an increase in iGABASnFR.F102G fluorescence is seen in type II SGNs after electrical stimulation of MOCs, indicating that endogenous release of GABA from MOCs is able to act at type II SGNs. These results provide the first visualization of GABAergic signaling in the cochlea and the first functional evidence for a GABAergic synapse between MOCs and type II SGNs.

TU131. Traveling Wave Amplification in the Short-Wave Region by Changes in Organ of Corti Area That Exert Forces on Scala-Media Fluid That Amplify the Pressure Wave

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Category: Inner Ear: Cochlear Mechanics
Background: There are two regions of cochlear amplification: the traveling-wave amplification (TWA) region at and basal of the best frequency (BF), and the non-propagating amplification (NPA) region extending basally from the TWA region. The amplification “changeover frequency” between these regions corresponds approximately to the long-wave to short-wave transition. In both regions outer-hair-cell (OHC) vibrations produce similar increases in organ of Corti (OoC) motions, but in the NPA region, amplification is only local, whereas in the TWA region, amplification is coupled to the traveling wave. Here we consider what produces this difference.
Methods: A hypothetical conceptual model based upon published results and physical reasoning.

Results: In the short-wave region, TWA is hypothesized to be produced by an "OoC area pump" consisting of: (1a) OHC vibrations that squeeze the OoC producing a cyclic cortilymph flow from the peri-OHC region to longitudinally along the OoC tunnels, and (1b) Deiters-cell longitudinal motion that changes OoC area, that are (2) accompanied by changes in OoC area and vibrations of the reticular lamina (RL), and (3) RL motion exerts forces on scala media (SM) fluid that add energy to the SM traveling-wave pressure, with (4) the net effect of producing TWA. In the short-wave region, traveling-wave fluid motion and pressure become focused and radially centered along the top and bottom edges of the OoC, which increases the effectiveness of RL motions in producing TWA. In the long-wave region, OoC area pump effectiveness is reduced by (1) there is no traveling-wave focusing so out-of-phase movement of the RL and the OoC lateral region partly cancel, reducing the net force on SM, (2) the longer wavelength of cortilymph flow along the tunnels produces a smaller cyclic flow from an OoC cross-section into the tunnels and a smaller area change, with the net effect of much less traveling-wave amplification in the NPA region, and (3) when OHC squeezing causes less flow into the tunnels, there is more cyclic bulging at the Hensen-cell region. OoC transverse motions measured in the gerbil base (Cho and Puria, 2022) show OHC motion phases consistent with TWA from the OoC-area-pump method but not consistent with TWA from the OHCs acting directly on the basilar membrane.
Conclusions: The OoC-area-pump hypothesis produces TWA from measured OHC motions in live, sensitive gerbils, and works for the phase of RL motion produced by a simple, classic view of cochlear mechanics, i.e., that BM motion toward scala vestibuli rotates the OoC and produces RL radial motion toward the modiolus that (with little tectorial-membrane radial motion) deflects OHC stereocilia in the excitatory direction and causes OHC contractions at a phase that produces TWA.
Acknowledgements: Supported in part by NIH NIDCD R01 DC007910 (to Sunil Puria).

TU132. Vibration Pattern of the Cochlear Bone With Bone Conduction Stimulation
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Category: Inner Ear: Cochlear Mechanics
Background: During bone conduction hearing, sounds are transmitted as vibrations through the structures of the head that ultimately results in a vibration of the basilar membrane inside the inner ear. This basilar membrane vibration is believed to originate in the pressure difference between the scala vestibuli and scala tympani. Experimental, clinical, and numerical studies suggest that this pressure difference is mainly caused by the vibration of the bone encapsulating the inner ear. However, it is not clarified what type of vibration modes that contribute to the hearing and their contributions as a function of frequency.
Methods: The vibration of the bone surrounding the inner ear is categorized in three modes: translational, rotational, and compressional motion. Each mode as three spatial dimensions and the vibration is described by nine parameters. The parameters were assessed by vibration measurements of the cochlear bone in three human cadaver heads (five ears) using a three-dimensional laser Doppler vibrometer. The 3D vibrations on the visible bony surface of the cochlea, and area of approximately 4 by 5 mm, were measured during bone conduction stimulation at 15 to 20 positions. The coordinates of the measurement points in relation to the cochlear geometry were obtained by high-resolution cone-beam CT. The measurement positions and their associated 3D velocities were fed into a minimum square algorithm to estimate the nine parameters of the bony motion surrounding the inner ear.
Results: The translational motion dominates for the entire frequency range investigated, 0.3 to 10 kHz. When the rotational and compressional components are computed as their magnitudes at the outer surface of the cochlea, they are approximately 20 dB below the translational motion at 0.3 kHz but improves with around 10 dB per decade. The rotation seems to be nearly 10 dB greater around the axis in-line with the internal auditory canal compared to the two other axis. The compression is significantly lower in the direction of the internal auditory canal compared to other directions. When the vibration mode analysis is converted to wave speed of translational sound transmission in the bone, the speed is around 100 m/s at 0.3 Hz increasing with frequency to 400 m/s at 2 kHz. At higher frequencies, the wave speed fall around 500 m/s.
Conclusions: The measurement of bony vibrations at multiple points close to the cochlea revealed translational motion as dominant for frequencies up to 10 kHz. Estimates of both wave speed and compression of the bone is similar to previous estimates in the literature.

TU133. Estimation of Hearing Loss Caused by Fistula and Air Bubble in Cochlea by Using Cochlear Fluid Dynamics Model Assuming Compressible Perilymph

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Category: Inner Ear: Cochlear Mechanics

Background: Though cochlear fistula is rare in hearing diseases, fistula would be unavoidable in the process of surgery for cholesteatoma. Also, rapid ascent of divers from deep water may cause air bubbles in cochlear duct. All of these cases, since the cochlear perilymph touches directly to the air, the pressure level of the sound waves traveling in the cochlea need to be zero at the boarder between the air and perilymph. As a result, we might suffer from significant hearing loss. In this paper, we discuss how much the hearing loss causes depending on the position of the air using the cochlear fluid dynamics model assuming compressible perilymph.

Methods: Since our cochlear model assuming compressible perilymph treats the sound waves traveling in the cochlea more strictly, the simulation results obtained from there are expected to be closer to the real clinical data. For demonstration, two cochlear models are designed; one is a “fistula model” which has a circularly opened fistula on the wall of the cochlear duct, out of which is covered by air. The other one is an “air bubble model”, in which a spherical air bubble floats inside the scala vestibuli or the scala tympani. Then, in each models, an acoustic plane wave is excited at the base of scala vestibuli, and the maximum displacement of the basilar membrane is measured and compared to that of the healthy ear.

Results: In the fistula model, when a fistula is given near the oval window, hearing loss occurs significantly at all audible frequencies. However, when the fistula exists on the scala bestibuli, as the location of the fistula approaches the apex of the cochlea, hearing recovers at higher frequencies and hearing loss remains only at lower frequencies. On the other hand, even if the fistula exists on the scala tympani, there is almost no effect on hearing. The similar feature can be confirmed in the air bubble model, and when the air bubble floats near the base of the cochlea in the scala vestibuli, the damage to the hearing becomes significant.

Conclusions: In our previous research, we succeeded in separating the sound waves excited in the cochlea into “even-mode sound waves” and “odd-mode sound waves” and reported that only the odd-mode sound waves generate traveling waves. As the odd-mode sound wave proceeds along the cochlear ducts, it is gradually converted to the traveling wave on the basilar membrane. However, if the air region exists on the course of mode conversion from the odd-mode sound wave to the traveling wave, it is considered that the conversion is interfered and hearing loss occurs at the corresponding frequencies.

TU134. Evaluating Shifts in the Human Cochlear Tonotopic Map Using Binaural Pitch Matching

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Category: Inner Ear: Cochlear Mechanics

Background: The stiffness gradient along the basilar membrane determines where a traveling wave peaks, thereby creating the frequency-place map or tonotopic organization of the cochlear partition. In ears with endolymphatic hydrops, the increased fluid volume has been hypothesized to stiffen the cochlear partition, thus altering the tonotopic map, particularly in the apical half of the cochlea, where hydrops is most pronounced (Tonndorf, 1976).

Diplacusis is the perceptual anomaly where the perceived pitch of a tone is different between ears. Binaural diplacusis has been attributed to differences in the cochlear frequency-place map between ears (Tonndorf, 1976). Place theory predicts that different pitch percepts will occur if the traveling waves evoked by the same pure tone peak at different physical locations along the cochlear partition in the two ears. In normal ears, with a frequency range of ~10 octaves, binaural pitch differences are small, typically less than one semitone (Albers and Wilson, 1968; Brännström and Grenner, 2008; Colin et al., 2016).

Methods: This study will describe and test a tool for assessing inter-aural pitch differences in normal ears and in a small group of individuals with unilateral endolymphatic hydrops with the goal of identifying potential shifts in tonotopic organization. We developed (and will describe) an adaptive paradigm for the measurement of diplacusis in human ears based on the protocol of Colin et al. (2016). We will assess diplacusis using stimuli of the same sound pressure level in both groups (which will produce differing sensation levels) as well as stimuli adjusted for sensational level (to compensate for existing hearing loss).

Results: We expect our results will confirm the degree and direction of perceptual pitch differences between right and left ears of normal hearers and characterize pitch differences in the affected and non-affected ears

of individuals with endolymphatic hydrops. We expect, based on previous results, that pitch shifts will be small in normal hearers and larger in those with unilateral endolymphatic hydrops, consistent with an altered frequency-place map. This type of shift has previously been observed in guinea pigs with induced hydrops (Guinan et al., 2021).
Conclusions: Perceptual tests that are sensitive to shifts in the cochlear tonotopic map would be of value when assessing and monitoring the consequence of unilateral hearing pathologies and formulating intervention strategies.

TU135. Human Cochlear Masking and OAE Suppression Measured Using Dynamic Stimuli

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Category: Inner Ear: Cochlear Mechanics
Background: Previous recordings of mouse basilar-membrane (BM) motion in response to exponential frequency chirps have shown that upward sweeps (i.e., from low to high frequency) are more effective suppressors of tonal probe signals than downward sweeps (high to low) (Charaziak, ARO 2022). That study found that the differential suppressive effects of sweep direction depend strongly on sweep rate, being stronger for faster sweeps. These results in mice are reminiscent of findings from human perceptual studies using so-called "Schroeder-phase complexes," which are stimuli that contain multiple harmonic frequency components of equal amplitude but variable starting phase, summing to produce a series of periodic linear frequency sweeps. Behavioral studies find that negative Schroeder-phase maskers (e.g., upward sweeps) are more effective maskers of tonal signals than positive Schroeder-phase maskers (e.g., downward sweeps). The psychoacoustic literature on Schroeder-phase masking interprets the dependence on sweep direction as arising not primarily from suppression, but from cochlear dispersion (phase curvature), an interpretation supported by animal measurements and physiological models.
Methods: The current study investigates the effects of sweep rate and direction in normal hearing humans, both behaviorally and physiologically. We use exponential frequency sweeps, as in Charaziak (2022), rather than the linear sweeps produced by Schroeder-phase complexes, hypothesizing that the use of exponential sweeps will provide new opportunities to relate masking and suppression to cochlear processing, which is roughly exponential with frequency. For the psychoacoustic experiments, detection thresholds for tonal signals masked by upward and downward sweeps are measured as a function of sweep rate and direction. In the same subjects, stimulus-frequency otoacoustic emissions (SFOAEs) at the tone frequency are recorded using the sweep combinations from the psychoacoustic paradigm as suppressors. In addition to providing a measure of the suppression produced by the various sweep waveforms, the SFOAE measurements provide estimates of cochlear dispersion and delay that can be used to interpret sweep rates (e.g., as "fast" or "slow").
Results: Differences in psychoacoustic masking and OAE suppression for various sweep combinations will be explored and compared. To the extent that the two phenomena share common generation mechanisms, we expect behavioral masking and OAE suppression to depend similarly on sweep characteristics.
Conclusions: The long-term goal of our study is to better understand dynamic aspects of nonlinear signal processing in the cochlea. Here, we expand on previous work in mice (Charaziak 2022) to explore the effects of chirp sweep rate and direction on signal suppression and masking in normal hearing human listeners.

TU136. The Impact of Targeted Ablation of One Row of Outer Hair Cells and Deiters' Cells on Cochlear Amplification

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Category: Inner Ear: Cochlear Mechanics

Background: Normal cochlear function involves force production from three rows of outer hair cells (OHCs) to amplify and tune the traveling wave. The mechanisms that underlie OHC force summation to create the high gain and sharp frequency tuning associated with cochlear amplification are important to understand because most forms of hearing impairment are caused by OHC loss. While current models predict that OHC forces add energy to the traveling wave as it propagates along the cochlea, it is unclear how much impact removing a percentage of OHCs will have on cochlear amplification. To study the effect of OHC loss on amplification, noise or ototoxic drug exposure, or aging have been used to decrease the number of OHCs throughout the cochlea, but it is difficult to interpret the results due to the variability in OHC loss that results from these methods. Here, we sought to overcome this limitation by generating a selective cell ablation model with a well-defined reduction in OHCs and corresponding supporting cells. To this end, we used a genetic approach to target and ablate the third row of Deiters' cells (DCs) in Lgr5DTR-EGFP/+ mice with a mature cochlea.
Methods: We injected Lgr5DTR-EGFP/+ and wild type mice with diphtheria toxin (intraperitoneal) at P21, and at P28 used immunohistochemistry and confocal microscopy to localize and quantify the extent of DC and OHC loss in the cochlea. We also probed the effects of OHC loss using in vivo measurements of hearing (ABRs and DPOAEs), endocochlear potential (EP), and volumetric optical coherence tomography vibrometry (VOCTV).
Results: In Lgr5DTR-EGFP/+ mice, cell loss was well-defined and limited to the third row of OHCs and DCs throughout the apical and middle cochlea at P28. By P56, the third row loss of OHCs and DCs was more severe due to increased degeneration in the basal cochlea. Although the EP was similar in Lgr5DTR-EGFP/+ mice and wildtype mice, Lgr5DTR-EGFP/+ mice had a 79% reduction in cochlear amplification at the ~11 kHz region, and an increase of ~35 dB and ~20 dB in ABR and DPOAE thresholds, respectively.
Conclusions: These results suggest that the loss of a relatively small number of OHCs, when evenly distributed along the tonotopic axis of the cochlea, causes a substantial reduction in cochlear amplification. Furthermore, the Lgr5DTR-EGFP/+ mouse is an excellent model for further study of the effects of cell loss due the uniform reduction in the third row of OHCs.
The research is supported by NIH DC014450, DC017741 (JSO), DC013774 (JSO and BEA), EB017113 (BEA), DC013910 (AGC).

TU137. 3-D Vibrometry of the Mouse Cochlear Apex

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Category: Inner Ear: Cochlear Mechanics
Background: Optical coherence tomography (OCT) has become an important tool for measuring the vibratory response of the living cochlea. Nevertheless, it is only capable of measuring motion along the optical axis. Hence, measurements are 1-D. To overcome this limitation and provide a measure of the 3-D vector of motion in the cochlea, we developed an OCT system with three sample arms in a single interferometer. We hypothesized that the 3-D vibratory pattern is important to the generation of cochlear amplification.
Methods: Software to co-register the three channels and transform the data into Cartesian coordinates was developed. We then used the system to study living anesthetized CBA mice by imaging through the otic capsule bone. We measured 3-D vibrational motion throughout a volume of the organ of Corti of the mouse apical turn. To interpret these data, we averaged radial cross-sections within the volume and aligned the averaged data to the cellular structure of this region of the mouse cochlea. Data from multiple mice could thus be averaged and used to create a movie in 3-D.
Results: Our data show that the organ of Corti vibrates in a multi-modal fashion, with a pattern of vibrations that involve the transverse, radial, and longitudinal directions. The structure does not vibrate as a single unit when stimulated at the characteristic frequency, resulting in different cells vibrating with different angular displacements and magnitudes. We are continuing to measure 3-D vibrational data over a wide range of sound frequencies and intensities to characterize vibratory patterns within the mouse cochlear apex.
Conclusions: We conclude that the vibratory pattern of the organ of Corti is complex. This complexity likely both reflects and underlies the production of cochlear amplification. Further 3-D vibrometry studies in transgenic mice with altered cell mechanics will help elucidate how the structures within the organ of Corti work together to produce gain and sharpened tuning.

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TU138. Rapid Mitochondrial Calcium Uptake is Critical for Normal Function of Stria Vascularis in FVB/NJ Mice

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Category: Inner Ear: Damage and Protection

Background: Cochlear hair cells (HCs), spiral ganglion neurons, and cells of the stria vascularis (SV) are prone to irreversible damage in sensorineural hearing loss. These regions have also been known to contain cell types that are metabolically active and therefore are enriched in mitochondria when compared to surrounding tissue. Predictably, evidence implicates mitochondrial dysfunction as a lead cause in several forms of deafness. Like in many other cell types within an organism, mitochondria in the cochlear cells are responsible for vital cellular functions, including energy production, apoptosis, cell signaling, and Ca2+ storage. These events are dependent on the ability of mitochondria to capture Ca2+ ions: mitochondria shape cellular Ca2+ signals by acting as a Ca2+ buffer and responding to Ca2+ elevations by increasing the cell energy supply or by triggering the cell death program of apoptosis. The major specific channel for rapid Ca2+ uptake by mitochondria is the multi-protein mitochondrial Ca2+ uniporter (MCU) complex or "uniplex". Excessive cytosolic Ca2+ can quickly enter the matrix through MCU. MCU uniplex thus contributes to Ca2+ buffering, ensuring cytosolic Ca2+ homeostasis, and is posited to have a critical role in cochlear function. MCU uniplex consists of MCU, which forms the main Ca2+ permeant pore that is incumbent for rapid Ca2+ uptake, and other main protein components, such as EMRE and MICU1. Previously, we demonstrated that FVB/NJ mice without functional MCU (Mcu–/–), a model for deficient rapid mitochondrial Ca2+ uptake, show early onset hearing loss. To determine the cause, we tested if cochlear HCs function normally without rapid mitochondrial Ca2+ uptake and assessed the function of the SV.

Methods: We crossed MCU-floxed mice (Mcu fl/fl) with Atoh1-Cre line to obtain hair cell-specific Mcu fl/fl; Atoh1 Cre+ mice. SV function was tested in the the constitutive Mcu–/– mice.

Results: Preliminary data from Mcu fl/fl; Atoh1 Cre+ mice showed that ABR thresholds were comparable to control mice, suggesting a non-hair cell etiology for the induction of early onset hearing loss we observed in the constitutive Mcu–/– mice. Next, we assessed the function of the SV in Mcu–/– mice. Mcu–/– mice show statistically significant reduction in EP values when compared to heterozygous littermates. In addition, reduced thickness of the SV in both apical and basal cochlear turns were observed in Mcu–/– compared to control mice.

Conclusions: Our data implicate SV pathophysiology as the preponderant mechanism responsible for hearing loss in the Mcu–/– mice. Further experiments using conditional knockout mice are needed to identify which cells of SV are responsible for the observed hearing loss in mice lacking MCU.

TU139. Oseltamivir (Tamiflu) Mitigates Hearing Loss in Mouse Models of Acoustic Trauma

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Category: Inner Ear: Damage and Protection

Background: Affecting an estimated 10-15% of all people, noise-induced hearing loss (NIHL) remains a major cause of disability worldwide. Despite this high prevalence, there are no FDA-approved drugs to treat or prevent NIHL, a problem further compounded by the expense and risk associated with the novel drug development process. To circumvent these obstacles, we performed a high-throughput screen (HTS) of previously FDA-approved drugs for otoprotection and identified oseltamivir (Tamiflu), a neuraminidase inhibitor currently approved for the treatment of influenza, as a promising candidate. Oseltamivir was additionally shown to reduce cisplatin-induced hair cell death in an ex vivo assay of P3 FVB mouse cochlear explants with low toxicity and high potency, further bolstering its hypothesized otoprotective potential. Given these results, we hypothesized that oseltamivir treatment in vivo could mitigate hearing damage following traumatic noise exposure.

Methods: Baseline hearing ability was established in 6-8 week old FVB mice through measurements of auditory brainstem response (ABR). Mice were then subjected to a noise challenge consisting of a 2 hour period of exposure to 100 dB SPL noise covering a 8-16 kHz octave band. Twice daily treatment with oseltamivir (10 mg/kg, 50 mg/kg, and 100 mg/kg) or carrier via oral gavage was initiated either 24 or 48 hours following noise exposure and continued for either three or five days. ABR and wave 1 threshold shifts were measured 14 days following treatment.
Results: Oseltamivir, a high ranking candidate from HTS screening, exhibited low toxicity (TI >220) and high potency (IC50 450 nM) in cochlear explants. In vivo, 100 mg/kg oseltamivir treatment significantly protected hearing in noise-exposed mice with an average reduction of ABR threshold shift of 20-25 dB SPL when initiated 24 hours post-noise exposure; no protection was seen when treatment was initiated 48 hours following exposure. Mice treated for five days exhibited no improvement over the three day treatment group. Moreover, oseltamivir-treated mice experienced significant protection of wave 1 amplitude at 80 and 90 dB SPL.
Conclusions: Oseltamivir emerged as a possible otoprotective compound in our initial HTS and cochlear screens. In vivo, oseltamivir treatment rescued multiple indicators of hearing function in mice when given for three days starting 24 hours after traumatic noise exposure, suggesting a window of opportunity for treatment following acoustic trauma. While its mechanism of action as an otoprotective agent requires further research, favorable ABR and wave 1 amplitude results suggest that oseltamivir treatment may protect against cochlear synaptopathy in the setting of traumatic noise exposure. Combined with its widespread availability and regulatory approval, our results suggest oseltamivir is a potential candidate for drug repurposing to prevent noise-induced hearing loss.

TU140. Comparison of Middle Ear Muscle Reflex Measures in Carboplatin-Treated Chinchillas
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Category: Inner Ear: Damage and Protection
Background: The middle ear muscle reflex (MEMR) is an involuntary contraction of the stapedial muscle in response to moderately loud acoustic stimuli. In mammals, this contraction stiffens the ossicular chain, briefly reduces the admittance of the middle ear in a frequency-dependent manner and decreases sound input to the inner ear. In routine clinical protocols, the MEMR is assessed using a single frequency probe tone with multiple elicitor stimuli that provide information regarding the status of the middle ear, inner ear, and central auditory pathway when combined with other tests. The MEMR has recently been suggested to be an effective measure of cochlear synaptopathy, the noise- or age-induced degeneration of inner hair cell (IHC) synapses. Specifically, MEMR sensitivity to synaptopathy appears to increase when measured with a wideband probe stimulus. IHC damage is believed to underlie suprathreshold deficits such as tinnitus, hyperacusis, and speech-in-noise difficulties that occur even when auditory thresholds are in the normal clinical range. Previously, we found that MEMR thresholds measured using a 226 Hz probe tone were unaffected by severe and selective loss of IHC in chinchillas treated with carboplatin, an anticancer drug that destroys IHC in this species. To explore potential differences among MEMR probes on the effect of selective IHC loss, the MEMR was measured using both a wideband probe and a single-frequency probe in chinchillas with moderate to severe IHC loss.
Methods: Young adult chinchillas with free access to food and water were used for this study. All MEMR measurements were conducted in awake animals. Wideband MEMR was measured using an ER10X dual probe system (Etymotic Research) using click stimuli in the measurement ear and broadband noise (BBN) elicitors in the contralateral ear. 226 Hz probe tone MEMR were measured with a Tympstar clinical analyzer. 226 Hz MEMR were obtained using multiple elicitor stimuli, including pure tone bursts and BBN. In addition to MEMR measurements, auditory brainstem response thresholds (ABRT) were evaluated from 1-12 kHz as a measure of hearing sensitivity. Distortion product otoacoustic emissions (DPOAE) were collected (1-10 kHz) to assess the presence of OHC-dependent cochlear non-linearities. Following baseline data collection, chinchillas were treated with 50-75 mg/kg carboplatin (i.p.), a dose range that has been shown to produce 40-80% IHC and little-to-no OHC loss. ABR thresholds, DPOAE, and MEMR measures were re-assessed following a 3-week recovery period.
Results: Carboplatin treatment induced 50-80% IHC loss, but did not affect OHC, DPOAE or ABRT; results that are consistent with previous studies using this dose.

Conclusions: Preliminary data suggest that MEMR threshold, as measured by either 226 Hz probe tone or wideband probe, were not significantly affected by selective IHC loss; however, at high elicitor presentation levels, IHC loss may weaken the magnitude of the MEMR response.

TU141. Differential Effect of Peroxynitrite Decomposition Catalyst on Cisplatin-Induced Cytotoxicity in Organ of Corti Versus Tumor Cells

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Category: Inner Ear: Damage and Protection

Background: Ototoxicity is a major limiting factor in cisplatin chemotherapy. Intervening with broad spectrum antioxidants, such as sodium thiosulfate, reduces hearing loss but compromises the anti-cancer activity of cisplatin in the tumor cells. As cisplatin hydrolyzes and transforms into an electrophile and interacts with the DNA resulting in single and double strand DNA breaks and also triggers oxidative DNA damage to eventually stall replication of the tumor cells, targeting oxidative stress could interfere with its anti-cancer activity. In mitotically quiescent cells like the inner ear hair cells, cisplatin-induced nitrative stress plays a major role in causing cell death. Previous studies have demonstrated that selective targeting of nitrative stress using peroxynitrite decomposition catalysts (PNDCs) that do not inhibit oxidative stress prevents cisplatin-induced ototoxicity. However, it is not known whether PNDCs could prevent ototoxicity without compromising the anti-cancer activity of cisplatin. Therefore, in this study, we test the hypothesis that co-treatment with Manganese (III) tetrakis (4-benzoic acid) porphyrin (MnTBAP), a PDNC, will reduce the cytotoxic effect of cisplatin in organ of Corti cells but will not inhibit the cytotoxic effect in tumor cells.
Methods: Effect of MnTBAP chloride was evaluated in UB/OC1 (embryonic mouse inner ear), HCC1937 (triple negative breast cancer) and LnCAP FGC (prostate cancer) cell cultures treated with cisplatin. Cell viability was assessed using MTT assay. Nitrative stress was assessed using immunocytochemistry assay with anti-nitrotyrosine. Cell migration was assessed at 6, 12, and 24 hours using wound healing assay.
Results: MTT assay indicated that cisplatin's IC50 was 20 μM for UB/OC1 and HCC1937, and 1.5 μM for LnCAP FGC cells. Co-treatment with 100 μM of MnTBAP promoted cell survival in UB/OC1 ($p<0.05$, $n=6$) but not in HCC1937 and LnCAP FGC cells. Immunofluorescence study indicated that 20 μM of cisplatin increased nitrative stress in UB/OC1 (3.3-fold) and HCC1937 (3.1-fold) cells and MnTBAP co-treatment significantly ($p<0.0001$) reduced the cisplatin-induced nitrative stress in both cell types. More importantly, assessment of cell migration indicated that MnTBAP co-treatment decreased the wound width in UB/OC1 cells by 16% relative to cisplatin treated cells. In contrast, the wound width was increased by 8% in MnTBAP co-treated HCC1937 cells suggesting the differential effect in UB/OC1 versus tumor cells.
Conclusions: Collectively, these results suggest that MnTBAP co-treatment protects the UB/OC1 cells from the cytotoxic effect of cisplatin and does not interfere with cisplatin's cytotoxic effect in the tumor cells.

TU142. Macrophages and MHCII Expression in the Spiral Ganglion of Deafened Mice and Rats

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Category: Inner Ear: Damage and Protection

Background: Spiral ganglion neurons (SGNs) receive auditory information from cochlear hair cells, the auditory receptor cells, and transmit it to the CNS. SGNs gradually degenerate and die after hair cell death, potentially adversely affecting the efficacy of cochlear implants. The reason for post-deafening SGN degeneration remains unclear. Gene expression profiling shows dramatic upregulation of immune response-related genes in the spiral ganglion following deafening. Suppression of immune response by anti-inflammatory drugs prevents SGNs loss, implying that the immune response contributes to SGN death. Transcriptome analyses also demonstrated that deafening results in upregulation of MHCII, a molecule involved in antigen presentation to lymphocytes and adaptive immune response. Here we show by immunofluorescence that the increase in macrophage number following deafening in two animal deafness models is accompanied by increased expression of MHCII on these cells, implicating the adaptive immune response in SGN death post-deafening.
Methods: We used two different animal models for our experiments. Rat model: Male and female Sprague-Dawley rats were injected with kanamycin in the second postnatal week to kill hair cells and euthanized at postnatal day 70 (P70). Mouse model: DTR (Pou4f3huDTR/+) mice can have hair cells killed by injection

with diphtheria toxin, which was injected on P5. Here, we used mice generated from crossing MHCII–/– (B6.129S2-H2dlAb1-Ea/J) mice to DTR mice. MHCII+/+, MHCII+/–, and MHCII–/– mice were harvested at postnatal day 60. Sections were immunolabeled with following antibodies: anti-myosin 6/7 to confirm loss of hair cells, anti-MHCII antibodies to identify MHCII-expressing potential antigen presenting cells, and anti-IBA1 or anti-F4/80 to label macrophages in rats or mice, respectively.
Results: In deafened rats and mice, macrophage number increased in the deafened spiral ganglion relative to hearing control. In mice, the increase depended on MHCII: MHCII+/+ mice had the largest increase in post-deafening macrophage density while MHCII–/– mice had the smallest. A subset of these infiltrating mononuclear phagocytic cells is MHCII+APCs and the expression of the MHCII molecule was upregulated after deafening.
Conclusions: In both rats and mice, inflammation in spiral ganglion was associated with SGN degeneration post-deafening. We previously showed that MHCII knockout significantly reduces SGN death post-deafening. Consistent with that, we show here that MHCII KO reduces macrophage number in the deafened spiral ganglion, implicating macrophages in SGN death. The data suggest that MHCII-mediated antigen presentation, involved in the adaptive immune response, plays a significant role in SGN degeneration after deafening.

TU143. Inactivation of Cochlear Macrophages by Minocycline Ameliorates Noise Induced Hearing Loss in CBA/CaJ Mice

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Category: Inner Ear: Damage and Protection
Background: Cochlear macrophages are long-lived resident immune cells that mediate inflammation within the peripheral auditory system. Activation of cochlear macrophages can worsen damage to sensory hair cells following noise exposure through the release of pro-inflammatory secreted factors. Immediately after acoustic trauma, cochlear inflammation occurs and continues for days reaching a maximum between 2-3 days following noise. The resolution of inflammation is an active process that promotes tissue repair and regeneration, which contributes to the recovery of hearing during a time window of 1-2 weeks following noise exposure. In this study, we sought to test the hypothesis that inactivation of macrophages with minocycline treatment during the pro-inflammatory phase of cochlear inflammation would improve ABR thresholds in CBA/CaJ mice following acoustic trauma.
Methods: To test this hypothesis, CBA/CaJ mice were either given minocycline or vehicle for 5 consecutive days beginning 1 hour before 2 hours of 112 dB broad-band noise exposure. Untreated CBA/CaJ mice were also tested as a baseline control. Hearing status was evaluated at 2 weeks after noise-exposure.
Results: Noise exposure resulted in an increase of approximately 40 dB in ABR threshold to clicks in vehicle treated mice at two weeks post exposure. Treatment with minocycline, however, reduced ABR thresholds to clicks by approximately 15 dB compared with vehicle treatment in CBA/CaJ mice at two weeks post exposure.
Conclusions: These results confirm that reducing the pro-inflammatory activity of cochlear macrophages with minocycline treatment ameliorates noise induced hearing loss in CBA/CaJ mice. Better understanding the contribution of cochlea macrophage activation to the progression of hearing loss following acoustic trauma will allow for the development of treatments that promote the resolution of inflammation to improve outcomes for patients with hearing loss.

TU144. A New Trick of an Old Drug: Mitigating Aminoglycosides-Induced Ototoxicity

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Category: Inner Ear: Damage and Protection
Background: Aminoglycosides are one of the most commonly-used classes of antibiotics worldwide. They are a highly effective treatment against sepsis and chronic infections, particularly among cystic fibrosis and tuberculosis patients. However, permanent hearing loss and/or balance disturbance occurs in up to 20% of patients taking aminoglycosides. Despite this debilitating side effect, currently there are no FDA-approved therapies to prevent aminoglycosides ototoxicity.
Methods: An approved drug (DXU696212) has been identified in our hit-to-lead expansion drug screening campaign. Preclinical characterization of its otoprotective efficacy is carried out using in vivo zebrafish

assays. DXU696212 is tested against the entire aminoglycosides drug class, including gentamicin, neomycin, kanamycin, tobramycin, amikacin, streptomycin, sisomicin, and geneticin (G418) in acute and chronic exposure models. Additional experiments such as MET channel functional and Texas Red-conjugate aminoglycosides uptake assays are also performed to elucidate the mechanisms of DXU696212 otoprotective actions. E. coli growth inhibitory assays were performed to test its effect on aminoglycosides antibacterial activity.
Results: Our in vivo zebrafish assay results show that DXU696212 offers substantial otoprotection against the entire class of aminoglycosides and it does not attenuate aminoglycosides antibacterial activity in vitro. DXU696212 mitigates aminoglycosides-induced hair cell damage via both MET channel blockade and intracellular mechanisms. The combination of the two modes of action are dose-dependent.
Conclusions: We have demonstrated the potential preclinical efficacy of DXU696212 in our studies and determined the optimal dose against the entire class of aminoglycosides. In vivo rodent ABR/DPOAE studies are underway to establish its efficacy in mammalian models. Given its excellent clinical safety profile, DXU696212 holds the promise to be a safe and effective therapy for aminoglycosides ototoxicity prevention.

TU145. Proof of Principle of Extracellular Vesicle Application in a Cochlear Implantation Trauma Model With Guinea Pigs Preventing Fibrosis and Residual Hearing Loss

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Category: Inner Ear: Damage and Protection
Background: Application of extracellular vesicles (EVs) derived from bone marrow and umbilical cord mesenchymal stem cells (MSC) are a promising tool for the modulation of pathways and targeting cochlear cells in order to prevent or treat immunological processes elicited by cochlea implantion trauma. Previously we have shown the efficacy of EVs in spiral ganglion cell cultures and prevention of noise-induced hearing loss in mice.
Methods: EV application associated with CI- insertion trauma was performed in a group of guinea pigs (n= 8) with a CI right after administration of EVs isolated from human umbilical cord MSC (UC-MSC- EVs). The control group (n=11) was implanted with a CI exclusively. Hearing was observed pre and post implantation and after 4 weeks via auditory brainstem response and compound action potential measurements. Weekly impedance measurement and confocal laser scanning microscopy (CLSM) on optical cleared whole cochleae were conducted monitoring fibrosis development.
Results: Our findings revealed no negative effect and the tendency of protection of residual hearing after UC- MSC-EVs administration 4 weeks post implantation in the EV group compared to the control group (10 dB at 32 kHz). The extension of fibrosis within the cochlea in the EV group was statistically less compared to the control group.
Conclusions: EV application associated with cochlear implantation seems to be a safe and solid combination to prevent post implantation trauma and preserve residual hearing. For precise immune modulation to achieve a targeted inner ear cell protection or even restauration further investigations are needed.

TU146. Isolation and Characterization of Extracellular Vesicles and Their Micro RNA Content From Murine in Vitro and Ex Vivo Inner Ear Models

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Category: Inner Ear: Damage and Protection
Background: We lack reliable inner ear biomarkers to guide diagnosis and treatment of hearing loss. MicroRNAs (miRNA), small single-stranded RNA molecules, affect post-transcriptional gene regulation and may be detected at sites distant from their cells of origin via transport by extracellular vesicles (EVs). miRNA and EVs both may be altered in disease states, modify disease progression, and serve as biomarkers.

We previously demonstrated differential miRNA expression in perilymph of humans with different inner ear pathologies and, more recently, have successfully isolated sensory hair cell-specific EVs from human perilymph. In this study, we demonstrate isolation and characterization of EVs and their miRNA content from murine ex vivo and in vitro inner ear models of oxidative stress, a key pathophysiologic mechanism in age-related hearing loss and various ototoxic insults.
Methods: House Ear Institute-Organ of Corti 1 (HEI-OC1) auditory cells and organ of Corti (OC) explants harvested from P3 C57B16/J mice were cultured with or without a low concentration of hydrogen peroxide to induce oxidative stress without causing significant cell death. Using a commercial kit, EVs and their miRNA content were isolated from the culture medium in each experimental group; the miRNA content was characterized with the Agilent microarray system. EVs from the HEI-OC1 control and treated cell culture medium were then incubated with P3 OC explants. Total RNA was extracted from the explants of each experimental group and profiled with the Agilent microarray system. All experiments were repeated in triplicate. A machine learning computational analysis was performed to identify miRNA or messenger RNA (mRNA) with significantly different expression levels between the treatment and control groups. Ingenuity pathway analysis was performed to identify implicated proteins, and targeted immunohistochemistry was performed for validation.
Results: Exposure of HEI-OC1 cells and OC explants to oxidative stress resulted in, respectively, 102 and 105 miRNAs significantly differentially expressed between the control and treatment groups. The majority of differentially expressed miRNA were downregulated in the HEI-OC1 treatment group and upregulated in the OC treatment group. EVs from conditioned HEI-OC1 culture medium induced changes in the mRNA expression profiles of OC explants, with 32 mRNA significantly differentially expressed between the two experimental groups.
Conclusions: EV-derived miRNA expression was altered under conditions of oxidative stress for murine HEI-OC1 cells and OC explants. Different populations of EVs also appear to alter the transcriptome of OC explants. These findings suggest that ototoxic insults (eg, medication toxicity, cochlear implant insertion trauma, noise exposure) may alter EV and biomolecular expression within the inner ear. EVs may also serve as drug delivery vehicles to mediate otoprotection following inner ear trauma. Future work to validate these findings in a murine in vivo sound trauma model is ongoing.

TU147. The Role of Calcium, Akt, and ERK Signaling in Cadmium-Induced Hair Cell Death

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Category: Inner Ear: Damage and Protection
Background: Studies have found a correlation between higher levels of blood or urinary heavy metals, including cadmium, and hearing loss. It has also been shown that cadmium can kill hair cells in both mammalian and fish models, however, the mechanisms by which cadmium kills hair cells are largely unknown. We investigated if signaling pathways implicated in cadmium-induced cell death in other cell types, namely calcium, Akt and ERK, were also playing a role in cadmium-induced hair cell death.
Methods: We used the zebrafish lateral line to assess the amount of hair cell death seen in response to varying doses of cadmium and calcium, Akt and ERK signaling inhibitors. Fish were also treated with cadmium and stained for either pAkt or pERK to assess activation of those signaling pathways.
Results: We found that while calmodulin inhibition did protect against cadmium-induced hair cell death, inhibition of CaMKII, the IP3 receptor, or mitochondrial calcium uptake failed to protect. Inhibition of the latter two have been shown to be able to protect against aminoglycoside-induced hair cell death. The calmodulin inhibition result may be due to calmodulin's role in the mechanotransduction process, something we have previously shown to be important for cadmium-induced hair cell death. We also observed an increase in pAkt levels in hair cells and pERK levels in supporting cells following cadmium treatment. Inhibiting these signaling pathways showed a slight increase in the amount of hair cell death observed suggesting they are being activated by cells as a protective mechanism, which is different than some other cell types where their inhibition actually protects cells from cadmium-induced cell death.
Conclusions: Overall our results show that calcium, Akt and ERK signaling pathways are not playing the same role in cadmium-induced hair cell death that they do in cadmium-induced cell death in other cell types and that cadmium and aminoglycosides appear to kill hair cells through distinct mechanisms.

TU148. Open Board

TU149. The Diagnostic Utility of Electrocochleography as an Indicator of Cochlear Noise Injury

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Category: Inner Ear: Damage and Protection

Background: Damage to the inner ear arising from noise is insidious and delayed in clinical presentation. There exists a need for sensitive clinical tools to detect cochlear noise injury in at-risk populations. Electrocochleography (ECochG) is a technique to measure the electrical response arising from the cochlea and the distal portion of the auditory nerve evoked by an auditory stimulus and include 1) cochlear microphonic (CM), 2) cochlear summating potentials (SP) and 3) cochlear nerve compound action potentials (CAP). Studies of noise overexposure in animals demonstrate a disturbance of the synaptic junction between cochlear inner hair cells and afferent nerve fibers, as well as reduced neural responses, even in cases when thresholds are normal or have recovered to pre-exposure levels. The cochlear hair cells are considered the major generators of the SP, while the CAP represents the summed post-synaptic firing of the auditory nerve fibers. Suprathreshold amplitude of the CAP is reduced in animal models of noise-induced cochlear synaptopathy. Noise overexposure in animal studies is characterized by reduction in CAP amplitudes, while SP amplitudes either increase or remain unaffected, suggesting that the relationship between the SP and CAP may be a sensitive measure of cochlear synaptopathy. This study investigated the extratympanic ECochG in noise-exposed firefighters to explore the diagnostic utility of ECochG as an indicator of cochlear noise injury.

Methods: Cochlear function was assessed in 70 noise-exposed firefighters using EcochG and distortion product otoacoustic emissions (DPOAEs) via the Intelligent Hearing Systems (IHS) Smart EP/DPOAE system (Miami, FL). DPOAEs were recorded using f2 tone frequencies with a fixed primary ratio (f2/f1) of 1.22, L1 = 65 dB SPL, L2 = 55 dB SPL for the frequency range of 1.5 – 10 kHz. SP and CAP of the EcochG was used evaluate presynaptic versus postsynaptic differences in cochlear neural output. Responses were recorded using a wave I optimizing montage for both a 100 μs rarefaction click stimuli and a 4000 Hz tone burst at 113 dB peak SPL at a stimulus repetition rate of 11.1/sec. Results were compared to age-matched controls. Absolute SP and CAP amplitudes, and SP/CAP ratios were computed and compared between groups.

Results: DPOAE amplitudes were significantly reduced in noise-exposed firefighters compared to controls ($p < 0.01$). EcochG resulted in reduced mean amplitude of the CAP in the noise-exposed group compared to controls ($p < 0.05$). Significant differences are also observed for SP/CAP ratio in noise-exposed firefighters compared to controls ($p<0.05$), consistent with differential changes in post-synaptic firing.

Conclusions: There are significant differences in EcochG responses for noise-exposed firefighters compared to age matched controls, marked by reduced CAP amplitudes. Results suggest the SP/CAP ratio may serve as a potential marker of cochlear synaptopathy arising from noise injury.

TU150. A Transcriptomic Analysis of Noise Damaged Cochlea for Evaluating Late Phase Response

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Category: Inner Ear: Damage and Protection

Background: Exposure to noise can evoke sensorineural hearing loss. Excessive noise makes damage to hearing organ such as cochlea and auditory nerve. This peripheral damage causes changes in the central nervous system. Cochlear damages after noise exposure were wide and various, therefore a lot of studies were reported in aspect of molecular, structural and auditory functional changes. The authors tried to evaluate the late phase change of biological process after noise exposure.

Methods: C57BL/6N mice of 12-week-old were introduced this study. Animals were divided to experimental (n=3) and control group (n=3). Experimental group was exposed to white noise of 120dB SPL for two hours. After 12 weeks, all animals were sacrificed, and cochleae were harvested. Difference of expression levels of mRNAs were evaluated by RNA sequencing analysis between two groups. Hearing level was measured using auditory brainstem response.

Results: In the experimental group, auditory brainstem response showed permanent threshold shift. RNA sequencing identified 102 DEGs (57 upregulated and 45 down regulated). Transcriptomic analysis reveals that regulation of cellular metabolic process and molecular function were decreased, and response to

fibroblast growth factor and regulation of lipid biosynthetic process were increased. GO functional analysis showed several transcriptional factors were increased significantly in the noise exposed cochleae.
Conclusions: Among these, TNF signaling pathway and MAPK signaling pathway are robustly induced. Even after 12 weeks, it is showed that the molecular biological changes continued, and it is suggesting that noise damage induces long-term changes in cochleae.

TU151. ULK1 Activator Protects Against Noise Induced Hearing Loss by Activation Autophagy

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Category: Inner Ear: Damage and Protection
Background: Noise-induced hearing loss (NIHL) has the highest incidence in environmental related hearing loss, however there is no effective therapeutics for patients. It's known that autophagy plays an important role in facilitating cellular normal functions in different cells of the cochleae under stress condition, including noise trauma, aging and ototoxic injury. However, no study proved whether activation autophagy is an effect therapeutic strategy for NIHL.
Methods: Local administration of ULK1 activator was conducted in C57BL/6 mice and CAG-RFP-EGFP-LC3B mice aged 4 weeks old. Cochleae of the C57BL/6 mice were collected 2 days after local supplementation for western blot. Drug treatment was followed 2 days later by an 8-16kHz octave-band noise at 104 dB SPL for 2 hrs. ULK1 activator gavage administration began at 5 days before noise exposure, and continuously maintained for another 2 days, when weight monitor was conducted and different organs were collected 5 days after gavage administration for western blot. Autophagic flux was observed in different organs of CAG-RFP-EGFP-LC3B mice. ABR test and immunofluorescence were conducted in mice at 3, 7 and 14 days post noise (dpn).
Results: ULK1 activator enhanced ULK1 phosphorylation dramatically 2 days after local administration. LC3B expression level increased in ULK1 upregulated cochleae. There were more autophagosomes and autolysosomes in treated versus untreated SGNs. After treatment, presynapses of IHC were distinctly preserved 14 pdn, compared to contralateral cochleae. ULK1 activator preserved the ABR threshold shifts and maintained until 14 dpn. Weight loss was not observed during gavage administration, when phosphorylated ULK1S555 evaluated in cochlea, brain and heart and maintained in kidney and liver. Autophagosomes in SGN was significant evaluated with gavage administration. Auditory function was rescued comprehensively at 14 dpn by systemic administration, except high frequency (32 kHz.)
Conclusions: ULK1 activator local supplementation successfully activated ULK1 and protected against neuroexcitatory toxicity by enhancing autophagy flux in the cochleae, through preservation synapses and auditory function. Oral administration of ULK1 activator was safe, also activated ULK1 and enhance autophagy flux in neural system specifically, demonstrating that the drug was able to penetrate blood-brain-barrier and activate autophagy. Additionally, ULK1 activator treatment by systemic administration exhibited similar protection effect from NIHL as local delivery, exhibiting superior potency in protection of NIHL.

TU152. Temporal and Cellular Characterization of the Unfolded Protein Response in the Cochlea After Sound Exposure

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Category: Inner Ear: Damage and Protection
Background: Endoplasmic reticulum (ER) stress has been implicated in genetic and acquired hearing loss. ER stress induces the Unfolded Protein Response (UPR), in which misfolded proteins induce homeostasis or apoptosis depending on the burden of injury. Expression of BiP and S-XBP1 marks the homeostatic response, whereas CHOP expression is associated with apoptosis. Treatment with ISRIB, a drug that suppresses the pro-apoptotic arm of the UPR, can prevent noise-induced hearing loss (NIHL) and cochlear synaptopathy (NICS). Mice deficient for the gene TMTC4, which exhibit cochlear cells that are especially susceptible to ER stress, have normal hearing at developmental onset but then rapidly develop progressive

deafness. In this study, we sought to better understand the temporal and cellular pattern of UPR-associated hearing loss.
Methods: To investigate the temporal evolution of the UPR after acoustic overstimulation, we exposed 8-week-old male and female wild-type CBA/J mice to 8-16 kHz octave-band noise for 2 hours at 98 or 106 dB SPL, levels that respectively induce temporary threshold shift (TTS) with NICS, or permanent threshold shift. Cochlea were harvested for qPCR measurement of Bip, S-XBP1, and CHOP mRNA expression at multiple timepoints after noise exposure using the 2-delta/delta CT method. To investigate the cellular specificity of UPR-associated hearing loss, we used TMTC4-knockout mice as a model of UPR-associated progressive hearing loss. We generated hair-cell and supporting-cell specific TMTC4 knockout lines using Myo15Cre and Prox1-Cre-ERT2, respectively, in conjunction with TMTC4loxP, and measured auditory brainstem response thresholds.
Results: Cochlear expression of Bip, S-XBP1, and CHOP was measured at 0, 2, 6, 12 and 24 hours after 98 or 106 dB SPL noise, levels that cause NICS or NIHL, respectively. In both conditions, gene expression changes were present at 0 and 2h, and returned to baseline by 6h. Both noise exposure levels caused upregulation of Bip, but only the 106 dB SPL exposure caused upregulation of CHOP. Male animals exhibited greater CHOP expression than females. Myo15Cre/TMTC4loxP, but not Prox1-Cre-ERT2/TMTC4loxP, mice exhibited rapid postnatal hearing loss and hair-cell death.
Conclusions: Upregulation of UPR genes immediately after noise exposure implicates the UPR in the early cochlear response to acoustic overstimulation. Louder noise, which results in permanent hearing loss and hair-cell death, is associated with upregulation of CHOP, the marker of the pro-apoptotic arm of the UPR, whereas moderate noise, which causes cochlear synaptopathy but not hair-cell death, does not exhibit CHOP changes. CHOP changes also correlate with sex differences in noise susceptibility. Finally, ER stress susceptibility in the TMTC4 deafness model is a hair-cell-specific effect. Taken together, these findings suggest that the UPR is involved in the early cochlear response to noise, and that the temporal and cellular patterns of UPR activation and ER stress correlate well with hearing loss and hair-cell death.

TU153. Do You Want Pepper on That?: Natural Alkaloid From Indian Long Pepper as Protection Against Noise-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection
Background: Noise-induced hearing loss (NIHL) affects more than 300 million people worldwide. The degradation of the inner ear from acquired hearing loss produces a total cost of $980 billion annually. Thus, it is imperative that we develop effective therapies to avoid this financial toll and mitigate the reduction in the quality of life for millions. There are no FDA-approved drugs that protect the inner ear against NIHL. However, preliminary data from our lab suggests that piperlongumine (PG) – a natural alkaloid derived from the Indian long pepper (Piper longum) – has therapeutic potential for the treatment of NIHL. In a zebrafish model for NIHL, piperlongumine protected zebrafish hair cells and presynaptic ribbons against glutamate-like excitotoxicity, suggesting it may restore hearing function after noise exposure. Experiments with the NF-kB reporter zebrafish line suggested PG protects by inhibiting the NF-kB pathway. Therefore, we hypothesize that PG treatment has a protective effect against NIHL via regulation of specific signaling pathways in the inner ear. We will first investigate PG's otoprotective capacity against noise, and then determine its mechanism of action.
Methods: To determine PG's therapeutic effect against noise, we assessed 7- to 8-week-old CBA/CaJ mice exposed to 94 dB SPL, 8-16 kHz octave band noise for 2 hrs., to damage the synapses without hair cell loss. Animals received PG (50mg/kg b.w. IP) one day before noise exposure, immediately after noise, and 24 hours after noise. Auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) were recorded before (baseline), 24 hours, 7, and 21 days post-noise. The inner ears were processed for ribbon synapse quantification.
To determine PG's mechanism of action in vitro, mouse embryonic fibroblasts (MEFs) were incubated with PG and TNF-a and assessed for NF-kB and AKT activities.
Results: Noise exposure resulted in significant ABR threshold shifts 24hrs and 7 days after exposure. Conversely, mice treated with PG showed comparable ABR threshold levels to the baseline readings. Both groups (noise and noise + PG) returned to baseline thresholds 21 days after exposure. DPOAE threshold significantly increase in both groups 24 hours after noise and went to baseline levels at days 7 and 21 post-

noise, thereby validating our model for synapse damage without hair cell loss. In MEFs, PG significantly inhibited both NF-kB and AKT pathways. We currently assessing ribbon synapses for the different treatments and we will continue to assess PG's therapeutic effect and mechanism of action in the mouse model for NIHL.
Conclusions: Treatment with PG returned ABR thresholds to normal values more quickly than observed in noise-only mice. PG may be conferring protection through inhibition of NF-kB and AKT pathways.
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TU154. The Role of Macrophages in Chronic Suppurative Otitis Media Sensorineural Hearing Loss

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Category: Inner Ear: Damage and Protection
Background: Chronic suppurative otitis media (CSOM) is the most common cause of permanent hearing loss in children in the developing world. We have created a validated Pseudomonas Aeruginosa (PA) CSOM mouse model and observed outer hair cell (OHC) loss in the cochlear basal turn. We also revealed that macrophages are associated with this OHC loss. In the current study, we investigate the macrophage's role in CSOM sensorineural hearing loss (SNHL).
Methods: We manipulated the resident macrophages using the colony stimulating factor 1 receptor (CSF1R) inhibitor PLX 5622, causing about 92% of resident macrophage elimination. Macrophage depletion does not result in hearing loss showing that resident macrophages are not required to maintain hair cells in the cochlea. Macrophage depletion also does not cause the cytokine profile change. Then we created CSOM in both the control and the macrophage depletion groups.
Results: A few OHC loss occurs at 7 days (7d) CSOM in both groups. There are both more macrophages and more OHC loss in the control group compared to the macrophage depletion group at 14 days CSOM. Interestingly, the NLRP3 inflammasome associated factors including NLRP3, PYCard, Caspase1 and IL-1β are elevated in the control group compared to the macrophage depletion group at 7d CSOM.
Conclusions: Taken together, the data implicates that the increased macrophages may cause OHC loss through NLRP3 inflammasome activation.

TU155. AC102 – Phase I Results and Phase II Initiation for a New Drug Candidate for the Treatment of Sudden Sensorineural Hearing Loss

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Category: Inner Ear: Damage and Protection
Background: Sudden sensorineural hearing loss (SSNHL) is a serious condition that often has severe psychosocial consequences. While its causes are often unknown, there is consensus that these include permanent loss of outer hair cells (OHCs) and synaptic disconnection of inner hair cells (IHCs) from the auditory nerve. Currently, SSNHL is mostly treated with steroids which lack reliable efficacy data and therefore remain unapproved for the treatment of SSNHL. Here we present clinical safety and tolerability results for the novel drug candidate AC102. This small molecule almost completely reversed noise-induced hearing loss in preclinical models by counteracting apoptotic death of OHCs and reversing synaptic disconnection of IHCs, making it a promising drug candidate for the treatment of ISSHL.
Methods: In the Phase I clinical trial, AC102 was compared to placebo in healthy volunteers receiving either AC102 formulated in a thermosensitive gel by a single intratympanic injection into one ear at increasing concentrations and volumes. The other half of the subjects received a placebo injection. End points were safety and tolerability of AC102, including assessment of audiological and vestibular function.
Results: Treatment emergent adverse events (TEAEs) occurred almost equally in both treatment groups. TEAEs included vertigo, bleeding at the injection site, ear discomfort, ear pain, and throat irritation after gel injection. However, most TEAEs were mild and resolved within one week. No serious adverse events (SAEs) or adverse events of special interest (AESIs) were observed. In both groups, there was a transient increase in hearing threshold mainly at higher frequencies resolving within 2-4 days after gel injection. However, no clinically relevant changes in OHC- and auditory neural pathway function were observed. AC102 concentrations in human plasma were dose-dependent and decreased over the 24h observation

period. However, peak plasma concentrations and total amount of the active drug after 24h were below 10% of the values observed in rats and dogs at the no-observed-adverse-effect-level (NOAEL) after receiving oral AC102.
Conclusions: In this Phase I clinical trial, AC102 elicited only mild and transient TEAEs with only low systemic drug concentrations. Therefore, the compound was safe and well tolerated by healthy volunteers, allowing its application in patients. Consequently, AC102 is currently being evaluated in SSNHL patients in a randomized and blinded two-arm study Phase II clinical trial. This study will enroll patients with moderately-severe to profound idiopathic SSNHL at up to 50 study sites in Europe. Efficacy, safety, and tolerability of AC102 will be compared to standard oral steroid treatment, allowing a comparison to the current treatment of choice. AC102 may provide a promising therapeutic approach to address the significant, unmet medical need for patients with SSNHL.

TU156. Virus-Infection in Cochlear Supporting Cells Induces Hair Cell Death by Trail-Induced Necroptosis

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Category: Inner Ear: Damage and Protection
Background: Viral infection such as Family Paramyxoviridae (mumps virus and measles virus), Family Matonaviridae (rubella virus), Family Herpesviridae (herpes simplex virus, varicella zoster virus, cytomegalovirus, and Epstein-Barr virus), Family Orthomyxoviridae (influenza virus), and Family Picornaviridae (enterovirus and encephalomyocarditis virus) has shown evidence to cause sudden sensorineural hearing loss so far, but its mechanism is still veiled.
We previously reported that supporting cells (SCs, Hensen's cells and Claudius' cells) and greater epithelial ridge cells (GERCs) acquire hallmarks of macrophages such as migration and phagocytosis expressing macrophage markers during viral infection (we refer to these cells as macrophage-like cells), then protect hair cells (HCs) from viral infection by secreting cytokines including type I interferons (IFNs) (Hayashi et al., 2020). The aim of this project is to understand the mechanism of HC death during viral infection to the organ of Corti (OC).
Methods: The OC of postnatal day 2 mice was cultured overnight for stabilization. Then Theiler's murine encephalomyelitis virus (TMEV), which belongs to picornavirus and has high affinity to nerve tissues, was administered to the cultured tissue (3.0 x 107 pfu/ml). The virus-infected tissue was subjected to protein or gene expression assay.
Results: HC death was observed by virus administration to the WT OC, although viral infection to HCs was not prominent. HC death was also observed in the Il6 KO or Ifnar1 KO mouse cochlea infected with viruses, indicating so-called cytokine storms induced by macrophage-like cells did not cause the HC death. Interestingly, the dying HCs were negative for cleaved caspase-3 (an apoptosis marker). GO analysis using comprehensive gene expression profile from mock, LPS-treated, and TMEV-infected cochleae detected a necroptosis-related gene set, Trail, Tlr3, and Mlkl. Trail, a TNF superfamily protein, is known to induce cell death by binding to its receptors, DR4 (death receptor 4) and DR5 (death receptor 5). Expression of DR4 and DR5 was found specifically in HCs, which indicates that HCs receive Trail secreted by macrophage-like cells. Anti-Trail antibody administration with TMEV rescued HC death, while recombinant Trail administration harmed HCs. HCs in the TMEV-infected cochlea were positive for phospho-Mlkl, an executor of necroptosis. Based on these findings, it is presumed that HC death is executed by necroptosis via the Trail/death receptor (DR4 and DR5) signaling pathway.
We also examined the efficacy of necroptosis inhibitors on HC protection against secreted Trail during viral infection to the OC. We administered nectostatin-1 or ponatinib to the virus-infected OC with various concentrations, then observed each efficacy on HC protection in a dose-dependent manner.
Conclusions: During viral infection to the OC, HC death is induced by not apoptosis but necroptosis without prominent viral infection to HCs, which is dependent on the Trail/death receptor intercellular signaling pathway. The HC death can be rescued by necroptosis inhibitors.

TU157. Ultra-Low Biofouling Zwitterionic Hydrogel Coatings Reduce Intracochlear Impedance and Fibrosis 4 Weeks After Cochlear Implantation in Sheep

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Category: Inner Ear: Damage and Protection
Background: Following cochlear implantation, fibrosis from insertion trauma and the foreign body response affect device function and longevity. Zwitterionic hydrogel coatings, when applied to cochlear implants provide a highly lubricious surface and have been shown to significantly reduce maximal insertion force, insertion force variation, and overall work of cochlear implantation in a human cadaveric model. Furthermore, zwitterionic hydrogel coatings have ultra-low biofouling properties and have been shown to reduce fibrosis associated with the foreign body response when implanted subcutaneously in rodents. We hypothesize applying zwitterionic hydrogel coatings to cochlear implants can significantly reduce cochlear fibrosis by mitigating both insertion trauma as well as the foreign body response. Complex impedance has been used to assess the electrochemical environment of a cochlear implant after implantation, with increased access resistance suggesting the presence of fibrosis around the cochlear implant. By implanting functional human cochlear implants with a zwitterionic coating in the cochlea of live sheep, this study aims to assess electrode function as well as cochlear response to zwitterionic hydrogel coatings of cochlear implants.
Methods: Functional human cochlear implants were implanted in sheep bilaterally, one ear with a zwitterionic coated cochlear implant, and the contralateral ear with an uncoated control. Implants were incubated in vivo for 4 weeks. Complex impedances were measured across 8 cochlear implant electrodes in saline preoperatively, immediately after implantation, and at the time of animal sacrifice. Whole cochlear imaging was performed with 3D Xradia, with image segmentation and volumetric analysis completed with 3D Slicer software. Samples also underwent histological evaluation.
Results: Total impedance was reduced after 4 weeks implantation, with significant reductions in both polarization impedance and access resistance components as compared to uncoated controls. Volume of neoossification and fibrotic scar tissue was significantly reduced in zwitterionic hydrogel coated cochlear implants as compared to uncoated controls.
Conclusions: Ultra-low biofouling zwitterionic hydrogel coatings of cochlear implants, successfully reduce intracochlear impedances and neoossification after 4 weeks incubation in sheep.

TU158. Food Supplement Induced Residual Hearing Preservation in Partial Insertion -Cochlear Implantation (FIPPI-CI)

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Category: Inner Ear: Damage and Protection
Background: The indications for cochlear implantation (CI) continue to expand and an increasing number of patients with good low-frequency residual hearing are being considered for a CI. With the introduction of partial insertion at our clinic, the residual hearing of these patients can be well preserved. However, some patients continue to lose their residual hearing as it is observed in classical cochlear implantation. During times of environmental stress, reactive oxygen species levels can increase dramatically due to increase of metabolic rate and decrease of cochlear blood flow. This can result in significant damage to cell structures known as oxidative stress and release of pro-inflammatory processes that can lead to the initiation of programmed cell death. Antioxidant systems may either prevent these reactive species from being formed, or remove them before they can damage vital components of the cell, and thus protect the remaining hearing.
Methods: In this double-blind randomized placebo-controlled clinical trial the effect of antioxidant, a specific combination of vitamins β-carotene (converted in the body to vitamin A), ascorbic acid (vitamin C),

trolox (vitamin E) and the vasodilator magnesium (Mg), together named ACEMg, is investigated regarding its effect on residual hearing.
Patients with residual hearing in the low frequencies take the oral supplement twice a day for a period of 105 days, starting one-day prior to cochlea implantation. Our primary objective is to compare the residual hearing 3 months after initial fitting versus the preoperative residual hearing in the low frequencies, between the ACEMg treated and a placebo group. Additionally, speech recognition tests as well as electrophysiological measurements of implant function are assessed.
Results: As this is a double-blind study and recruitment is ongoing, we do not know at this time which patients have received the ACEMg combination and which have received the placebo and can therefore present analysis on the residual hearing but not on the ACEMg effects. To date we have recruited nearly half of the patients needed for a reliable analysis and a first interim analysis shows good residual hearing at all insertion depths for the first 41 patients. Patients with an insertion depth of less than 23.9 mm completely retained their residual hearing and had a mean residual hearing loss of 11.5 dB (±7.4) over the frequencies 125 Hz to 1.5 kHz, Patients with greater insertion depths have a mean residual hearing loss of 22 dB (±11.8). Considered across all insertion depths, residual hearing could be preserved in 80% of cases.
Conclusions: The partial insertion approach is successfully preserving residual hearing. Data on ACEMg effects to improve hearing preservation with this surgical procedure will be presented after finishing the recruitment.

TU159. Exploring the Use of Locally Administered Near-Infrared Light to Mitigate the Effects of Noise Exposure

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Category: Inner Ear: Damage and Protection
Background: Temporary threshold shifts (TTS) can occur with as little as 12.5% of a safe, allowable noise dose from continuous ambient noise. This is thought to occur, at least partially, through biochemical pathways that initiate an oxidative stress response following sound exposure. Near-infrared (NIR) light has been successfully utilized in disease processes where oxidative stress plays a central role and shows promise as an otoprotective therapeutic. In pre-clinical models, NIR light has been shown to dampen undesirable biochemical responses to noise exposure and enhance innate cellular repair mechanisms. Here, we sought to determine the effect of safe noise exposures on auditory health and assess whether pre-noise NIR light therapy can mitigate the effects of noise exposure.
Methods: Following baseline hearing health assessments, participants (n=30) with normal hearing (<25 dB hearing level in each ear from 500 – 6 kHz) were exposed to open ear, continuous, pink noise at 94 dBA for 15 minutes. Participants underwent a total of 4 sessions and received either active or sham NIR therapy for 30 minutes prior to or following noise exposure. Sessions 1 and 2 were separated by >24 but <48h in order to maximize NIR therapy. After a minimum of 2 weeks, participants returned for Session 3 and 4 where they received the opposing device (active vs. sham in crossover fashion). Pre-and post-test audiograms were collected at 3000, 4000, and 6000 Hz.
Results: Participants in the experimental group experienced TTS, with a mean shift of 6.79 dB (SD: 6.24), 10.61 (SD: 6.89), and 7.30 dB (SD: 7.25) at 3000, 4000, and 6000 Hz, respectively. Analysis of variance (ANOVA) demonstrated no significant difference in TTS observed at any frequency following active NIR therapy and subsequent noise exposure. However, administration of the sham NIR device prior to noise exposure resulted in significant variation between frequencies. Post-hoc comparisons revealed that TTS at 4000 Hz differed significantly from 3000 Hz, suggestive of a possible protective effect at 4000 Hz. Age and gender significantly predicted TTS in simple linear regressions. All participants' post-noise audiograms returned to baseline at 3000, 4000, and 6000 Hz within 60 minutes of noise exposure.
Conclusions: Although even safe levels of noise can induce TTS, these changes are transient in nature and may indicate healthy sensorineural inner ear systems. Locally administered NIR prior to noise exposure does not appear to mitigate noise-induced TTS in totality but may lessen the magnitude of the shift. Given our significant finding at 4000 Hz, further exploration is needed to determine the effect of NIR therapy in the context of noise-induced hearing loss and other otologic indications.

TU160. Inner Ear Drug Delivery Using Cochlear Catheter During Cochlear Implantation: A Feasibility and Safety Study

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Category: Inner Ear: Drug Delivery

Background: Intracochlear drug delivery poses unique challenges due to anatomical structure of the inner ear. Although systemic and intratympanic methods have been investigated for inner ear drug delivery, no consistent benefits have been observed. To achieve greater and consistent drug levels, intracochlear delivery is the key, but achieving high perilymphatic drug concentrations in all regions of the cochlea, particularly the furthest apical regions is still problematic. The objective of this study was to evaluate the feasibility and safety of a custom designed cochlear catheter (cannula) for inner ear drug delivery using a rat model of cochlear implantation. This system has a great potential for a combined use with cochlear implantation to prevent loss of residual hearing.

Methods: A preclinical rat animal model of cochlear implantation was used. The animals were divided into different groups: 1) Control; 2) animals implanted with a cochlear implant (CI); 3) animals implanted with a dexamethasone eluting CI; 4) Canula elution of phosphate buffer saline (PBS); 5) Canula elution of Ringer lactate; 6) Canula elution of Ringer lactate and implantation of animals with CI; 7) Canula elution of Ringer lactate solution and animals implanted with a dexamethasone eluting CI. Hearing thresholds were determined in each group pre-operatively, day 7 and day 30 post-cochlear implantation, using auditory brainstem responses (ABRs). Animals were euthanized at day 30 post-cochlear implantation, and organ of Corti dissections were performed for each group. Immunostaining was performed to determine hair cell (HC) damage.

Results: Hearing threshold shifts at day 7 and day 30 were significantly higher in the implanted animals in all frequencies as determined by ABRs. On the other hand, hearing threshold shifts were significantly lower in the canula elution groups (PBS and Ringer) as well as implanted with dexamethasone eluting CI at day 7 and day 30 for all frequencies. Cannula effusion does not affect the efficacy of dexamethasone eluting CI. HC count was significantly higher in cannula effusion groups as well as animals implanted with dexamethasone eluting CI compared to rats receiving non-drug eluting CI.

Conclusions: The result of our study suggests that cochlear catheter can be used efficiently for inner drug delivery during cochlear implantation for the preservation of residual hearing without having adverse effects on cochlea. Cannula effusion can be used to deliver pharmaceutical interventions along with dexamethasone eluting CI without affecting its efficacy. Future studies involving inner ear delivery of otoprotective agents using this cochlear catheter will facilitate in its potential translation from bench to bedside.

TU161. Polymer Fiber-Based Neuronal Guidance Scaffold for Cochlear Implants to Improve the Electrode-Nerve Contact

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Category: Inner Ear: Drug Delivery

Background: In the case of sensorineural hearing loss, the most effective therapy is the implantation of a cochlear implant. However, a critical issue is that the distance between the electrode of the implant and the spiral ganglion neurons (SGNs) is relatively large which results in a loss of signal transmission. Therefore, an improvement of the electrode-nerve contact for enhanced auditory impression is needed. To achieve this, a neuronal guidance scaffold and drug delivery system which is applied to the cochlear implant is designed. This scaffold will bridge the gap between the cochlear electrode and the SGNs. The basic structure of this scaffold is made of biodegradable polymer fibers consisting of polyglycolide and poly-ε-caprolactone. For a better regeneration of the inner ear, the fibers shall be decorated with growth factors (brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3)) which are released from the fibers. This should lead to an increased survival of the SGNs and should stimulate the outgrowth of neurites. In addition, the

fibers are coated with components of the extracellular matrix (laminin and heparan sulfate (HS)) to offer growing neurites a favorable environment for their extension to the electrode.
Methods: For the experiments unmodified and amino-modified fibers were used to test different surface properties for the desired application. First, HS was attached to the fibers covalently. After this, these fibers, as well as fibers without HS, were incubated in a growth factor solution with BDNF/NT-3. To test the release behavior of the different fiber-types release experiments were carried out. The supernatants of the release experiments were used for in vitro studies to test the neuroprotection of the growth factors and the effect on the neurite length. In another experiment, laminin was attached to the fibers covalently. These fibers were used for cell culture investigations with cochleae of neonatal Sparague-Dawley rats.
Results: After the modification the fiber types differ in their chemical composition at the surface and their surface roughness. The release experiments with BNDF and NT-3 revealed that there are differences in the release behavior when the fibers are coated with HS in contrast to fibers which are not coated with HS. In addition, there is a difference between the unmodified and amino-modified fibers. The cell culture investigations with laminin showed that explants with SGNs can grow on laminin-coated fibers. The neurons form extended neurites which grow preferably along the fiber, thus using the fiber as a guidance structure.
Conclusions: The experiments show promising results concerning the use of these fibers as a drug delivery system for growth factors. They show a neuroprotective effect and induce an outgrowth of neurites. Additionally, these fibers can be used for guiding the neurites of the SGNs.

TU162. Open Board

TU163. Development and in Vitro and in Vivo Testing of a Guinea Pig round Window Niche Implant for Controlled Cochlear Pharmacotherapy
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Category: Inner Ear: Drug Delivery
Background: Inner ear disorders are significantly affecting people worldwide and there is an unmet need for effective cochlear pharmacotherapies. Intratympanic delivery is a compromise between minimally-invasive application in the middle ear cavity and local application which may lead to an effective diffusion of the active ingredient into the inner ear avoiding systemic side effects. We aim to develop an individualized drug eluting round window niche implant (RNI) to achieve a controlled drug delivery into the cochlea. The RNI is firstly developed for guinea pigs (GP-RNI) as an established animal model for proofing the safety and efficacy of the concept.
Methods: A one-size-fits-all GP-RNI model was created to reduce the variabilities in the animal model. The temporal bones of 12 Dunkin-Hartley guinea pigs were scanned by µCT. Using 3D Slicer the round window niches (RWN) were segmented and reconstructed and an average model was built. The GP-RNI models were printed by a 3D-Bioplotter® using silicone containing dexamethasone (1%). The implantability of the GP-RNI was evaluated in fresh guinea pig cadavers (N = 6) performing µCT imaging and CLSM (confocal laser scanning microscope). Biocompatibility and bio-efficacy were tested by MTT assay and a tumor necrosis factor-alpha (TNFα)-reduction test. Drug release was measured performing HPLC-MS. Finally, guinea pigs received a cochlear implant electrode insertion trauma to induce inflammatory response. Animals were divided into two groups, being implanted with a CI (N = 7) or a CI+GP-RNI (N = 7) for four weeks. Auditory brainstem response (AABR) and impedances were measured, µCT imaging and histology were performed to evaluate the implant position in vivo and to analyse fibrous tissue growth around the GP-RNI and the CI.
Results: The GP-RNI containing 1% DEX was biocompatible and reduced TNFα production in vitro. The drug release showed a burst within the first hour subsequently slowed down with average release rates ranging from 2.9 ng/h (from 1 – 6 h) to 0.2 ng/h (from day 21 – 29).The GP-RNI was implantable and fitted in all tested guinea pig cadavers. The in vivo experiments are ongoing but interim analysis indicates that no complications or side effects are observed after RWN implantation. The µCT and CLSM scans reveal that

the GP-RNIs stay well attached to the round window membrane 28 days after implantation. The analysis of the full data sets are pending since not all animals are finalized yet.
Conclusions: The 1% DEX containing GP-RNI is biocompatible and anti-inflammatory in vitro. It is easily implantable and stays in situ within the experimental period of 28 days. The analysis of the anti-inflammatory potential concerning fibrosis inhibition and hearing preservation is ongoing. The GP-RNI is a well-suited and tolerable tool for intratympanic drug delivery, but its beneficial effects have yet to be demonstrated.

TU164. Evaluating Autophagy Compounds With an in Vivo Model to Deliver Potential Therapies for Noise-Induced Hair Cell Loss

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Category: Inner Ear: Drug Delivery
Background: Autophagy allows cells to safely degrade and recycle dysfunctional components. There is evidence of autophagy participation in hair cell (HC) damage. In a previous study (Draf et al., Frontiers in Cell and Developmental Biol 2021) we screened 154 autophagy compounds in an in vitro mouse model and identified 18 compounds that exhibited a significant and protective effect in aminoglycoside - induced HC damage. In order to apply this finding in an in vivo setting, we have developed a model that enabled continuous delivery to the cochlea. Our goal was to combine autophagy compounds targeting similar aspects of autophagy for their effects on HC loss after noise exposure.
Methods: Five sets of six adult female C57BL/6J mice ranging from 5-6 weeks were included. Using a postauricular approach a hole was drilled into the medial aspect of the posterior semi-circular canal and a catheter was attached to a micro-osmotic pump (1ul per hour, 3 days) was placed into the posterior canal. The opening was sealed with fibrin sealant and fascia. The pump which contained either an autophagy compound combination or dimethyl sulfoxide (DMSO) as a control was placed into a subcutaneous pouch on the back of the mouse. 100dB SPL noise was presented for 2 hours. Post-noise exposure and 14 days postoperative auditory brainstem response (ABR) was conducted in a sound attenuating chamber with 4, 8, 12, 16, 24 and 32 kHz tone bursts for both ears. Perfusion fixation was performed after day 14, the organ of Corti was dissected and immune-staining with Myosin VIIa, Phalloidin and CtBP2 was performed in order to quantify inner and outer HC, and ribbon synapse counts with Image J software.
Results: ABR thresholds pre-and post-noise were compared between mice which were treated with an autophagy combination plus DMSO versus mice treated with DMSO alone. Significantly more recovery thresholds were observed post-noise for mice treated with a specific autophagy combination. This finding was also strengthened when comparing HC and ribbon synapse survival in treatment versus the control group.
Conclusions: Certain autophagy compound combinations protect against HC loss after noise exposure. The semi-circular canal model presents a reliable and reproducible approach for continuous drug delivery transport to the cochlea. Moreover, our study evaluated autophagy compound combinations that have not been tested previously on HCs and ribbon synapses. Our findings could serve as basis for further studies with these compounds as potential drug targets.

TU165. Open Board

TU166. Using Tones in Wideband Noise to Investigate Effects of Neural Fluctuations and Local Off-CF Inhibition in the Inferior Colliculus: Models and Physiology

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Category: Midbrain: Structure and Function
Background: Many mechanisms in the auditory periphery and midbrain impact the representation of complex sounds in the inferior colliculus (IC). This project focuses on two mechanisms: neural fluctuations and off-characteristic frequency (CF) inhibition. Neural fluctuations are low-frequency variations in auditory-nerve fiber rate that are shaped by peripheral nonlinearities, such as inner-hair-cell transduction and

cochlear compression. Most IC cells are excited (band-enhanced, BS) or suppressed (band-suppressed, BS) by these fluctuations. For tone-in-noise (TIN) stimuli, fluctuation amplitudes in auditory-nerve fibers tuned near the tone frequency are reduced. A recent narrowband TIN study found that BE IC rates decreased when tone levels increased, and BS IC rates increased when tone levels increased (Fan et al., 2021, HearRes 409:108328). These results are consistent with neural fluctuation sensitivity and could be explained by an IC model featuring same-frequency inhibition and excitation (SFIE). In this study, a different pattern of responses was observed for wideband TIN. These new results suggested a role for off-CF inhibition, which has been proposed to explain other response properties, such as sensitivity to frequency modulation. Here, we tested the hypothesis that off-CF inhibition in combination with fluctuation sensitivity can account for both narrowband and wideband TIN responses in IC.
Methods: Extracellular single-unit responses were recorded from the IC in awake Dutch-belted rabbits. Wideband TIN stimuli consisted of a four-octave Gaussian noise band with a tone that varied in frequency across three octaves centered on the CF of the target neuron. For comparison, to narrowband TIN stimuli consisting of 1/3-octave noise and a tone centered at CF were also recorded. Stimuli were varied in spectrum level and signal-to-noise ratio and were presented binaurally or through the contralateral ear. Computational models for IC responses consisting of same-frequency inhibition and excitation were expanded to test the hypothesis that off-CF inhibition can account for the wideband TIN responses.
Results: When a tone near CF was added to a wideband noise, both BE and BS cells responded with increased rates. Responses decreased with the addition of an off-CF tone. The SFIE model did not predict this result accurately, instead predicting the trends observed in response to narrowband TIN. The SFIE model was modified by adding off-CF inhibitory inputs to from other IC cells. BE responses simulated by this model accurately predicted both the decrease in rate when tones were added to narrowband noise and the increase in rate for wideband TIN responses.
Conclusions: Responses in the IC to wideband TIN were consistent with a model that contains both off-CF local inhibition and sensitivity to neural fluctuations. Ongoing work will expand the computational model to include BS cell responses.

TU167. Brainstem Speech Encoding is Dynamically Shaped Online by Fluctuations in Cortical α State

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Category: Midbrain: Structure and Function
Background: Experimental evidence in animals demonstrates cortical neurons innervate subcortex bilaterally to tune brainstem auditory coding. Yet, the role of the descending (corticofugal) auditory system in modulating earlier sound processing in humans during speech perception remains unclear.
Methods: Here, we measured EEG activity as listeners performed speech identification tasks in different noise backgrounds designed to tax perceptual and attentional processing. We hypothesized brainstem speech coding might be tied to attention and arousal states (indexed by cortical α power) that actively modulate the interplay of brainstem-cortical signal processing.
Results: When speech-evoked brainstem frequency-following responses (FFRs) were categorized according to cortical α states, we found low α FFRs in noise were weaker, correlated positively with behavioral response times, and were more" decodable" via neural classifiers.
Conclusions: Our data provide new evidence for online corticofugal interplay in humans and establish that brainstem sensory representations are continuously yoked to (i.e., modulated by) the ebb and flow of cortical states to dynamically update perceptual processing

TU168. Modulation of Medial Geniculate Neurons by VIP Signaling: Roles of VIP Receptors and Sources of VIP Input

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Category: Midbrain: Structure and Function
Background: Neurons in the medial geniculate (MG), the thalamic relay nucleus of the ascending auditory pathway, express receptors for vasoactive intestinal peptide (VIP), a neuropeptide known to play important neuromodulatory roles in several brain regions. We uncovered that VIP neurons in the inferior colliculus (IC), a class of glutamatergic principal neurons, project to the MG and express VIP mRNA, suggesting that these neurons are a source of VIP signaling to MG neurons. VIP signaling potently influences neuronal

excitability in the somatosensory thalamus, but whether VIP signaling plays a similar role in the MG remains unknown. Based on these data, we hypothesized that VIP signaling modulates the excitability of MG neurons via VIP receptors (VIPRs), and that IC VIP neurons are a major source of VIP signaling in the MG.
Methods: To test this hypothesis, we used brain slice electrophysiology, pharmacology, immunofluorescence, and retrograde tracing in MG slices prepared from C57BL/6J, VIP-IRES-Cre x Ai14, and VIP-IRES-CRE x Ai32 mice of both sexes.
Results: We found that puff application of 2 µM VIP elicited depolarization in many MG neurons. Using pharmacology during current-clamp recordings, we found that bath application of PG 99-465, a VIPR2 antagonist, led to a decrease in the effect of VIP in MG neurons, suggesting that VIP signaling in the MG is mediated in part by VIPR2s expressed by MG neurons. In addition, we are using retrograde tracers to determine the sources of VIP signaling to the MG. Our preliminary retrograde tracer data confirm that the MG receives input from VIP neurons in the IC, suggesting that IC VIP neurons are a major source of VIP signaling in the MG.
Conclusions: Our data show that VIP elicits depolarization in many MG neurons, and that this effect is mediated in part by VIPR2s expressed by MG neurons. Furthermore, our preliminary retrograde tracer data suggest that IC VIP neurons are a major source of VIP signaling in the MG. Overall, our results suggest that VIP signaling from the IC and possibly other brain regions is well positioned to modulate auditory processing in the MG. Using VIP-IRES-CRE x Ai32 mice to express Channelrhodopsin2 in VIP neurons, we are now working to determine whether optogenetic stimulation of VIP terminals elicits endogenous release of VIP in the MG.

TU169. T-Stellate Neuron Synapses Onto NPY and VIP Neurons in the Inferior Colliculus Undergo Short Term Depression

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Category: Midbrain: Structure and Function
Background: The inferior colliculus (IC) is the auditory processing hub of the midbrain and is important for the processing of speech and other vocalizations. T-stellate neurons within the ventral cochlear nucleus (VCN) represent one of the major projections to the IC. T-stellate neurons send information to the IC about sound frequency and amplitude have been implicated in the processing of vocalization cues. However, specific neuronal populations that T-stellate cells target in the IC, and how T-stellate cells contribute to IC cell excitability, are still widely unknown. Recently, our lab identified two novel classes of IC neurons: glutamatergic VIP neurons and GABAergic NPY neurons that together represent ~55-75% of stellate cells within the IC.
Methods: With whole cell path clamp recordings and optogenetic circuit mapping, we tested the prevalence and dynamics of T-stellate cell projections to both VIP and NPY neurons to determine how T-stellate neurons influence distinct neuronal populations in the IC.
Results: We show for the first time that both VIP and NPY neurons receive functional synaptic input from T-stellate cells. Optogenetic stimulation of T-stellate terminals within the IC elicits excitatory post-synaptic potentials (EPSPs) in NPY and VIP neurons, and these EPSPs undergo short term synaptic depression. Interestingly, the EPSPs evoked by T-stellate input to NPY neurons are typically larger in amplitude than those evoked in VIP neurons. In addition, activation of T-stellate terminals elicited both direct excitation and feedforward inhibition in both NPY and VIP neurons, suggesting that T-stellate afferents may recruit local inhibitory circuits in the IC.
Conclusions: Together, these results show that T-stellate neurons functionally project to both excitatory and inhibitory populations within the IC, and these projections may also involve local circuitry. These T-stellate projections to the IC may be involved in IC selectivity for sound cues, and future steps include in vivo modulation of T-stellate projections to determine how these VCN neurons contribute to sound frequency and amplitude selectivity within NPY and VIP neuron populations.

TU170. Generation and Validation of Synthetic Data for Simulation-Based Inference of Middle-Ear and Conductive Pathologies

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Category: Middle and External Ear
Background: Conductive loss stems from a diverse set of possible pathologies, such as ossicular fixation (OF), ossicular disarticulation (OD), or superior-canal dehiscence (SCD), each of which requires a different clinical treatment. Noninvasive measures would be valuable to assess the middle-ear (ME) status, to reduce uncertainties about the diagnosis prior to surgery, and to monitor postoperative outcomes. Wideband tympanometry (WBT) could become a cost-effective tool for noninvasively diagnosing ME pathologies. However, the task of mining complex WBT datasets for a reliable indicators of ME pathologies has proven challenging. Machine learning (ML), with its powerful pattern-recognition and classification capabilities, may provide a reliable tool for doing this. However, only very limited attempts have been made thus far to incorporate ML into ME assessments, mainly due to the lack of large labeled WBT datasets with confirmed pathologies required to train ML algorithms.
Methods: To account for the lack of sufficient pathology-identified training data, we propose to use synthetic WBT responses from anatomically realistic finite-element (FE) models of the human ear with verified mechanistic behavior. Randomly varying the material properties of the FE model within normal and beyond-normal ranges mimics normal and pathological conditions while accounting for inter-subject variability, age-related changes to ME structures, and measurement noise. An inference neural network (NN) was trained on this range of model parameters and responses to produce probability distributions for parameter values. Since the model parameters are explicitly mapped to ME anatomical components, their values provide quantitative assessment of the ME status.
Results: Preliminary results show the capability of the NN to provide probability distribution of the model parameters corresponding to the normal and pathological ME conditions. For instance, the NN can identify in the FE model simulated cases for the mallear-ligament stiffness higher than the normal OF case, the ossicular-joint stiffness lower than the normal range OD case, and cochlear load lower than the normal range for an SCD case. The pathological cases so far have been obtained from FE simulation of known ME parameters and we plan to confirm the validity of these cases in comparison with confirmed labeled clinical data available (e.g., https://www.science.smith.edu/wai-database/).
Conclusions: The inference NN method is a promising tool to quantify the ME parameters which represent its status. From the probability distribution of these parameters, it should be possible to determine whether the ME is normal or pathological and, if pathological, which anatomical structure(s) the pathology stems from. Validation of synthetic data used to train such ML application is crucial to perform transfer learning to the limited available clinical WBT data of confirmed pathological cases.
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TU171. Mesoscale Selective Plane-Illumination Microscopy for Thickness Map of Sub-Layers of the Human Tympanic Membrane

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Category: Middle and External Ear
Background: Acoustic stimuli are converted into vibrations of the middle-ear ossicular chain by the tympanic membrane (TM). The human TM is characterized by a cone shape and multi-layered histological architecture with epidermal, fibrous, and mucosal layers. The vibrational 3D-motion pattern of the TM is inextricably tied to the geometrical and mechanical properties of the TM. Two fiber layers, each of which features a different orientation of collagen fibers, i.e., along the radial or circumferential direction, give rise to the characteristic orthotropic material properties of the TM. The thickness of the sub-layers, and hence the local radial and circumferential stiffnesses, are not uniform across the TM. The thickness maps of the sub-layers of the entire TM would therefore benefit improvement of numerical model simulations as well as surgical reconstructions of the TM. However, it has been proven either impossible or impractical to acquire the sub-layer thickness maps of the TM with previously employed imaging techniques. In this work, we present a novel imaging approach based on mesoscale selective plane-illumination microscopy (mesoSPIM) to obtain the sub-layer thickness maps of the entire, intact human TM.

Methods: The TM with the malleus and a small portion of peripheral bone surrounding the tympanic annulus was extracted from fresh-frozen human cadaveric temporal bones, to maintain the integrity and shape of the isolated TM. Novel tissue-clearing protocols were applied to the sample, followed by immunolabeling of collagen fibers. The TM was subsequently imaged with a custom-made mesoSPIM setup, which provided near-isotropic sampling (~5 µm in all directions) via an axially scanned light sheet at three excitation wavelengths (488 nm, 561 nm, and 640 nm). Image processing was performed on a high-performance computing platform to accommodate the large datasets in the order of ~1 terabyte.
Results: The slice images showed a clear distinction of the epidermal, fibrous, and mucosal layers of the TM. Morphometric parameters which could be obtained from the imaging datasets included the total thickness, sub-layer thickness, and fiber orientation. The data indicated a variation of the sub-layer thicknesses across the TM, which is consistent with previous investigations reporting data from different sub-regions of the TM.
Conclusions: The proposed methodology establishes a suitable approach to obtain sub-layer thickness maps of the entire, intact human TM. The sub-layer thickness maps are used to develop more accurate and precise numerical model simulations and will be compared to vibrational 3D-motion data of the same isolated TM samples under acoustic stimulation. This will provide a more comprehensive understanding of the link between the morphometry and the vibrational 3D-motion of the TM, which is key for the improvement of surgical reconstructions of the TM.

TU172. Manufacturability of a Tetraethyl Orthosilicate-Based Thixogel for Use as a Single Application Otitis Externa Therapeutic

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Category: Middle and External Ear
Background: Otitis externa, also known as outer ear infection, is a frequent affliction in both humans and animals. The most prevalent treatment for otitis externa is ear drops, but it is difficult to adhere properly to this treatment, causing poor patient compliance and the potential for complications. As a result, we have developed a tetraethyl orthosilicate-based thixogel for use as a single application treatment for otitis externa to increase ease of use and improve patient outcomes. We investigated the manufacturability of the thixogel, focusing on several key aspects: formulation repeatability and reproducibility, material source and tolerances, release of a variety of model drugs, and impact of application-specific physiological factors, specifically local pH and enzymatic activity on drug release.
Methods: Thixotropic thixogels were prepared by activating tetraethyl orthosilicate (hTEOS) with hydrolysis and combining with either aqueous hyaluronan (HA) or phosphate buffered saline and adjusting the pH of the solution to ~7.65. Thixogel properties (elastic modulus, thixotropy, drug release, dry substance) were tested for thixogels made with TEOS and HA from different manufacturers, different concentrations of aqueous HA, and thixogels made by adjusting the pH to a range of values. Elastic moduli and thixotropy were both measured using a rheometer. Dry substance was measured using a moisture analyzer. Drug release was determined using in vitro models, wherein the amount of drug released into PBS at 37 °C was measured using absorbance or fluorescence, depending on the drug. Fluorescein, fluorescein disodium, green fluorescent protein, and blue dextran (5000, 20000, and 500000 g/mol) were all used as model drugs. The effect of drug encapsulation versus entrapment on drug release profiles form thixogels with fluorescein and fluorescein disodium was also tested. The impact of ear canal-specific physiological factors, pH and enzymic activity, on drug release was evaluated using fluorescein. A standardized commercial in vitro skin irritation test was used to evaluate the biocompatibility of the thixogels.
Results: Thixogel properties remained consistent regardless of HA or TEOS manufacturer, aqueous HA concentration, and over a wide range of pH levels (7.3 to 8.0). Model drug properties, including molecular weight, and logP, impacted their release. The effect of the loading method varied depending on model drug, with fluorescein having no difference in release between the methods, while fluorescein disodium displayed distinct release profiles, likely due to the differences in logP. Physiological factors did not seem to have an impact on model drug release profiles.
Conclusions: Our results indicate that these thixogels are well suited for production and scalability, as they have a robust manufacturing process, have a wide tolerance for pH level, release a variety of model drugs (small molecule, protein, and polymer), and are not impacted by outer ear canal-specific physiological factors.

TU173. Spontaneous Healing of Acute Total Tympanic Membrane Perforations in Rats With or Without Excision of the Mallear Handle

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Category: Middle and External Ear

Background: We identified the keratinocyte proliferation centers of ears with acute total perforations with and without excision of the mallear handle in Sprague-Dawley (SD) rats, and explore the effect of excision of the mallear handle on hearing.

Methods: Bilateral, acute, total tympanic membrane (TM) perforations were created in 33 male SD rats; the mallear handle was removed from the right ears but preserved in the left ears. Each TM was endoscopically photographed daily until the perforations closed and auditory brainstem response thresholds were determined in18 rats immediately pre- and post-perforation, and 1 month after perforation. Three rats were sacrificed on days 3, 5, 7, 9, and 11 after perforation and both TMs were histopathologically evaluated.

Results: Endoscopically, all 18 perforations closed completely in both ears. The mean time to closure was 6.83 ± 0.85 days in preserved ears and 8.50 ± 0.71 days in excised ears; the difference was significant ($P < 0.001$). Strong keratinocyte proliferation commenced at the annulus and the side of the mallear fold in excised ears but at the annulus, the mallear handle, and the side of the mallear fold near the perforation in preserved ears. Thus, proliferation was evident near the mallear fold in both groups. The upper halves of the perforations healed more rapidly than the lower halves in all rats, and the posterior halves more rapidly than the anterior halves in 28 ears of both groups (77.8%, 28/36). No significant difference was found among two ears regardless of pre- or post-perforation. However, after the closure of perforation, the ABR thresholds in the handle-excised ears were significantly higher than that of the handle-preserved ears at 16 kHz ($P=0.029$) and 32 kHz ($P =0 .017$), while the difference wasn't significant at the remaining frequencies among the two ears. In addition, significant difference of the threshold shift between pre-perforation and perforation closure was found at 16 kHz ($P =0 .011$) and at 32 kHz ($P = .017$) among the two ears.

Conclusions: The mallear fold region may be the principal keratinocyte proliferation center that plays the major role in TM healing in SD rats.The injury of mallear handle prolong the closure time of perforation with worse recovery of high frequencies hearing but don't affect the closure.In addition, the mallear fold should be avoided during myringoplasty in clinic.

TU174. Altered Secretome by Diesel Exhaust Particles and Lipopolysaccharides in Primary Human Nasal Epithelium and Its Correlation in Otitis Media: A Secretome Analysis and Cohort Validation Study

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Category: Middle and External Ear

Background: Airway epithelial cells can be active participants in the defense against environmental pathogens by producing signaling and regulatory molecules to elicit local or systemic inflammation. Our goal in this study was to investigate the effect of diesel exhaust particles (DEPs), a main component of urban air pollution with particulate matter, alone or in combination with lipopolysaccharide (LPS), a known stimulant from Gramnegative bacteria, on the secretome in the primary human nasal epithelium (PHNE) and otitis media (OM).

Methods: Primary human nasal epithelial cells were cultured at an air–liquid interface (ALI) to create a differentiated in vivo-like model of the epithelium at ALI day12 and then exposed to DEPs (particulate matter <4 μm) or lipopolysaccharide (LPS) alone (mono-exposure) and DEPs plus LPS (co-exposure) at the apical side of the PHNE. The bottom media of the PHNE exposed to DEPs and/or LPS were collected to be defined the alteration of secreted proteins (secretome) using label-free based quantitative proteomics. We further confirmed expression of proteins selected from the secretome data in independent experiments using ELISA, immunocytochemistry and Western blot. Middle ear effusion samples from children with Otitis media were obtained. We obtained regional air pollution data of PMs from the National Ambient Air Monitoring Information System (NAMIS) which is provided to the public through Air Korea (http://www.airkorea.or.kr/web)(KECO, 2020a)

Results: In total, 658 specific human proteins were quantified in the secretome of PHNE, of which 32 were differentially expressed in response to DEP exposure alone and 58 were differentially expressed in response to DEP plus LPS co-exposure. LPS shifted the DEP-induced secretome alterations toward an increase in cell adhesion, movement, migration, and motility. Some canonical pathways related to inflammation and cancer were involved in the secretome alterations induced by DEPs and/or LPS . β-catenin and ERK were downregulated and p53 was upregulated by exposure to DEPs plus LPS. Among the differentially expressed secreted proteins, leukemia inhibitory factor (LIF) was also detected at a high level in the MEEs of OM patients, and LIF levels in IL17C-negative MEEs of children with OM were positively correlated with the average daily atmospheric particulate matter concentration 8 days before sample collection.
Conclusions: We demonstrated that the apical stimulation with DEPs and LPS can significantly alter the basal secretome in PNHE, and the alteration of secretome can be reflected by surrounding inflammation with effusion of fluids in vivo such as MEEs in OM patients.

TU175. Crossmodal Compensation in Partial Hearing Loss: An Eeg Comparison Between Verbal and Non-Verbal Visual Processing

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Category: Multisensory Processing/Interactions
Background: Auditory deprivation is associated with intra-modal plasticity that modifies connections in visual cortex and crossmodal plasticity where auditory cortical neurons are repurposed to respond to visual stimuli. These changes are thought to underpin improvements for visual abilities in deafness such as motion discrimination, face discrimination, and visual localization. However, less is known about visual plasticity under conditions of partial or incomplete hearing loss, such as age-related hearing loss (ARHL), where auditory cortices still receive some level of afferent input. While some studies have shown evidence of crossmodal plasticity in adults with partial hearing loss that negatively relates to speech-in-noise (SIN) listening, it is unclear whether cortical activation to visual stimuli is dependent on stimulus type (e.g., verbal, non-verbal). Should SIN ability and hearing loss scale differently with verbal compared to nonverbal stimuli, this might reveal functionally relevant effects of visual plasticity on speech communication. The present study aims to determine if changes in visual evoked potentials (VEP) amplitudes and latencies in individuals with partial hearing loss depend on the verbal or non-verbal nature of the stimulus. We predict that individuals with poorer SIN perception will have larger and earlier VEPs for verbal stimuli opposed to non-verbal stimuli.
Methods: Participants aged 40 to 80 underwent pure-tone audiometry to 8 kHz, and QuickSIN was used to measure SIN listening. Participants were then fitted with a 64-channel EEG montage and seated in a sound-attenuated room to limit distractions. Electrocortical activity was recorded while participants viewed visual verbal and non-verbal motion stimuli. The verbal stimuli included alternating frames of two Preston Blair visemes to mimic a single-syllable utterance. The non-verbal motion stimuli consisted of a radially modulated circle-star pattern that gives rise to apparent motion. For each stimulus type, stimuli were shown in five blocks for a total of 250 repetitions.
Results: Data collection is ongoing.
Conclusions: These findings will potentially clarify how cross-modal plasticity expresses under conditions of hearing loss depending on stimulus content, and how it relates to speech-in-noise outcomes. For instance, research in adults with ARHL has shown a strong negative correlation between VEP N1 latency and speech-in-noise perception scores (Campbell and Sharma 2014, PLOS ONE, 9(2), e90594), indicating that visual plasticity may interfere with speech intelligibility or that listeners with ARHL and poor SIN rely on visual cues more frequently. We hope to determine whether these relationships depend on the relevancy of the stimulus for speech communication.

TU176. Intra-Cortical Mechanisms for Integration of Auditory and Olfactory Information

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Category: Multisensory Processing/Interactions
Background: In complex environments, the brain must integrate information from multiple sensory modalities, including the auditory and olfactory systems. However, little is known about how the brain

integrates auditory and olfactory stimuli. Here, we investigated the mechanisms underlying auditory-olfactory integration using anatomy, electrophysiology, and behavior.
Methods: We first used viral tracing strategies to investigate the circuits underlying auditory-olfactory integration. We next developed an experimental system for delivering combinations of auditory and olfactory stimuli during in vivo electrophysiology, and tested the effect of odor stimuli on auditory cortical responses to sound in awake mice. Finally, we trained mice on a sound-detection Go/No-Go task, as well as a sound- and odor-detection task, to assess how odor stimuli affect auditory perception and behavior.
Results: Our results demonstrate direct inputs to the auditory cortex (ACx) from the piriform cortex (PCx), mainly from the posterior PCx, suggesting an anatomical substrate for olfactory integration in ACx. In awake mice, odor stimuli modulate the responses of ACx neurons in a stimulus- and sound level-dependent manner, suggesting a neural substrate for olfactory integration in ACx. In behaving mice, odors modulate sound detection thresholds in a stimulus intensity-dependent manner.
Conclusions: Together, our findings reveal novel mechanisms for auditory-olfactory integration involving the ACx.

TU177. Measuring the Impact of Auditory System Impairments on Eye-Movement-Related Eardrum Oscillations (EMREOs)

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Category: Multisensory Processing/Interactions
Background: The addition of visual cues improves auditory processing abilities, particularly in spatial tasks. Accounting for eye movements is critical to linking vision and spatial hearing because every eye movement shifts the relative relationship between visual and auditory reference frames. Each eye movement requires an update of incoming sensory information in order to integrate the two sensory inputs. Numerous neurophysiological studies have revealed eye movement-related modulation of the auditory pathway. We previously identified a unique type of low frequency otoacoustic emission that accompanies eye movements. These eye movement-related eardrum oscillations (EMREOs) can occur in the absence of external sound and carry precise information about saccade magnitude, direction, and timing (Gruters et al 2018, Murphy et al 2020).
Methods: Here we evaluate the degree of reproducibility of different EMREO response parameters within the normal hearing population. For example, what are the differences and similarities between left and right ears? Is response reproducibility altered when ear canal recordings are analyzed based on location of the visual targets (instead of left vs right ear)? In other words, do saccades to some spatial targets generate more consistent changes in ear canal pressure than others?
Results: We compare the results of EMREO analysis from our normal hearing population to responses in individuals with different hearing pathologies to assess the impact of hearing loss on the EMREO. Two candidate auditory motor systems may be involved in generating EMREOs: the middle ear muscles and/or the cochlear outer hair cells. Examining EMREO responses in hearing systems with different anomalies can aid in understanding how these eye movement-related effects in the auditory periphery contribute mechanistically to hearing.
Conclusions: Our previous work showed EMREOs are abnormal in subjects with hearing impairment, most commonly being abnormally small in individuals who have impaired outer hair cell or stapedius function. Here we extend this work by comparing responses from a larger pool of subjects with auditory system impairments. In addition to determining the physiologic drivers of EMREOs, future work is needed to assess if individuals with abnormal EMREOs have specific impairments in the perceptual process of integrating visual and auditory spatial information.

TU178. Distinct Timescales of Audiovisual Speech Statistics in the Delta and Theta Frequency Bands

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Category: Multisensory Processing/Interactions
Background: Auditory cortical activity tracks speech modulations in the delta (1-4 Hz) and theta (4-7 Hz) frequency bands, but the roles and roots of these privileged neural frequencies remain unclear. Speech modulations in the theta range are evident in the envelope spectrum of natural speech signals and coincide with the periodicity of open-close cycles of the mouth during natural speech. Yet, energy in the speech

envelope spectrum falls off below ~3 Hz and acoustic features corresponding to delta synchronisation are less obvious.
Methods: Here, we analysed audiovisual speech statistics in a large video dataset (>4000 speakers) of natural speech (LRS3). Specifically, we used regularised canonical correlation analysis (CCA) to relate bandpass speech envelopes and facial landmarks extracted from the speech video. A cross-validation scheme was used to identify audiovisual correlations that generalise across speakers in the dataset. By linearly recombining envelope frequency bands, CCA can be used to perform a decomposition of the envelope spectrum conditioned on correlation with the visual signal. The weighted combination of modulation filters learned by CCA can then be visualised as a set of modulation transfer functions for the speech envelope.
Results: The analysis revealed different sources of audiovisual correlation in different parts of the speaking face at distinct temporal scales. One set of correlation components captured envelope fluctuations in the 3-4 Hz range correlated with mouth movements, as expected. A second set of components captured lower envelope frequencies in the 1-2 Hz range correlated with global head and face movements. These head and face movements performed during speech were thus consistently correlated with envelope fluctuations in the 1-2 Hz range across speakers. The lower frequency components were only consistent in natural audiovisual speech and were not apparent in simpler audiovisual speech material of matrix sentences (GRID).
Conclusions: Energy around 3-4 Hz dominate the envelope spectrum of natural speech signals. Here, a lower frequency mode was revealed by considering audiovisual speech statistics. The two distinct timescales of audiovisual speech statistics align notably with the delta and theta frequency bands and could indicate that cortical envelope sensitivity at these rates relate to motor constraints.

TU179. Cortical Responses Reflecting Comprehension Abilities Evoked With Auditory, Visual, and Visual-Then-Auditory Stimuli: Normal-Hearing Participants Vs. Cochlear Implant Users

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Category: Multisensory Processing/Interactions
Background: Cross-modal cortical reorganization is known to occur in individuals with untreated hearing loss. However, the research literature is still unclear about the effects that the regular use of hearing devices such as cochlear implants (CIs) have on this phenomenon. Here, we explored the effects of cross-modal plasticity on comprehension-related abilities in experienced CI users.
Methods: Using multichannel electroencephalography, we tested individuals who had used CIs for at least two years as well as age-matched controls (N = 2 × 13). Using digit triplet stimuli, we evoked cortical responses known to reflect comprehension abilities, that is, the N400 and Late Positive Complex (LPC) responses. The participants were tested in auditory, visual, and visual-then-auditory stimulus conditions.
Results: Comparison of the response amplitudes and latencies from three stimulus conditions is expected to provide insights into cross-modal plasticity. The absence of any group differences would indicate that regular CI use can reverse the effects of cross-modal reorganization caused by untreated hearing loss. Alternatively, if group differences are observed, this would indicate no or only a partial reversal due to CI treatment. In the case of observable group differences, we would expect larger amplitudes and longer latencies in the CI group. Such differences would be indicative of more cognitive processing related to stimulus 'repair' required to compensate for the degraded auditory input from the CI device.
Conclusions: The results are expected to provide insights into how CI treatment affects late cortical processes reflecting the comprehension of auditory and visual input signals.

TU180. Electric Acoustic Integration in Bimodal Cochlear Implant Users

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Category: Other, Auditory and Cognitive Processing
Background: Bimodal subjects receive electric stimulation through a cochlear implant (CI) and acoustic stimulation on the opposite side through their residual acoustic hearing. Bimodal CI users can combine electric and acoustic information to improve speech understanding. However, the observed benefit presents high variability across subjects. This variability may be associated with the effectiveness of the integration between electric and acoustic stimulation. The effectiveness of bimodal integration might be affected not

only by individual characteristics of the user but also by the time processing delay or frequency mismatch between the CI and the acoustic side. Moreover, there are still debates whether bimodal CI users can obtain binaural integration by combining electric and acoustic stimulation across opposite ears.
In the first study, we investigated methods to assess bimodal integration. A behavioral paradigm based on speech understanding performance and an electrophysiological paradigm based on selective attention decoding were conducted in bimodal CI users. Speech material for both paradigms were presented to the CI side (CIS) alone, the acoustic side (AS) alone and to both sides simultaneously (CIS+AS).
We proposed a novel integration measure between electric and acoustic stimulation in bimodal CI users based on electroencephalographic (EEG) cortical evoked potentials obtained from a selective attention decoding paradigm. This measure is based on subtracting the correlation coefficients of selective attention decoding obtained with CIS+AS from the sum of correlation coefficients obtained with CIS only and AS only. This integration measure showed a significant correlation with speech understanding performance calculated in the same way.
In the second study, we investigated the effect of temporal delay and frequency mismatch between electric and acoustic stimulation on cortical auditory evoked potentials (CAEPs). It was hypothesized that bimodal CI users might obtain binaural interaction component (BIC) measured through CAEPs and that the amplitude of the measured BIC changes with interaural delay or with frequency allocation changes of the CI.
Methods: First, CAEPs were measured when listening with CIS alone, AS alone and with both sides together (CIS+AS). When listening with CIS+AS, different delays between the stimuli presented electrically and acoustically were presented. Second, CAEPs were measured across different frequency allocation maps for CIs. Three maps were created based on 1) the clinical map, 2) the CI tonotopic frequencies estimated from computed tomography and 3) a pitch matching paradigm between acoustic stimuli and each individual CI electrode.
Results: To validate the hypothesis that BIC of CAEPs changes significantly with interaural delay and frequency allocation, pilot measurements were conducted in normal hearing listeners. The results of the pilot study demonstrated a reduction of BIC with an increase of the delay between both listening sides. Moreover, BIC is reduced when frequency mismatch between listening sides is introduced.
Conclusions: Measurements in Bimodal CI users are ongoing.

TU181. Investigating the Effect of Cognitive Abilities on Auditory Processing

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Category: Other, Auditory Processing
Background: Auditory processing disorders (APD) is one of the most intriguing topics in the field of communication disorders. One of the primary concern with using the traditional diagnostic test batteries for APD is that they are affected by language and cognition (Maggu et al., 2021; Vermiglio, 2018). As a result, recently there have been suggestions on using basic behavioral tests that may limit the effect of cognitive aptitude on auditory processing (Maggu et al., 2021). In the current study, we seek to understand the effects of cognitive abilities on the basic auditory processing abilities.
Methods: In the current study, we are aiming at recruiting 30 adult subjects (age range: 18-30 years) with hearing abilities within normative limits. For testing cognitive abilities, we are conducting the following subtests of the NIH cognition toolbox (Weintraub et al., 2013): picture vocabulary test, Flanker inhibitory control and attention test, list sorting working memory test, dimensional change card sort test, pattern comparison processing speed test, and picture sequence memory test. For testing of basic auditory processing abilities, we are using the following subtests from the Portable Automatic Rapid Testing (Gallun et al., 2018): gap discrimination task, spatial release from masking, frequency modulation, spectrotemporal modulation, and dichotic sentence identification.
Results: The study is currently ongoing, and we will have the final results analyzed by the end of the year. We will conduct correlations between the cognitive tasks and the tasks of auditory processing to understand the relationship between the two.
Conclusions: At the completion of the study, we will be able to comment upon the effects of cognitive abilities on auditory processing. The findings from the current study will have direct implications in making recommendations on whether a detailed testing of cognitive abilities is needed when evaluating for auditory processing via behavioral tasks.

TU182. Dimensionality of Natural Auditory Scene Perception: A Factor Analysis Study

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Category: Other, Auditory Scene Processing

Background: Theories of auditory and visual scene analysis have long suggested our perception of a scene relies on the identification and segregation of multiple objects within it, resembling a detail-oriented processing style. However, it is possible that a more global-oriented process may occur in parallel when we evaluate auditory scenes. There is evidence in the visual domain for global properties that enable scenes to be rapidly recognized, even in the absence of recognizing the individual objects comprising the scene. It is our understanding that a similar line of research has not been explored in the auditory domain. Therefore, we aimed to evaluate the contributions of high-level information (global, schematic information about scenes) and low-level information (acoustic properties of scenes) to auditory scene perception. A secondary aim is to increase the field's ecological validity by utilizing our collection of high-quality auditory scenes, which will be made available to other researchers via an online database.

Methods: To examine global processing of auditory scenes, participants were presented with 200 scenes and asked to rate each scene on a series of 8 global properties (e.g., open vs. enclosed, outdoor vs. indoor, natural vs. human-influenced, temperature, season, transience, navigability, and sparseness). An extensive acoustic analysis was also conducted on each scene to determine which low-level features may predict different global property ratings.

Results: To evaluate the dimensionality of scene perception, we submitted the acoustic measures and average global property ratings of each scale to separate exploratory factor analyses (EFAs). The EFA of the acoustic measures revealed a seven-factor structure explaining 57% of the variance in the data, while the EFA of the global property measures revealed a two-factor structure explaining 64% of the variance in the data. Eight linear regression analyses were calculated to predict average performance on each global property rating scale based on the acoustic measures, which revealed each global property rating was significantly predicted by at least one acoustic variable (p's $\leq .05$), but the acoustic variables did not completely predict global property ratings (R-squared = 0.33-0.87) .

Conclusions: These results provide preliminary evidence for the ability to perceive auditory scenes from a global perspective. Furthermore, some of the acoustic measures predicted performance on the global property rating task but not completely, indicating global variables may be processed at a high level where acoustic features have been abstracted out or have non-linear relationships with global variables, which is suggested to occur in the ventral visual stream. The results of this study and the open availability of our scene collection will make it possible for us and other researchers to conduct further behavioral, computational modeling, and neural studies on all facets of perception, attention, and memory for natural auditory scenes.

TU183. Bone Conduction Thresholds of Normal Hearing Subjects at Extended High Frequencies

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Category: Other, Bone Conduction

Background: Measurement of bone conduction (BC) hearing thresholds at extended high frequencies (EHF; above 8 kHz) is of clinical interest but is technically complicated by limitations in standard BC transducer output, a lack of calibration standards and sparse prior human subject data with hearing level referenced to a small cohort of 'normal' subjects. Herein, we measure EHF BC thresholds using a Tascam HP-F200, a magnetostrictive BC transducer, with sufficient high frequency output at frequencies as high as 16 kHz. Using a novel BC transducer calibration paradigm (Remenschneider et al. JASA 2022; 151(5):2945-66), we report results in terms of BC transducer force output in dB re 1μN.

Methods: Subjects with standard frequency air (AC) and bone (BC) conduction thresholds ≤20dB, normal otoscopy and no history of middle-ear disease were recruited for extended high frequency AC testing with Sennheiser HDA 200 earphones, and BC testing with a calibrated Tascam BC transducer. A clinical audiologist obtained AC thresholds at 8, 9, 10, 11.2, 12.5, 14 and 16kHz in both ears and unilateral unmasked, unoccluded BC thresholds in either the left or the better hearing ear. BC threshold force levels (in

dB re 1μN) were derived from computed audiometer output voltages via a previously described calibration scheme.
Results: Fifteen subjects, 6 female, mean age 32 years (range 18-45) met inclusion criteria and were enrolled. We identified median force thresholds in dB re1μN at 8kHz: 42dB, 9kHz: 41dB, 10kHz: 44B, 11.2kHz: 43dB, 12.5kHz: 33dB, 14kHz: 41dB and 16kHz: 58dB. The full range of EHF BC thresholds increased with increasing frequency, but the interquartile range (±25% around the median) generally was between 10 and 20dB. AC thresholds showed a similar increased range with increasing frequency between subjects. In four subjects who underwent repeat EHF BC testing on separate days, test re-test variability was within 5-10dB at all frequencies, including 14 and 16khz.
Conclusions: EHF BC thresholds obtained with a Tascam BC transducer are reported in terms of transducer force output in dB re 1μN, enabling threshold comparisons across studies and transducer types. A range of EHF AC and BC thresholds are observed across subjects with normal standard frequency hearing. Test-retest variability of EHF BC thresholds appear similar to standard frequency AC and BC threshold testing. Our reported EHF BC thresholds are consistent with historical measures of EHF BC thresholds in similar subjects using an alternative BC transducer.

TU184. Successful Central Auditory Testing in Young Tanzanian Children and Its Relationship to Cognitive Function

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Category: Other, Central Auditory Function
Background: Results on central auditory tests (CATs) correlate with cognitive function in adults with HIV. This has not yet been demonstrated in children living with HIV (CLWH). This study evaluated a) the feasibility of using CATs in young children, and b) the relationship between CATs and cognitive performance in CLWH and controls. Traditionally, drawing conclusions from CATs administered to children younger than 7 is discouraged. Understanding if young children can complete these tests reliably and whether the test results correlate with neurocognitive function is important for using these tests to evaluate children. We examined completion rates and performance on CATs at the first visit in a cohort of both CLWH and HIV-negative children and related this to cognitive function.
Methods: 486 children (ages 4.5 to 7.5 years at first visit) performed CATs and neurocognitive tests as part of a larger longitudinal study in Dar es Salaam, Tanzania. CATs included the Triple Digit Test (TDT), the Hearing in Noise Test (HINT), the Gap Detection Test (Gap), and the Staggered Spondaic Word Test (SSW). All participants also completed a test of nonverbal intelligence and overall cognitive function (the Leiter International Performance Scale—3rd Edition (Leiter-3)). Data from the first visit were analyzed. Binomial logistic regression examined the likelihood of completing each of the four CATs and included age, HIV status, and Leiter-3 composite scores as predictors. Multiple linear regression using those same predictors assessed factors associated with performance on these CATs amongst those children who were able to complete each test. Socioeconomic status and gender were not included in the models because they were not correlated with any central auditory measures.
Results: Completion rates for the CATs were generally high. On their first visit, 362 children (74.5%) completed the TDT, 345 (71.0%) completed the HINT, and 348 (71.6%) completed the SSW. Gap completion rates were much lower—only 3 children (0.6%) completed the gap at their first visit. Completion rates increased with age. Completion of the SSW was related to HIV status; CLWH were slightly more likely to complete this test. Cognitive domains associated with completion of each specific CAT varied; however, completion of each CAT was associated with at least one neurocognitive domain. Factors associated with better performance on CATs included increased age, HIV negative status, higher nonverbal IQ, and faster processing speed.
Conclusions: Based on the high completion rates on the TDT, HINT, and SSW, administering these tests to children aged 4.5-7.5 years appears feasible. Each central auditory test was strongly related to cognitive performance, although the relationship to cognitive domains varied by test. Young children can complete most CATs reliably in Dar es Salaam and their CAT results correlate with measures of cognitive function.

TU185. Developing a Calibration Method for Reducing Wave Peak Amplitude Variability in Auditory Evoked Potentials

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Category: Other, Electrophysiology

Background: It is well known that current auditory evoked potentials (AEP) such as auditory brainstem response (ABR) and electrocochleography (ECochG) have limited diagnostic sensitivity, mainly due to high waveform variability. Previously, a permanent loss of cochlear synapses without a change of hearing threshold was found in noise-exposed or aging animal models. This cochlear synaptopathy can be detected by significant decreases in compound action potential/ABR wave-1 amplitudes in animals using sedation and subdermal electrodes. However, these effects translate poorly for clinical diagnosis mainly due to the use of non-invasive surface recording electrodes which yield high variability of ABR wave-1 amplitudes. Here, based on calibration methods used in other electrophysiological equipment, we designed an electronic circuit that generates a pulse signal during each time-locked ABR recording. The pulse signal is designed to be used as a calibration reference to adjust the measured amplitudes of AEP wave peaks and reduce amplitude variability.

Methods: The circuit was implemented in AEP recording montages using five different electrode configurations. ABRs were recorded from 14 adult CBA/CAJ mice (7 male) using the five electrode configurations in two different impedance conditions: good interelectrode impedances, where impedance values of non-inverting and inverting recording electrodes were 1 kΩ or less; and poor interelectrode impedances (i.e., >1 kΩ). To create a poor impedance, subdermal electrodes were inserted so that the electrode just pierced the skin. Calibrated ABR wave-1 amplitudes were compared between the five electrode configurations by measuring the correlation between the calibration pulse amplitudes and the interelectrode impedance for each configuration. We then measured the variability of raw wave-1 amplitudes and calibration pulse adjusted ABR wave-1 amplitudes across each electrode configuration.

Results: We identified one electrode configuration with a strong positive correlation between calibration pulse amplitude and interelectrode impedance, which also shows a significant reduction in the variability of adjusted ABR wave-1 amplitudes compared to raw wave-1 amplitudes.

Conclusions: The data demonstrate the feasibility and validity of our calibration approach to reducing the variability of repeated ABR recordings; however, further testing involving human patients and surface electrodes is required before it can be implemented in clinical practice.

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TU186. Readability of the American, Canadian, and British Otolaryngology- Head and Neck Surgery Societies' Patient Materials.

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Category: Other, General Otolaryngology

Background: Patient education materials across 3 national English otolaryngology-head and neck surgery (OHNS) societies: the American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNS), the Canadian Society of Otolaryngology-Head and Neck Surgery (CSOHNS), and Ear, Nose, and Throat United Kingdom (ENT UK) were examined to determine whether they are written at a level suitable for patient comprehension.

Methods: Readability was calculated using the Flesch-Kincaid Grade Level, Flesch-Kincaid Reading Ease Score, and Simple Measure of Gobbledygook Index. All public patient education materials available through the CSOHNS, AAO-HNS, and ENT UK websites were assessed. Patient education materials were grouped into categories by subspecialty.

Results: In total, 128 patient materials from the 3 societies were included in the study. All 3 societies required a minimum grade 9 reading comprehension level to understand their online materials. According to Flesch-Kincaid Grade Level, the CSOHNS required a significantly higher reading grade level to comprehend the materials presented when compared to AAO-HNS (11.3 vs 9.9; 95% CI, 0.5-2.4; $P < .01$) and ENT UK (11.3 vs 9.4; 95% CI, 0.9-2.9; $P < .01$). Patient education materials related to rhinology were the least readable among all 3 societies.

Conclusions: This study suggests that the reading level of the current patient materials presented through 3 national OHNS societies are written at a level that exceeds current recommendations. Promisingly, it highlights an improvement for the readability of patient materials presented through the AAO-HNS.

TU187. Harmonizing Independently Collected MRI Datasets for Greater Statistical Power in Hearing and Brain Imaging Research

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Category: Other, large datasets of brain imaging and hearing in humans
Background: The proliferation of machine learning and associated techniques has advanced our ability to gain insights and make inferences from datasets in a data-driven and model-free manner. Model free analysis offers significant advantages compared to traditional linear or structural modeling-based approaches because inherent limitations that accompany the adoption of a model are avoided entirely. Conversely, absent an assumed and testable model, both inference and validation must be performed from within the same dataset. This necessitates that the cardinality of the dataset be sufficiently large. Moreover, inferring robust, repeatable, and statistically significant results in a purely data-driven manner requires far more sizeable datasets than traditionally collected in a magnetic resonance imaging (MRI) based clinical study.
Methods: The Hearing Health Institute (HHI) has put together one of the first repositories to adopt this approach with its dataset constituted from four different MRI centers – University of Illinois at Urbana-Champaign (UIUC), Washington University at St. Louis (WASHU), Wilford Hall Ambulatory Surgical Center (WHASC), and Northwestern University (NU). Replete with brain imaging, demographic, behavioral and hearing health related data (threshold and supra-threshold audiometry, tinnitus-related questionnaires, etc.) this is one of the largest repositories of its kind dedicated to hearing health research. To account for differences in protocols and scanners, the HHI data has been processed using the most up to date and open-sourced technologies as prescribed by Brain Imaging Data Specification (BIDS) standards and Neuroimaging Preprocessing Tools (NIPREPS) community. This multimodal dataset is comprised of T1 and T2 weighted anatomical scans, functional scans, diffusion weighted images as well as scans corresponding to resting state and task-based functional MRI paradigms. Furthermore, the dataset's participants encompass healthy controls, tinnitus, and hearing loss populations as well as participants in whom the two conditions are co-morbid.
Results: To date we have completed harmonization on 289 participants' data from across UIUC, WASHU, NU and WHASC. These include imaging quality control, confound identification, regression and functional MRI preprocessing followed by normalization, standardization, parcellation and functional connectivity analysis. Additionally, we have included behavioral and audiological data linked with multimodal brain imaging data in a fashion directly compatible with BIDS standards. We are in the process of analyzing the impact of hearing loss and/or tinnitus on the combined data set allowing us to visualize differential impact across the lifespan. The basic dataset will eventually be publicly available.
Conclusions: While exceptionally large repositories like UK Biobank or Human Connectome Project offer candidate datasets for such data-driven analysis, they sometimes lack detailed clinical assessments needed to understand the neurobiology of health and disease. An ability to harmonize and amalgamate different data sources, including such clinical assessments, often from multiple independent studies, offers another, more economical way to increase the statistical power of inferred results while avoiding renewed spending.

TU188. Assessment of the Medial Olivocochlear Reflex Using Electrocochleography in Humans

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Category: Other, Olivocochlear Efferents - Electrocochleography
Background: The medial olivocochlear (MOC) reflex increases the conductance of the outer hair cells (OHCs), which results in a decrease in cochlear amplifier gain. This decrease in gain may protect the inner ear from noise-induced damage and improve the neural coding of speech in noisy backgrounds. Research in laboratory animals shows that the cochlear microphonic (CM) and compound action potential (CAP) – responses associated with electrocochleography (ECochG) – are influenced by MOC reflex activity. Specifically, CM amplitude increases and CAP amplitude decreases for probes presented in a quiet background when the MOC reflex is elicited. Further, an “anti-masking” effect is observed for CAPs

measured in the presence of ipsilateral noise when the MOC reflex is elicited. This study determines the sensitivity of ECochG to MOC reflex activity in young, normal-hearing humans for CM and CAP measurements made in the presence of a contralateral broadband noise (CBBN) elicitor.
Methods: We assessed the effects of presenting CBBN on the CM and CAP measured from a custom-made tympanic membrane electrode in normal-hearing young adults. Our primary outcome variable was the change in CAP or CM amplitude resulting from the introduction of CBBN. Experimental manipulations involved changing click/chirp rate, probe frequency, or CBBN level, and including/excluding ipsilateral noise.
Results: We observed a consistent increase in the amplitude of the CM when CBBN was presented at lower (45-55 dB SPL) levels, consistent with evoking the MOC reflex. These enhancements in CM amplitude were largest for probe frequencies between 200-2000 Hz; however, the peak frequency associated with the largest enhancements was unique for each participant. At higher CBBN levels (65-75 dB SPL), we observed a decrease in CM, consistent with evoking the middle ear muscle reflex. CBBN had no effect on CAP amplitudes regardless of stimulus type (click or chirp), or click/chirp rate, which is inconsistent with contralateral suppression of CAP amplitudes observed from studies in laboratory animals. Further, an anti-masking effect was not observed for CAP amplitudes when CBBN was presented in a condition where the probe was mixed with low-level (40 dB SPL) ipsilateral broadband noise.
Conclusions: The CM is sensitive to MOC reflex activity for CBBN levels below ~60 dB SPL. The probe frequency associated with the largest MOC-related enhancements on CM amplitude was unique for each participant, suggesting that a swept tone will detect MOC activity that would otherwise be missed by a single-frequency probe. The CAP is not sensitive to MOC reflex activity for the measurements made in this study. This insensitivity may be due to challenges associated with obtaining a robust signal-to-noise ratio (e.g., >20 dB) when measuring CAPs in response to a low-level probe.

TU189. Vomeronasal Pits in Baleen Whales (Mysticeti): Vestigial or Functional?
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Category: Other, Rhinology: Chemoreception, Vomeronasal Organ
Background: Chemoreception (smell and taste) is a poorly studied sense in cetaceans (whales, including dolphins and porpoises). Olfaction is very reduced in baleen whales (mysticetes) and absent in toothed whales (odontocetes). Similarly, taste may be limited to detecting salinity.
Methods: Specimens were obtained post mortem from beach-stranded carcasses of mysticetes, including five rorqual species: blue whale (Balaenoptera musculus), fin whale (Balaenoptera physalus), sei whale (Balaenoptera borealis), minke whale (Balaenoptera acutorostrata), humpback whale (Megaptera novaeangliae), and three non-rorqual species: gray whale (Eschrichtius robustus), right whale (Eubalaena glacialis), pygmy right whale (Caperea marginata). Comparison were made with previously collected odontocetes. Distal third of rostrum was explored through transverse cuts along the mesorostral cartilage, immediately dorsal to vomer (two juvenile fins). Rostrum was examined after removal of mesorostral cartilage (two juvenile minkes). Tissues of suspected vomeronasal pits (juvenile humpback) and cribriform plate region (juvenile fin) were examined microscopically: formalin-fixed, paraffin-embedded, 5micron-sectioned, hemotoxylin-eosin stained.
Results: Paired indentations ("pits") were observed only in rorqual baleen whales. Pits were found on the ventral surface of the upper lip midline, immediately rostral to the smallest baleen plates. Pit depressions were noticeable on palpation, recessing approximately 0.5-1cm at the deepest point (medially). Pits were rounded (ovoid) in humpbacks and C-shaped (concavity facing laterally) in other rorquals. Curved pit depressions surrounded a raised oval region in humpbacks. Pits did not have any obvious gross opening. Gross dissections of the hard palate and vomer did not reveal a vomeronasal organ, or any tubular structure that could channel chemical signals. Microscopic analysis revealed peripheral nerves (sensory?) embedded among dense connective tissue and adipocytes. No olfactory nerves were confirmed associated with the cribriform plate. Cartilage adjacent to the cribriform plate contained variably-sized vacuoles, lined with flat to plump cells that were sometimes detached into the clear space. Vacuoles were filled with varying amounts of amphiphilic material. Their function is unknown. There were no organized structures indicative of a vomeronasal organ (e.g., receptor neurons, duct, capsule walls lined with sensory or non-sensory/ respiratory epithelium).

Conclusions: The position of paired pits on the undersurface of the midline upper lip suggests homology with initial receptors of a vomeronasal organ. Despite the presence of paired pits, no vomeronasal organ or associated structures were observed along the mesorostral cartilage, vomer, or hard palate tissues of the rostrum. No microscopic evidence of vomeronasal sensory or non-sensory epithelium was found, although peripheral nerves were present (presumed sensory, possibly trigeminal?). These data suggest that pits are likely vestigial vomeronasal receptors in rorqual whales. Perhaps chemoreceptive function is compromised in an aquatic environment, as chemical signals (e.g., prey, or urine of females in estrous) may become too dilute and easily dispersed by currents, particularly when whales move quickly or travel long distances (e.g., during migrations).

TU190. A Comprehensive Theoretical Framework Within Which to Examine the Strained Voice Quality

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Category: Other, Voice pathology

Background: Voice plays an important role in identifying a speaker's age, gender, emotion, underlying pathology, and intelligibility. Marked changes in voice quality (VQ) may lead a person to seek voice assessment and treatment by laryngologists and speech-language pathologists. Strain is one of the primary VQ dimensions along with breathiness and roughness, yet is understudied due to its complex nature and its co-occurrence with other dimensions. We have classified strained VQ as being "clean" for dysphonic voices that are 'primarily strained' and "covary" for dysphonic voices that also have breathiness, roughness, or both breathiness and roughness VQs. Here we review a theoretical framework in which VQs are considered to be a subset of sound qualities. As such, when bio-inspired models of auditory processing are applied to voiced stimuli, model outputs correlate well to the perceived breathiness or roughness qualities compared to conventional indices.

Methods: Strain VQ was estimated in four tasks. First (Anand et al., 2019), listener perception of strain used a Likert rating scale from 1 (least strain) to 7 (most strain). Second, stimuli categorized as 'clean' and 'covary' strain were examined by a group of 10 listeners using magnitude estimation (ME) tasks where numbers between 1 and 1000 were assigned to indicate perceived strain magnitude. Third, a single-variable matching task, which overcomes some limitations of the ME task, was based on a novel comparison sound. Fourth, healthy young talkers were asked to simulate strain by varying their vocal effort. Computational analyses of these stimulus sets included multiple conventional and bio-inspired predictors of vocal strain (i.e., cepstral peak prominence, spectral moments, pitch strength, and spectral sharpness). Spectral sharpness was computed from the model of Fastl and Zwicker (2007).

Results: Ratings of perceived strain were strongly correlated with spectral sharpness computed from the voice stimuli. For the strain magnitude estimates, auditory-based analytic methods explained more variance in perceived strain than conventional acoustic measures and spectral sharpness was a strong predictor of 'clean' strain. Differences in specific objective correlates between classes of strain were observed. Such differences could be due to the nature and limitations of the ME task. The matching task resulted in high intra- and inter-rater reliability and the percept of strain was strongly related to spectral sharpness. For simulated strain, sharpness values systematically varied and were significantly different between typical (least), intermediate, and maximum (most) vocal effort.

Conclusions: Perception of strain voice quality is strongly related with spectral sharpness. To better quantify the VQ of 'covary' strain voices, a three-dimensional matching task and 3D computational model that allows for simultaneous measurement of all three VQs is proposed which likely improve the measurement accuracy of voice quality. [Work supported by NIH R01DC009029].

TU191. The Effect of Temporal Regularity on Medial Olivocochlear Reflex and Its Relationship to the Allocation of Attention

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Category: Otoacoustic Emissions

Background: Medial olivocochlear (MOC) fibers efferently project to the outer hair cells (OHCs) from superior olivary complex. The fibers are activated by sound and exert an inhibitory effect on OHC motility; this effect has been termed the MOC reflex (MOCR). We have reported that the MOCR and phase locking value (PLV) of delta oscillations showed a similar decreasing tendency with increasing the jitter added to the

preceding sound sequence. This suggests that processing of temporal expectation in the cortical regions is involved in the predictive control of MOCR. Delta-band oscillations were also suggested to be involved in expediting reaction time (RT) to temporary predictable targets. The expediting effect is reported to be less affected by interference from a working memory (WN) task, which demands attentional resources. To elucidate the role of attention in the predictive control of MOCR, we examine the effect of an interference by a WM task on the enhancement of MOCR and delta-band oscillations associated with temporal regularity.
Methods: The MOCR was evaluated by the suppression of otoacoustic emissions (OAEs) induced by noise presented to the contralateral ear. The size of the OAE suppression has been used as a measure of MOCR strength. OAEs were evoked by clicks presented at the rate of 40 clicks per second. A stimulus block for the contralateral ear was composed of target noise (MOCR elicitor) and three preceding tones whose duration were all 500 ms. Predictability of the timing of target noise was modulated by adding jitter (ΔT) to the inter-stimulus interval among the tones and target noise, whose default was 500 ms. ΔT was randomly selected from 50, 100, 150, and 200 ms for each stimulus block and fixed within the block. We calculated the PLV of the delta-band electroencephalogram oscillations during the noise presentation.
In the main task, participants responded to the target noise and answered the jitter of the stimulus block. The attentional resources allocated to the main task were adjusted by the load of the WM task. Six letters of the alphabet were presented before the main task, and participants answered whether the letters were included in the presented alphabets. Listeners conducted the WN task with low load, where the six letters were composed of the same alphabet, and with high load, where the six letters were composed of different alphabets.
Results: In the low load condition, MOCR strength and PLV of delta wave decreased with increasing the jitter as in our previous study. In the high load condition, by contrast, the jitter-dependent decrease of both responses disappeared.
Conclusions: This suggests that delta-band oscillations are involved in the MOCR enhancement associated with temporal regularity, and that the enhancement demands attention to the target.

TU192. The Effects of Hearing Loss on the Joint-OAE Profile: Preliminary Findings

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Category: Otoacoustic Emissions
Background: Measuring both distortion-product otoacoustic emissions (DPOAEs) and stimulus-frequency otoacoustic emissions (SFOAEs), which are classified by their generation mechanisms as distortion and reflection OAEs respectively, yields what we term a "joint-OAE profile". In normal hearers, this profile reveals that: SFOAEs are higher in level than DPOAEs (at low-to-mid frequencies and high stimulus levels), show more linear growth, and grow more steeply than do DPOAEs into their compressive region. DPOAEs, in contrast, are strongly compressed at relatively low-to-moderate stimulus levels and are generally lower in level than SFOAEs (Abdala et al., 2022). The joint-OAE profile has not been characterized in ears with hearing loss.
Methods: 2f1-f2 DPOAEs and SFOAEs were recorded at 8-12 stimulus levels across five octaves with rapidly sweeping tones in 225 subjects, with either normal hearing (n=117) or mild-to-moderate hearing loss (n=118).
Results: Hearing loss, in general, disrupts the characteristic joint-OAE profile: (1) Both reflection and distortion emissions show reductions in level; however, DPOAE levels are more reduced by hearing loss than are SFOAE levels; (2) "Source strength" (OAE level at its steepest growth re: stimulus level) mimics this pattern, showing a selective reduction of DPOAE strength; (3) The compression "knee" of the I/O function is elevated for DPOAEs only, and (4) the overall slope of SFOAE growth becomes steepened in impaired ears. These four effects occur, to varying degrees, with hearing impairment, indicating that the two classes of emissions are differently affected by cochlear damage. It is less clear whether impaired ears with distinct etiologies of hearing loss produce unique joint-OAE profiles. Preliminary analyses suggest that the joint-OAE profiles from a group with hearing loss due to noise-exposure (n = 28) differs from the profile obtained in a group of individuals with presbycusis (n = 67). The typical pattern of selective DPOAE level reduction (and conversely, the relative preservation of SFOAEs) is not manifest in the noise-exposed group; rather, SFOAEs and DPOAEs are equally reduced. In presbycusic ears, by contrast, DPOAEs are consistently more reduced than SFOAEs, which has also been observed previously (Abdala et al., 2018).

Conclusions: A group of individuals with cochlear damage due to noise exposure showed reflection OAEs that were more reduced in level than did a group with presbycusis, although the mean audiograms were comparable to within 5 dB. Reflection emissions may be uniquely sensitive to noise-induced hearing loss (from 2-6 kHz), as suggested by previous work (Marshall et al., 2009). Additional subject accrual is needed from varied etiologies of hearing loss to assess the potential application of these results to individuals. Our eventual goal is to distinguish between ears with hearing loss that look audiometrically similar but are of distinct etiology, for the purpose of targeted intervention.

TU193. The Strength of Medial Olivocochlear Reflex is Modulated by Auditory Task Difficulty

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Category: Otoacoustic Emissions

Background: The medial olivocochlear (MOC) system is thought to be responsible for modulation of peripheral hearing through descending (efferent) pathways. This study investigates the connection between peripheral hearing function and auditory attention tasks of different degrees of difficulty.

Methods: Peripheral hearing function was evaluated by analyzing the amount of suppression of otoacoustic emissions (OAEs) by contralateral acoustic stimulation (CAS), a well-known effect of the MOC system. Simultaneously, levels of attention were evaluated by event-related potentials (ERPs).

Results: The ERPs showed clear differences in processing tasks of different difficulty, but and there was also slight increase of OAE suppression with task difficulty. There was no effect on OAE latency or the presence of spontaneous OAEs, nor was there any difference in noise level or number of rejected trials. Significantly, however, we observed that the suppression levels of OAEs for easy and hard tasks were correlated with the magnitude of the P3 wave in the ERP.

Conclusions: The amount of the MOC involvement for tasks of increasing difficulty grows with the amount of cortical resources used in task processing. There appears to be a connection between cortical attentional processes devoted to hearing and the MOC reflex.

TU194. Differential Effects of Two-Tone Harmonics and Single Tone Tokens on Subsequent Sounds

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Category: Primary Auditory Cortex

Background: Mouse ultrasonic vocalizations (USVs), are of communicative significance. Analysis of context specific USVs provides a powerful tool to gain insight into mechanisms underlying dysfunctional social communication. Mouse USVs contain a variety of syllable classes with distinct spectrographic features. Two-tone harmonic complexes (TTHC, tones T1 and T2, T2 = 2*T1) are present in c57bl/6 male mouse USVs, particularly during courtship and mating. Since they are produced repetitively as whole sequence during courtship and more so during mating, it maybe that such a sequence is perceived as a single auditory object. Thus, to begin addressing such a possibility we ask the question – do repeating TTHCs (T1 and T2) and rms matched single tone (T1) have differential effects on subsequent tones or TTHCs in a sequence?

Methods: We use extracellular electrophysiology to record from single units using 4x4 microelectrode arrays, from Layer II/III of the mouse auditory cortex (ACX). Tone responses of single neurons were obtained first. Next, responses to a set of 3 sound tokens at varying intervals were obtained. The intervals between pairs of sounds were varied between 60 and 280 ms for different sets of stimuli. Each set consisted of 4 stimuli: i) T1-T1-T1T2, ii) T1T2-T1T2-T1, iii) T1T2-T1T2-T1T2 and iv) T1-T1-T1.

Results: We quantified the effect of the history of two tone tokens and two TTHC tokens on a subsequent tone or a subsequent TTHC. We found that based on the rate responses of the subsequent token there was no significant effect of tone or TTHC history. However, it was not the case for the silent interval between the second and third token and the duration following the third token (or for the gaps). Both the gaps had higher activity when the first two tokens were TTHCs compared to that when the first two tokens were tones. The above effect is seen for cases when the gap between tokens is greater than 90 milli-seconds. Thus while token history, in our stimuli, did not affect the response to the subsequent token, but the silent intervals preceding and following the third token had higher activity when TTHCs were repeated in the past.

Conclusions: We hypothesize that in case of TTHCs there is a release from inhibition due to the presence of an additional component, whose effect causes the increased activity. Thus, in the above manner, the activity during the silence interval may add to the overall response to a sequence of harmonics than its individual component and maybe a mechanism of tying a sequence of harmonics together into a unified sound object.

TU195. Cingulate Cortex Facilitates Auditory Perception Under Difficult Listening Conditions

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Category: Primary Auditory Cortex

Background: We often exert greater cognitive resources (i.e., listening effort) to understand speech under challenging acoustic conditions. This mechanism can be overwhelmed in those with hearing loss (HL), resulting in cognitive fatigue in adults, and potentially impeding language acquisition in children. However, the neural mechanisms that support listening effort are uncertain. Evidence from human studies suggest that the cingulate cortex is engaged under difficult listening conditions, and may exert top-down modulation of the auditory cortex (AC). Here, we asked whether the gerbil cingulate cortex (Cg) sends anatomical projections to the AC in the gerbil, and whether it mediates effortful listening.

Methods: To determine anatomical connectivity, retrograde and anterograde virus tracers were injected into gerbil AC and Cg, respectively. To assess effortful listening, an amplitude modulation (AM) rate discrimination task was used, and stimulus parameters (AM rate, sound duration) were varied to adjust the difficulty of listening conditions. Using an appetitive Go-Nogo paradigm, gerbils (n=13) were trained to discriminate between "Go" stimuli consisting of a range of AM rates (4.5-12 Hz, broadband noise carrier, 100% depth) and a "Nogo" AM stimulus (4 Hz). Trials were clustered into 'easy' or 'hard' blocks, where the sound duration was 1s or 0.25s, respectively. AM rate discrimination thresholds were determined from psychometric functions. To determine whether the Cg is required for task performance, trained gerbils (n=9) were implanted with bilateral cannulae in Cg, and muscimol was infused prior to testing to pharmacologically inactivate Cg and compared to saline-infused controls. To determine whether Cg1 neural activity is recruited during effortful listening, chronic electrode arrays were implanted into the Cg of behaviorally-trained animals (n=4), and wireless recordings from Cg neurons were obtained while gerbils performed the task. Neural firing rates were analyzed within trials and across blocks to assess whether Cg activity was selectively modulated when task difficulty is high.

Results: Anatomical tracing experiments revealed a strong, descending projection from layer 2/3 of the Cg1 subregion of the cingulate cortex to superficial and deep layers of dorsal and primary AC. When Cg1 was pharmacologically inactivated, perceptual performance was disrupted only for difficult listening conditions: thresholds for the 1s blocks (i.e., 'easy' blocks) remained the same across saline and muscimol conditions (~5 Hz AM), whereas thresholds for 0.25s blocks were elevated only for muscimol conditions (saline: ~5.5Hz AM; muscimol: ~7Hz AM). Analysis of Cg recordings from awake-behaving animals are currently underway to determine whether Cg neurons represent task difficulty.

Conclusions: Taken together, the results reveal a descending cortical pathway from Cg to AC that mediates perceptual performance during difficult stimulus conditions. This pathway is a plausible circuit that may be undermined by HL.

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TU196. Interactions of Developmental Stress and Hearing Loss on Gap Detection and the Auditory Cortex

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Category: Primary Auditory Cortex

Background: Early conductive hearing loss (eCHL) is linked with lasting speech perception deficits. These can be attributed in part to compromised processing of temporally varying sounds such as short gaps, linked to reduced inhibition throughout the auditory system, particularly the auditory cortex (ACx). Early-life stress (ELS), which is a risk factor for poorer outcomes in children with eCHL, affects inhibition in non-sensory regions of the brain, but not in primary cortices which have been examined: motor and somatosensory. However, we have previously demonstrated that ELS worsens perceptual and ACx sensitivity to gaps in sound, suggesting that inhibition in ACx may be susceptible to ELS. Maturation of parvalbumin (PV)

inhibitory neurons plays a critical role in the development of cortical response properties, in part via activity-dependent accumulation of perineuronal nets (PNNs). eCHL and ELS have separately been shown to alter expression of PV neurons and PNNs in a region dependent manner, suggesting that the dual impact of these two developmental disruptions may be visible in ACx.
Methods: Mongolian gerbils were divided into 4 groups: Controls, ELS, eCHL, and eCHL+ELS. ELS was induced via ten two-hour sessions of maternal separation and restraint during postnatal (P) days P9-24. Reversible CHL was induced using earplugs from ear-opening (P11) to P24. Animals were either tested on perceptual tasks or used for immunohistochemistry. For behavior, thresholds for detection of short gaps in ongoing sound were collected at P33, 36, and 39 (adolescence) and P83, 86, and 89 (adulthood) using gap-inhibition of the acoustic startle response. For immunohistochemistry, animals were perfused at either P35-40 or P85-95, and their brains were sliced and stained with an antibody to parvalbumin, and biotinylated Wisteria floribunda agglutinin to label PNNs and delineate primary ACx. In cortex, both labels were imaged at 20x and counted using ImageJ.
Results: Measured during adolescence, ELS, eCHL, and their combination all impaired behavioral gap detection, but with no difference in severity. By adulthood, ELS and eCHL deficits were reduced but still present. However, the interaction of ELS and eCHL impaired thresholds much more than either individual insult. In ELS adolescent animals, ACx had reduced densities of PV cells, PNNs, and PV cells with PNNs. The reduced PV cell density continued into adulthood. For animals raised with eCHL or ELS+eCHL, forthcoming labeling data will be presented.
Conclusions: Developmental CHL and ELS induce long-lasting deficits in gap detection, but their interaction is especially detrimental in adulthood when the effect of the individual insults has lessened. The long-lasting effects of ELS on cortical inhibition and PNNs, along with our previous results showing poorer gap sensitivity in ACx neurons, implicate altered inhibitory strength in ACx as an underlying mechanism for this temporal processing deficit.

TU197. Task-Optimized Models of Relative Pitch

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Category: Psychoacoustics
Background: Speech, music and other natural sounds are often harmonic, with frequency components that are integer multiples of a common fundamental frequency (f0). Information in sounds is often conveyed by changes in f0, perceived by humans as “relative pitch”. Relative pitch is believed to be extracted in two ways: by estimating the instantaneous f0 and then measuring changes in the f0 over time, or by measuring changes in individual frequency components. The first mechanism is specific to harmonic sounds, whereas the second is applicable irrespective of whether sounds are harmonic. Humans are believed to rely on the second mechanism in quiet – as evidenced by similar discrimination thresholds for harmonic and inharmonic sounds – but to rely on the first mechanism when hearing in noise. Thus far it has been unclear why relative pitch is structured in this way.
Methods: We optimized a neural network model to estimate relative pitch from audio signals. The model classified the change in f0 from one note of a melody to the next. The model was trained on melodies composed of harmonic notes played on a set of synthetic instruments. We then tested whether and when the model generalized to inharmonic notes. Inharmonic notes were generated by jittering the frequencies of harmonic notes. The jitter pattern could either be preserved across the notes of a melody, producing consistent frequency shifts between notes, or generated independently for each note.
Results: When trained in quiet, the model performed similarly for harmonic and inharmonic notes provided the inharmonic jitter pattern was fixed between notes. Performance was substantially worse when the jitter pattern varied from note to note, indicating that the model’s judgments were based on shifts between corresponding harmonics. Both these qualitative characteristics are observed in human pitch discrimination in quiet. When trained in noise, the model was more dependent on harmonic structure, showing better performance for harmonic than inharmonic notes.
Conclusions: Sensitivity to frequency shifts emerges in systems optimized to estimate relative pitch from harmonic sounds, but only in some conditions. These conditions mirror those in which humans are believed to rely on frequency shifts rather than representations of f0. These findings suggest that the dual mechanisms

of human relative pitch can be understood as an optimized solution to pitch change estimation in natural listening conditions.

TU198. Psychoacoustics of Auditory Memory for Natural Sounds

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Category: Psychoacoustics

Background: Auditory memory is integral to everyday life. Our ability to remember sounds is critical for everyday tasks ranging from localizing auditory objects to recognizing similarities between sonic experiences. However, the mechanisms and representations that underlie auditory memory for natural sounds remain unclear. Here we validated a paradigm to measure auditory memory for natural sounds. We then quantitatively characterized a number of memory phenomena in the auditory domain, demonstrating key results a model of memory should account for.

Methods: To measure auditory memory, we presented participants with a sequence of real-world auditory sound recordings. Some of the sounds were presented more than once. After each sound, participants judged whether they previously heard the clip during the experiment. Recognition memory was quantified as d'.

Results: Recognition was well above chance, but decreased with the number of intervening stimuli between the first and second presentations of repeated sounds (the interstimulus interval; ISI). When some stimuli were presented more than twice in the experiment, recognition performance increased with the number of repetitions. In one experiment, the stimuli were auditory textures, whose internal representation is believed to be well explained by statistics of standard auditory filter cascades. We used the McDermott and Simoncelli texture model to measure the similarity between stimuli in the experiment (via distance in the space of statistics). We found that false alarms made in the experiment could be predicted by the similarity of a stimulus to stimuli previously presented in the experiment.

Conclusions: Our results suggest that memory for natural sounds can be fairly accurate even across intervening stimuli; this memory worsens as ISI increases and improves as sounds occur repeatedly. The analysis of false alarms substantiates the phenomenon of false memories for sound, and shows that they can be quantitatively predicted by perceptual similarity. Future models of auditory memory must account for these phenomena.

TU199. Pitch Instability Enhancement Illusion and Its Relation to the Pitch Perception Mechanism

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Category: Psychoacoustics

Background: Humans perceive pitch based on temporal information of neural firing intervals and tonotopically encoded frequency information. However, mapping of primitive information to the psychological quantity of pitch is unclear. In this study, we focused on pitch instability enhancement illusion (PIEI), a phenomenon that cannot be explained using only extracted frequency information in the peripheral auditory system. PIEI occurs when a sound with a steady pitch is heard repeatedly and another sound with a pitch smaller than a semitone width is heard. When the pitch changes, the beginning of the note sounds unstable. Therefore, we investigated whether PIEI occurs in peripheral auditory processing by measuring frequency-following response (FFR).

Methods: First, a listening experiment was performed to confirm that PIEI occurs with the stimuli created for the experiment. In total, 50 normal-hearing adults participated in the listening experiment. Experimental stimuli were processed to decay exponentially. The duration of each stimulus was 700 ms, and the inter-stimulus interval was 300 ms. The first three reference tones were A4 (440 Hz), F#4, and C5. The pitch of the following two tones varied from the reference tones -100, -66, -33, 0, +33, +66, +100 Cent. The stimuli were presented randomly five times per stimulus, and the participants were asked to rate the pitch of the fourth tone of the presented stimulus on a six-point scale ranging from "not unstable at all" to "very unstable," and the FFR was measured. Five normal-hearing adults participated in the FFR measurement experiment. The experimental stimuli were used in the listening experiment. The reference tone was set to A4, and two types of following tones were used: A4+33 Cent. For comparison, a condition in which A4+33 Cent was presented continuously without PIEI was also created. Electroencephalogram was recorded at Cz, T3, and T4 in the 10–20 electrode system, with the earlobe as the reference electrode and the frontopolar midline electrode as the ground electrode. Stimulus sounds were presented using an audio interface adjusted

to approximately 80 dB sound pressure level. We confirmed that PIEI occurred in all participants in the experimental setup, except one.
Results: In the listening experiment, the main effects of the pitch were significant. The -33 Cent and +33 Cent changes were perceived as the most unstable. Time-frequency analysis of the measured FFRs showed no difference in FFRs with PIEI and without PIEI.
Conclusions: We found that PIEI was not observed in the auditory peripheral system; our findings support the hypothesis that PIEI is caused by higher-order auditory information processing in humans. The results provide a new promotion in pitch perception research by demonstrating the influence of higher-order auditory information processing on pitch perception. In the future, we will further clarify the effects of prediction and other factors.

TU200. Profile Analysis in Listeners With Sensorineural Hearing Loss

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Category: Psychoacoustics
Background: Profile analysis tests the ability of listeners to discriminate between sounds based on differences in their spectral envelopes. One remarkable feature of profile analysis is that thresholds are fairly robust to large (> 20 dB) random variations in sound level across intervals. Although this robustness suggests that listeners do not rely on energy-based cues from a single channel in the task, a satisfying alternative theory remains elusive. Development of alternative theories is limited in part because profile analysis has almost exclusively been measured at moderate frequencies (~ 1 kHz) and in listeners with normal hearing. To address these gaps in the literature, we measured profile analysis in listeners both with and without hearing loss at several stimulus frequencies.
Methods: Stimuli were inharmonic complex tones with equal-amplitude spectral components (5, 13, 21, 29, or 37 components) spaced logarithmically over ~4 octaves and centered at a target frequency (0.5, 1, 2, or 4 kHz). Listeners were tasked with detecting when the middle (target) component of the complex was incremented in level relative to its neighboring components, and the threshold increment size was measured. The tone complex either had an overall level (excluding the target increment) of 70 dB SPL (fixed-level condition), or the overall level was randomized across intervals over a range of 70 ± 10 dB SPL (random-level condition). To aid in interpreting the results, we simulated auditory-nerve and inferior-colliculus responses to our stimuli using an existing computational auditory model that includes the ability to model differing amounts of sensorineural hearing loss.
Results: Thresholds were lower (better) at low frequencies (≤ 2 kHz) than at high frequencies (4 kHz). This effect of frequency was larger for stimuli with many components than for stimuli with few components. Level randomization increased (worsened) thresholds in most conditions, but this elevation was largest in the 5-component condition. At 2 kHz, listeners with higher audiometric thresholds also had higher profile-analysis thresholds, particularly for higher numbers of components. However, at other frequencies, the relationship between audiometric thresholds and performance was less clear. Our simulated neural responses demonstrated that a recently proposed theory of profile analysis (Maxwell, Richards, and Carney, 2020; JASA, 147.5) could parsimoniously account for the observed trends. In lieu of more traditional energy-based cues, this theory is based on analysis of across-channel patterns of temporal fluctuations in auditory-nerve activity, referred to as neural fluctuations, which are transformed into a rate-based code in the inferior colliculus.
Conclusions: Profile analysis may rely on midbrain representations of auditory-nerve fluctuations. Future work will test the ability of this theory, in listeners with and without hearing loss, to account for performance in other behavioral tasks that include random level variation.

TU201. Detection Threshold of Regular and Irregular Successive Silent Gaps

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Category: Psychoacoustics

Background: Gap detection threshold (GDT) is one of the proposed indices to determine auditory temporal resolution. The GDT is the minimum length of detectable silent intervals embedded within a stimulus of a certain length (e.g., 500 ms). We have been developed an objective GDT measurement method using auditory steady state response (ASSR) elicited by broadband noise containing regular successive silent intervals [Kadowaki et al., 2022]. The noise stimulus has silent intervals every 25 ms, translating to 20 silent intervals when the stimulus length is 500 ms. We observed that GDTs using stimulus with regularly inserted multiple silent intervals were more sensitive than traditional GDTs, i.e., GDTs using single gap embedded within broadband noise. Understanding the reason for this difference in the point of view of detection sensitivity is important to confirm the validity of our proposed objective GDT measurement method. We hypothesized that this difference was due to: 1) multiplicity of silent intervals, and/or 2) regularity of inserted silent intervals. Therefore, psychophysical measurement was conducted to evaluate our hypothesis.
Methods: In experiment 1, we adopted the constant gap onset asynchrony (GOA) of 25 ms and varied the number of silent intervals. The number of silent intervals was set as 1, 2, 4, 8, and 16. In experiment 2, we adopted the constant number (eight) of silent intervals and varied the GOAs. GOA was set as 12.5, 25, 50, 100 ms in the regular GOA condition and GOA was set randomly in the irregular GOA condition. We hypothesized that the sensitivity increased as the repeat time is shortened and the GDT in the regular condition was shorter than that in the irregular condition. The stimulus carrier was a low-noise noise [Kohlrausch et al., 1997] to minimize the fluctuation of the carrier envelope itself. Three interval, three alternative, forced choice, 2-up and 1-down procedure was used for measurements. The thresholds were measured only once for each condition. Subjects with normal hearing participated in the preset study.
Results: The experimental results showed 1) the GDT with 16 silent intervals was approximately half the traditional GDT, 2) sensitivity increased significantly as the number of silent intervals increased, and 3) sensitivity did not significantly differ between regular and irregular successive silent intervals at least with long GOA.
Conclusions: To confirm the validity of the proposed stimulus for objective temporal resolution measurement, we measured the GDTs using broadband noise containing multiple silent intervals. The results showed that the GDT was significantly affected only by the number of silent intervals, at least for long-cycle regular successive silent intervals.

TU202. Exploiting Alternative Oxidase to Investigate Mechanisms of Decreased Hearing Performance in Glutathione Peroxidase-1 Knockout Mice

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Category: Psychoacoustics
Background: Sensorineural hearing loss has been linked to mitochondrial dysfunction. Mitochondria are not only critical for cellular energy supply but are also the primary source of reactive oxygen species (ROS), which may affect cochlear damage following noise exposure (NE). A promising approach to treat mitochondrial dysfunction in mice with impaired antioxidant enzyme defense resulting from a lack of glutathione peroxidase 1 expression (*Gpx1*-KO) may be the transgenic expression of alternative oxidase (*AOX*). Previous studies have demonstrated the beneficial effects of AOX on mitochondrial function and its potential to decrease ROS levels (e.g., Szibor et al., 2020, Bba Bioenergetics 1861: 148137). Here, we investigate whether AOX can rescue the degraded hearing performance of *Gpx1*-KO mice lacking a first-line antioxidant (Ohlemiller et al., 2000, JARO 1: 243-254). To this end, the hearing performance of C57BL/6.CAST-Cdh23Ahl+ mice with *Gpx1*-KO and additional expression of *AOX* was examined before and following NE.
Methods: Anatomical and physiological analyses were used to evaluate auditory nerve fiber (ANF) functionality. Further physiological analysis evaluated the integrity of the stria vascularis. Hair cell and afferent synapse loss were quantified at defined tonotopic regions in isolated organs of Corti (Braude et al., 2015, Hear Res 321: 52-64). Auditory brainstem response thresholds were measured before, 48 h following NE, and at the end of behavioral experiments using pure tones of 5, 10 and 20 kHz (0.5 ms) and clicks (20

µs). To determine whether potential ANF alterations were sufficient to affect perception, absolute and masked (2-50 kHz noise, 20 dB/Hz spectrum level) thresholds were assessed prior to and following NE using a reward-based Go/NoGo procedure. Based on hit and false alarm rates, thresholds were obtained for a d' of 1.8. To produce noise-induced hearing loss, ketamine-anesthetized animals were exposed to an octave-band noise (8-16 kHz) for 2 h at 104 dB SPL.
Results: *AOX* expressed in *Gpx1*-KO mice revealed both positive and negative effects on hearing performance. *AOX* expression rescued behavioral thresholds in *Gpx1*-KO mice. While number of synapses were similar in tested mouse lines, the decreased synapse volume in *Gpx1*-KO mice was not restored by *AOX* expression. Tested mouse lines had normal endocochlear potentials. Interestingly, *Gpx1*-KO mice expressing *AOX* were more susceptible to NE than *Gpx1*-KO mice with no *AOX* expression.
Conclusions: This observation suggests that AOX may interfere with the mitochondrial electron transport chain, affecting the cellular energy production leading to an insufficient ATP supply during NE that may be more crucial for ANF survival than the risk of damage by ROS. Our study emphasizes the importance of a better understanding of AOX effects when considering implementing *AOX* in gene therapy aimed at ameliorating auditory dysfunction.
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TU203. Pitch Perception Improves With Increasing Modulation Depth
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Category: Psychoacoustics
Background: Cochlear implants partially restore hearing in people with sensorineural hearing loss. Outcomes are impressive with most recipients attaining excellent speech comprehension in quiet without needing to rely on lip-reading cues, but musical pitch perception is much worse compared to normal-hearing peers. Cochlear implants have the capacity to provide precise fine-timing cues, but sound processing on existing devices generally only provides weakly modulated electrical stimulation, particularly for musical notes above middle C (i.e., ~262 Hz). This study examined the effect of modulation depth on pitch discrimination.
Methods: The ability to hear a shift in pitch as a function of modulation depth was measured in adults with no known hearing loss and in cochlear implant users. Discrimination thresholds were measured acoustically using sinusoidally amplitude-modulated tones and bandpass-filtered noise with modulation frequencies centered at 110, 220, and 440 Hz. Modulation depths included 25%, 50%, and 100% depth, as well an enhanced condition where modulation was a half-wave rectified sinusoid. CI users performed a follow-up procedure that instead used modulated pulsetrains delivered to a single-electrode. Computational models were used to test the predictive power of vector strength as a measure of synchrony in healthy physiology and in cochlear implant stimulation. Finally, vector strength to modulated pulsetrains were measured via neural telemetry in CI users at apical and basal regions of the electrode array. Stimuli were pulsetrains with modulation frequencies of 110, 220, and 440 Hz, with modulation depth set between comfort and 1, 2, 4, 8, and 16 clinical unit(s) under.
Results: In both groups of listeners, acoustic pitch discrimination improved with increasing modulation depth; however, cochlear implant users did not receive as large of a benefit. Neural synchrony to modulation frequency, as measured in a computer model of an auditory nerve, is a predictive metric of discrimination thresholds and explains between 74 and 94% of the variance for each stimulus type. Modulation detection thresholds were largely predicative of discrimination thresholds, with individuals most sensitive to modulation having the best pitch resolution. Preliminary results from the single-electrode pitch discrimination task show an improvement in thresholds compared to discrimination through the speech processor. Finally, early results measuring the synchronized response to modulated pulsetrains suggest that individuals with greater physiological capture of modulation frequency are those having the best pitch resolution.
Conclusions: Modulation depth had a pronounced effect on pitch resolution across a range of fundamental frequencies. These results were effectively explained by neural synchrony to modulation frequency, quantified as vector strength, both in terms of modeled and collected neural responses. Taken together, these results highlight modulation depth as a relevant factor for pitch and point to modulation enhancement as an avenue for improving pitch perception in cochlear implant users.

TU204. Discriminating Statistical Dependencies in Gliding-Tone Sequences

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Category: Psychoacoustics

Background: Listening in typical “cocktail party” scenarios involves the twofold process of sound segregation and source determination. To understand one specific talker among competing talkers, a listener must resolve a complex stream of sound into its meaningful parts, identifying which of these parts belong to the voice to be understood and which to the voice(s) to be ignored. Although the auditory/cognitive/linguistic mechanisms responsible for this process are not fully understood, evidence has been found that, while fluctuations in the amplitude envelope convey intelligibility, information in the rapid frequency fluctuations (roughly, those important for pitch perception) aids in the segregation of concurrent talkers. This study investigated the role of statistical output features spanning those typical of formant-frequency transitions. The underlying rationale was that sound sources may be distinguished by the statistical structure of the sounds they emit.

Methods: Stimuli were N concurrent sequences (N varied by duration) of logarithmically-spaced gliding tones whose slopes (temporal rates of frequency change) were determined in the initial time window by random-slope selection (from 5 possible "states"). The initial "burst" was thus a set of N synchronous tones spaced across frequency, the slope of each chosen randomly. The slopes of subsequent tones in each band were governed by probabilities defined in transition matrices (i.e., Markov chains). Each tone was allotted a frequency bin delimited by the tone’s maximum span in frequency and, for each sequence, bins were stacked to fill the frequency range of 200–2000 Hz. All sequences were equal in duration (1 sec), as were the constituent tones in each sequence (50, 100, or 200 msec, forming sequences of 20, 10, and 5 bursts, respectively). “Reference” sequences were generated via independent samples from the uniform-distribution matrix and formed one of the two stimuli presented in each trial. Target sequences were drawn from a number of matrices spanning the range between the uniform and identity matrices. The task was 2I-2AFC, with feedback, the subjects being instructed to choose the interval with a stronger statistical structure.

Results: Across tone durations, correct-response rates indicated rapid acquisition of task proficiency. Furthermore, the psychometric functions relating percent-correct discrimination performance to the degree of statistical structure of the target were orderly/monotonic over the range tested. The strength of the target (specified by the diagonal of the transition matrix) had a significant effect on performance levels ($p = 0.0005$), as did duration ($p = 0.028$).

Conclusions: These results confirm the ability of listeners to discriminate the statistical structure in broadband, time-varying acoustic signals. Although masked conditions were not tested, the sensitivity to frequency variation found here suggests that listeners could make use of transition probabilities presenting in spectral-peak movements (e.g., formant transitions) when segregating incoming sound mixtures into multiple talkers.

TU205. The Perception of Ultrasonic Vocalizations in Quiet and Noise by Noise-Exposed Mice

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Category: Psychoacoustics

Background: High-intensity noise exposures can lead to temporary or permanent increases in hearing thresholds for simple and complex stimuli in both young and old mice. Mice produce complex signals known as ultrasonic vocalizations (USVs) in a variety of behavioral contexts and it is thought these vocalizations serve a communicative function. However, it is unclear if high-intensity noise exposures affect perception of USVs similar to how speech perception is affected in humans with hearing loss. The effects of a two-hour exposure to a 100 dB 8-16 kHz narrowband noise on auditory thresholds for two types of USVs (complex and downsweep) in two different listening conditions (quiet and continuous 60 dB white noise background) were measured.

Methods: Thirty-one male and female CBA/CaJ mice ranging from 100 to 1000 days old were trained to detect USVs in a go/no-go operant conditioning paradigm with positive reinforcement. Each mouse participated in only one of the four conditions. Once consistent thresholds were obtained for a mouse, they were given the noise exposure. Thresholds were obtained daily for up to 120 days after the noise exposure and mean threshold deviations across the whole period were calculated.

Results: Similar to humans exposed to damaging noise, there was a lot of variability in the extent of the hearing loss following the noise exposures (ranging from -15 dB to 55 dB), with some mice experiencing large threshold shifts and other mice exhibiting only minor, temporary hearing loss. Before the noise exposure, mice listening in quiet had lower (better) thresholds than mice listening in noise. After the exposures, younger mice had smaller threshold deviations, or variability, compared to older mice, and mice detecting pure tones in quiet had lower variability than those detecting in noise. Threshold variability for downsweep USVs was greater than threshold variability for complex USVs, and threshold shifts differed for the two USV types.
Conclusions: The perception of USVs by mice deteriorated following exposure to intense noise, paralleling effects observed in humans and in mice for pure tone detection following noise exposures. Younger mice showed less noise induced deterioration than older mice, potentially highlighting central adaptations to peripheral damage. These results highlight the importance of studying communication signals in animal models of noise induced hearing loss.
[Supported by NIH DC016641]

TU206. Open Board

TU207. Perilymph Mass-Spectral Differences May Account for Response Variability in a Drug-Induced Hair Cell Regeneration Mouse Model
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Category: Regeneration
Background: Mammalian cochlear hair cells are unable to spontaneously regenerate following damage, leading to sensorineural hearing loss. The inability to regenerate means that hearing loss is permanent in humans. Therefore, it is important to investigate ways to induce regeneration of hair cells for potential future therapies. Overexpression of Atoh1, a key transcription factor during development, is capable of converting supporting cells (SC) to hair cells (HC) in mice; however, ectopic Atoh1 may not be sufficient in conversion of SC to HC in adult cochlea. Conditional knockout of p27Kip1 facilitates the conversion of SC to HC cells in an Atoh1-mediated pathway. Additionally, POU4F3, a downstream target of Atoh1 that remains present in mature HCs, along with Atoh1, was found to promote SC-to-HC conversion in adult mice, illuminating more potential targets for therapy. Through the use of high throughput screening we identified two compounds: A2CE (a potent p27Kip1 inhibitor) and Compound 18 (a POU4F3 agonist). When combined, these two drugs may be able to act synergistically in the conversion of SC to HC in adult mammals.
Methods: Adult (6-10 week-old) FVB mice were injected intratympanically with 5µL of a drug cocktail containing Alsterpaullone-2, cyanoethyl (A2CE) and Compound 18. Mice that were not injected with drug cocktail served as negative controls. After approximately 30 minutes, perilymph was collected from mice through the posterior semicircular canal for further analysis.
Diluted samples were analyzed via MALDI-MS. MS peaks corresponding to the two compounds were observed to account for presence of the drug in perilymph. Method was further validated via LC-MS. Additionally, cochlea from 6-10 week-old Sox2-CreER; TdTomato mice injected with the drug cocktail were harvested, decalcified, and dissected 4-12 weeks post-injection. The samples were further stained with Myosin VIIa, and DAPI and scanned via confocal microscopy. Images were then analyzed via Imaris to look for TdTomato and Myosin VIIa double positive cells, which indicated SC-to-HC conversion. Cells were counted and ratios were analyzed to determine the degree of conversion.
Results: In vivo treatment of adult mice with intratympanic drug cocktail injection exhibited varying detection peaks of A2CE and Compound 18 on analysis with MALDI which correlate with the morphological variation observed from mouse to mouse. These results were then correlated with the SC-to-HC conversion ratios obtained from treatment and analysis of cochleae via Imaris, which confirmed similar results of variation.
Conclusions: We demonstrate that intratympanic injection of a drug cocktail containing A2CE and Compound 18 exhibits differing degrees of variation when detecting the drug cocktail in the perilymph of different mice via MALDI. Further, we demonstrate that detection of A2CE and Compound 18 with MALDI correlate with the degree of variation seen in the conversion of SC to HC in mice.

TU208. The Effects of Combined Low-Level Laser Therapy and Neural Progenitor Cells on Facial Nerve Regeneration in Rat Animal Model

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Category: Regeneration

Background: Debilitating injuries involving facial nerve may progress to an individual's physical and mental decline. Although several studies evaluated the effects of stem cells and low-level laser therapy (LLLT) on facial nerve regeneration, there is little information available regarding in vivo application of LLLT in conjunction with neural progenitor cells (NPCs).

Methods: We developed an animal model of facial nerve damage to induce neurotmesis after resection, suturing, and crushing at the proximal portion of the left cervicofacial division. Rats with facial nerve damage were randomly assigned to 4 groups: (G1) no treatment; (G2) applying LLLT; (G3) seeding with NPCs; (G4) application of both NPCs and LLLT. LLL was applied on alternate days at wavelength of 810 nm and 633nm. The animals were assessed functional movement and compound muscular action potential (CMAP) of vibrissae after 4, 8, and 12 weeks.

Results: Through the improvement of peripheral nerve repair, there are no observable improvement of vibrissae compensation within 12-week post- neurotmesis. However, CMAP results showed an improvement of peak-to-peak amplitude after applying of both NPCs and LLLT.

Conclusions: The corresponding study shows promising results that combined therapeutic strategies of photobiomodulation and addition of progenitor cells showed regenerative properties that promote faster cellular repair which may provide faster functional recovery in future studies.

TU209. Evaluation of Inner Ear Progenitor Cell-Specific Exosome

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Category: Regeneration

Background: The permanence of hearing loss and imbalance is mainly due to the inability of the sensory epithelium to replace lost mechanoreceptor cells, or hair cells. So, hair cell replacement remains the ultimate goal in the treatment of damaged inner ears. Although cochlear hair cells are known to be not replaced in mammals, some neonatal mouse cochlear non-sensory cells including supporting cells are able to proliferate and differentiate into inner organoid. The purpose of our study is; (1) to effectively generate otic progenitor cells derived from mouse neonatal stem cells by isolating cells from the P2 organ of Corti, (2) to isolate exosome from the "proliferating state: sphere; and (3) to analyze exosomal miRNA to identify key genes related to inner ear regeneration.

Methods: We generated floating spheres from neonatal (P2) mouse supporting cells, and optimized the condition to harvest the most abundant number of solid spheres. The spheres were characterized by immunohistochemistry staining and western anaslysis on the proliferation day 7. For the control group, we harvested the cochlea from P2 mouse, and incubated them. The exosomes were isolated from two groups, and the exosomes were characterized with nanoparticle analysis, and western. Also, miRNA sequencing and data analysis was done to compare between two groups.

Results: Single cells were obtained from the dissected cochlea and after modifying the concentration of small molecules, culture plate, room temperature, and seeding cell numbers, seeding 2.5 x104 cells / well were proven to be most efficient number to generate the most abundant number of solid spheres in our experiment. Immunostaining of spheres demonstrated SOX2(+), PAX2(+), Myo7A partial (+) on the proliferation day 7. The exosome was isolated from two groups, and the average size of exosome was 173.8nm, the average number was 7.29 x108 particles /mL. In miRNA sequencing, 307 miRNAs were identified in sphere-origin exosome, and 217 miRNAs were identified the control group. A total of 122 miRNAs were more than 2 folds changed, and 12 miRNAs (10 miRNAs upregulated; 2 miRNAs downregulated) were statistically significant.

Conclusions: We've isolated exosome from inner ear organoid for the first time. Exosome research will lead to further understanding of the mechanisms related to inner ear proliferation, differentiation, and regeneration, which would hopefully guide us to the new therapeutic methods of hearing loss.

TU210. Comparing the Proliferative Capacity of the Young and Adult Stria Vascularis Using in Vitro Whole-Tissue Organotypic Explants

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Category: Regeneration

Background: The stria vascularis (SV) is a highly vascularized and specialized tissue responsible for maintaining the ionic environment within the cochlea needed for hearing. The SV also serves as the blood-labyrinth barrier, strictly controlling the movement of substances into and out of the cochlea similar to the function of the blood-brain barrier. Degeneration of the SV disrupts cochlear homeostasis, which results in progressive and irreversible hearing loss, yet there is little research investigating SV regeneration. To advance the development of treatments for SV-related hearing loss, a robust method to investigate the whole SV in vitro is required. Therefore, we have developed an organotypic explant technique to culture the whole SV at both young and adult stages to investigate its regenerative properties.

Methods: We used n = 10 CD1 mice from at least three independent litters at postnatal day (P) 0-1 for our neonatal stage, and P30-33 for our adult stage. We carefully dissected the SV from the lateral wall of the cochlea and cultured the explants on Matrigel coated plates for 72 hours in the presence of the proliferation marker, BrdU. To pharmacologically inhibit the Wnt/β-catenin signalling pathway, we used the TCF/LEF inhibitor, FH535.

Results: Using this technique, we investigated the proliferative capacity of the SV and we found that the neonatal SV is highly proliferative while the adult SV is not. This is consistent with results reported in vivo, demonstrating that we have generated a representative in vitro model of the SV. This also provides comparative models for the regenerative vs. non-regenerative SV. We hypothesized that the Wnt/β-catenin signalling pathway plays a role in the proliferation of the SV. We show that pharmacological inhibition of Wnt/β-catenin signalling in neonatal SV explants significantly reduces proliferation, which indicates that Wnt/β-catenin signalling may be a key player in the proliferative capacity of the SV.

Conclusions: We establish for the first time a whole-tissue in vitro system for the SV and provide evidence for a regenerative model of the SV in neonates which can be compared to a non-regenerative model in adults. We also demonstrate that we can introduce pharmacological intervention to our system and showed that Wnt/β-catenin signalling plays a role in SV proliferation. Together, our work contributes a novel experimental platform to study the SV and to develop regenerative therapies for SV-related hearing loss.

TU211. Deep Neural Networks Trained for Speech Recognition Do Not Generalize to Sine-Wave Speech

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Category: Speech Perception

Background: Sine-wave speech (SWS) is a sparse acoustic signal comprising 3-4 time-varying sinusoids. Naive listeners typically perceive SWS as noise, but readily perceive SWS as speech after priming. To better understand how humans learn to perceive SWS like natural speech (NS), we examined the ability of deep neural networks (DNNs) trained on NS words to recognize SWS words.

Methods: We tested DNN SWS word recognition both before and after fine-tuning the network based on SWS words derived from NS word counterparts. Deep learning network is trained based on the Speech Commands database and transferred to the SWS data set generated by Speech Commands.

Results: The DNN trained to recognize NS words did so with an accuracy of 0.91. However, such high word-recognition performance did not transfer to SWS (accuracy: 0.55), even after fine-tuning the network on the SWS words with variable learning rates (max accuracy: 0.67). In contrast, a naive model trained only to recognize SWS words was much more accurate (0.88), and better transferred to recognizing NS words (0.58, 0.75 after NS recognizing). Furthermore, representational similarity analysis showed that the outputs of the naive model trained only on SWS are closer to the outputs of the original speech model than the model transferred from NS to SWS.

Conclusions: This suggests that the accuracy of DNNs trained on NS typically derives from speech features other than those preserved in SWS. Furthermore, given that humans are readily able to perceive SWS as speech with minimal training, this, in turn, suggests that DNNs recognize speech in a manner fundamentally different from humans, and likely will require additional top-down mechanisms to be effective models for human speech perception.

TU212. Identification of Speech in Noise Deficits in Humans Using Speech-Induced MEG

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Category: Speech Perception

Background: Age-related deficits in speech-in-noise understanding pose a significant problem for older adults. Aging people often experience difficulties in perceiving speech in a noisy environment, even without elevated audiometric thresholds, a pathology that is assumed to be attributed to progressive cochlear synaptopathy. We here hypothesize that altered brain oscillations are triggered only by those speech sounds that are encoded deficiently. Thus, it could be that auditory fiber subtypes participate in different ways in the coding of sound classes relevant for speech (nasals, fricatives, liquids, or glides). Depending on articulation type, loudness, functional participation of higher or lower frequency spectra, the relevance of the auditory fiber type for the coding of e.g. fricatives or vowels could differ fundamentally and thus influence speech intelligibility in old age. Here, the cognitive ability to discriminate between new or rapidly changing stimuli could have a direct influence.

Methods: We used a frequency-tagging paradigm that tested the ability of speech intelligibility using MEG. Specifically, we asked young middle-aged and aged participants to attend to one speaker while being either distracted or not by a second speaker. Speakers were speaking at different syllable rates. Auditory language processing was studied using multi-channel MEG. This approach enabled us to identify language processing of individual speakers in auditory and prefrontal cortex with high-temporal resolution.

Results: We present first data indicating that MEG responses show reliable response change following the attended speaker. We moreover present initial data that relate the distinct speech response changes in young, middle aged and aged groups and compare the findings with differences in hearing thresholds, ABR waves, 5-word German sentence test (OLSA) and ASSR in individuals.

Conclusions: We hope to contribute to the development of first diagnostic methods for the early detection of speech coding problems in old age, and possibly also for the early detection of correlated cognitive deficits (up to dementia).

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TU213. Effects of Age and Musical Expertise on Perception of Speech in Single-talker Speech Maskers in Adults

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Category: Speech Perception
Background: Speech perception in interfering background speech relies on perceptual mechanisms such as segregating target speech from masking speech using the voice cue differences, and on cognitive mechanisms such as selective attention and inhibition of irrelevant information. These mechanisms have previously been suggested to be affected by both aging and musical expertise (typically referred to as "musician effect").
Two important voice cues that listeners can rely on to distinguish different speakers are mean fundamental frequency (F0), related to voice pitch, and the vocal-tract length (VTL), related to speaker size. Some studies have reported a decreased sensitivity to F0 differences in older adults, possibly affecting their ability to discriminate speakers. Furthermore, age-related cognitive changes may lead to difficulties in attention direction and inhibition. Compared to non-musicians, some studies reported musicians to show enhanced processing of acoustic features such as F0, as well as enhanced cognitive abilities such as those related to auditory attention and working memory.
Few studies further showed a musician advantage for speech perception in speech maskers, in both same or different voice cues for target and masker. However, reports of musicians outperforming non-musicians in such tasks have not always been consistent across both young and older adults. This could mean the effect is small and/or depends on the definition of musicianship, pointing to the need for further studies.
The aim of this study was to investigate the extent to which aging and musical expertise affects the benefit that listeners get from voice differences in a speech-in-speech perception task.
Methods: In this study, we included adults of varying ages and with various levels of musical expertise, all with self-reported normal hearing. All participants performed an online Coordinate Response Measure test with a single target speaker and a single-talker masker. Participants were asked to respond to keywords in the target speech, while target-to-masker ratios were varied, and differences in F0 and VTL voice cues between the target and competing voices were manipulated.
Results: Preliminary results showed a general effect of age, with accuracy scores decreasing with increasing age. Furthermore, listeners at all ages seemed to benefit from F0 and VTL voice cue differences for speech-in-speech perception. Data collection from listeners with musical expertise is still ongoing and results will be presented at the time of the meeting.
Conclusions: While overall speech perception in the presence of masker speech decreased as a function of age, listeners at all ages were able to use voice cues to distinguish target from masker speech. While a potential musician benefit is still under investigation, further effort may be needed to pinpoint what aspects of musicality are of particular relevance for speech perception.

TU214. Neurophysiological Markers of Sensory Gain and Their Relationship to Speech Perception in Noise in Young and Middle-Aged Adults

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Category: Speech Perception
Background: Human communication occurs in acoustically complex environments. Background noise decreases the amount and quality of available information to the listener. This interference results in reduced neural coding of spectrotemporally complex sounds and increased cognitive effort to overcome listening challenges caused by the acoustic degradation. Here, we explore the interaction between neurophysiological measures of bottom-up temporal processing and top-down listening effort, as it relates to speech-in-noise intelligibility in young and middle-aged adults with no overt increase in hearing thresholds. Bottom-up spectrotemporal representations are measured using envelope following responses (EFRs). EFRs are phase-locked neural ensemble responses that faithfully follow the stimulus amplitude envelope. The upper limit of phase-locking decreases along the ascending auditory pathway. Thus, we can assess temporal processing along the auditory pathway via EFRs evoked with decreasing modulation frequencies, while top-down listening effort is indexed by isoluminous task-related changes in pupil diameter.
Methods: Younger (18-25 years) and middle-aged (40-55 years) adults with clinically normal hearing thresholds (≤ 25dBHL at 250-8000 Hz) participated in this study. To index neural generators from cortical and subcortical regions, we calculated slopes across EFR amplitudes obtained to 3000 Hz tones, amplitude modulated at 40, 110, 512, and 1024 Hz. Speech-in-noise intelligibility was assessed using the Quick Speech-in-Noise (QuickSIN) test, a standardized audiologic assessment wherein listeners repeat target

sentences masked in four-talker babble under six signal-to-noise ratio (SNR) levels. Pupillary responses during QuickSIN were collected as a marker of listening effort, and a growth curve analysis was applied to measure changes in effort as a function of SNR.
Results: Speech in noise performance was near-ceiling for SNRs 25 to 10 dB, but listeners in both age groups showed an overall decrease in performance accuracy at the most difficult masking conditions of 5 and 0 dB SNR. Changes in pupil diameter also revealed an increase in listening effort with increasing task difficulty. Notably, middle-aged adults showed a greater increase in listening effort, especially at challenging SNRs, despite matched performance between the groups. Preliminary results suggest that EFR slopes indexing central gain show no age-related differences. However, middle-aged adults show overall reduced EFRs at faster AM frequencies, suggesting decreased peripheral neural representations of temporally complex stimuli.
Conclusions: These results indicate that middle-aged and younger adults with normal audiometric thresholds may rely on similar top-down and central gain mechanisms to disentangle competing speech streams. However, middle-aged adults may enlist more cognitive effort to obtain similar behavioral accuracy as younger listeners. These changes in effort may be underscored by decreased temporal processing in the auditory periphery. Ongoing work is aimed at comparing EFR slopes in adults with self-perceived hearing difficulties to those without, regardless of age, to better understand the mechanisms of bottom-up and top-down factors affecting speech perception in adverse listening conditions.

TU215. Investigating Categorical Perception in the Brain Using Machine Learning

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Category: Speech Perception
Background: Categorical perception of speech is a phenomenon where speech sounds that differ by the same acoustic magnitude are perceived as more similar to one another when they fall within the same category compared to different categories. This phenomenon can be studied using electrophysiological techniques, with one such technique being the frequency following response (FFR). Recently, machine learning algorithms, due to their efficiency in reducing experiment duration and participant fatigue, have become popular in analyzing FFR data. In this study, we aimed to evaluate (1) whether machine learning algorithms can accurately predict FFR recordings to a speech sound continuum, and (2) whether the classification patterns revealed evidence of categoricity at the level of the brainstem, the dominant source of the FFR to speech.
Methods: We analyzed FFR recordings to a 7-point /ba/ to /da/ sound continuum. For each 230-ms stimulus, 1500 trials were collected from 12 native adult speakers of American English. The raw 1-channel FFR recordings, collected in the BrainVision system, were pre-processed using a bandpass filter of 70-1000 Hz and averaged over 1500 trials. Linear support vector machine (SVM) learning algorithm was used to analyze two segments of each FFR trial, namely, the full FFR waveform (230 ms) and the formant transition region (first 50 ms) of the FFR waveform. A four-fold cross-validation strategy with 5000 iterations was implemented where three folds of the data were used for classification learning (e.g., FFRs of /ba1/ token were classified as one category) which was then used to predict the token category of the FFRs in the remaining one fold (e.g., FFRs of /ba1/ in the fourth fold were predicted as /ba1/ based on the classification learning). As a control condition, classification was also performed on the stimulus.
Results: We found that the endpoints of the /ba-da/ continuum were predicted with higher accuracy compared to the continuum points near the boundary for both full waveform and transition-only conditions. However, the prediction accuracy for the transition-only condition was overall higher than the full waveform condition. This might be because the steps in the /ba-da/ continuum differ only in the time window corresponding to the consonant-vowel transition. In the control condition, stimuli were predicted with 100% accuracy demonstrating confidence in our machine learning analysis technique.
Conclusions: Overall, the current findings indicate that categoricity, or rudiments thereof, may be indexed early in the speech processing pathway. In future, we plan to investigate other dimensions of the FFR (e.g., spectral information) to classify speech sounds using the machine learning algorithm.

TU216. Decoding of Infant Directed Speech Envelope in the Presence of Noise

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Category: Speech Perception
Background: Infants learn to perceive speech and acquire language under noisy, real-world conditions. Past research in adult listeners has shown that cortical tracking of the speech envelope is one method that can be used to study speech-in-noise perception. Infant-directed speech (IDS), in comparison to adult-directed speech, has been shown to facilitate cortical tracking of the speech envelope in quiet conditions for infant listeners. However, whether the infant brain can track the IDS speech envelope in the presence of competing speech is unclear. In this study, we recorded infant neural responses to continuous IDS using electroencephalography (EEG) in three conditions: Quiet, Co-located Noise, and Separated Noise.
Methods: Participants were 18 typically-hearing infants (9 seven-month-olds, 9 eleven-month-olds). The target stimuli consisted of naturally recorded IDS produced by two female native speakers of English from the Pacific Northwest. The noise stimuli consisted of four-talker babble and was constructed from four publicly available audiobooks read by American English speakers: 2 males and 2 females. The EEG data were recorded using a 32-electode Biosemi Active Two EEG system, with stimuli presented at an overall level of 70 dB SPL via speakers placed at 0°, +90°, and -90° azimuth. Each condition consisted of 10 one-minute IDS segments, for a total recording time of 30 minutes. During EEG recording, infants sat on a caregiver's lap in a sound-attenuated booth with an assistant showing them toys and books to keep them engaged and facing midline. EEG signals were pre-processed and analyzed offline using the decoding model of the Multivariate Temporal Response Function (mTRF) toolbox in MATLAB. This backward modeling approach assesses whether the stimulus envelope can be reconstructed based on the recorded neural responses. Pre-processing of the infant EEG signals involved filtering, down-sampling, re-referencing, and de-noising the data using Artifact Subspace Reconstruction.
Results: Preliminary analyses revealed that decoding prediction accuracies greater than 0 were observed in 15 of 18 infants in the Quiet condition, 12 of 18 in Co-located condition and 14 of 18 in the Segregated condition, suggesting that the speech envelope was represented in the recorded EEG signals in all three conditions for most infants tested.
Conclusions: These preliminary findings demonstrate the representation of the IDS speech envelope in EEG signals recorded from 7- and 11-month-old infants in quiet and in the presence of co-located and segregated noise. These results further demonstrate the feasibility of using the envelope model and mTRF method for investigating the development of speech-in-noise perception in infants and young children.

TU217. Sentence Intelligibility is Supported by Patterns of Spectral Covariation Between Frequency Bands

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Category: Speech Perception
Background: Speech signals are acoustically very redundant and this high level of redundancy could explain why sentence intelligibility is fairly robust even when speech signals are acoustically degraded [1-3]. In the present study, we investigated the contributions to sentence intelligibility of speech redundancy encoded as patterns of spectral covariation between frequency bands at the level of individual sentences.
Methods: Participants (N=16) transcribed 120 English sentences acoustically degraded to 5, 8, or 15 frequency bands derived from an ERB-scaled filter bank. Before the acoustic degradation, each sentence was expressed as a linear combination of principal component (PC) eigenvectors representing different patterns of spectral covariation between frequency bands. Half of the sentences preserved the frequency bands providing larger scores for the PC eigenvector accounting for more spectral covariance (high-covariance condition). These bands represented the spectral covariation patterns that were more dominant in the corresponding sentence. The other half of the sentences preserved the bands conveying larger scores for the eigenvector accounting for less spectral covariance (low-covariance condition). These bands represented the spectral covariation patterns that were less dominant in the corresponding sentence. We hypothesized that the frequency bands encoding larger proportions of spectral covariance would allow listeners to reconstruct a larger proportion of the missing signal than frequency bands encoding smaller proportions of spectral covariance.
Results: Participants yielded significantly better transcription accuracy scores in the high-covariance condition (mixed-effects, $ps<0.0021$). Critically, their accuracy in this condition was higher than 56% on average for as few as 5 frequency bands.

Conclusions: These findings indicate that sentence intelligibility is informed by patterns of spectral covariation between frequency bands. Specifically, they show that speech intelligibility strongly supported by the patterns of spectral covariation that are more redundant.

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TU218. Cortical and Subcortical Contributions to the Neural Response to Continuous Speech Measured With MEG

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Category: Speech Perception

Background: The neural response to speech is not only shaped by a comparatively slow tracking of the rhythm of words and syllables, but also by a fast tracking of the fundamental frequency of the voiced parts. EEG measurements of this fast response to natural continuous speech showed the presence of early subcortical contributions at a latency of about 8 ms that were modulated by selective attention (Forte et al., The human auditory brainstem response to running speech reveals
a subcortical mechanism for selective attention, elife, 6, (2017)). In addition, MEG studies found cortical contributions at the fundamental frequency of short speech tokens
(Coffey et al., Cortical contributions to the auditory frequency-following response revealed by MEG, nature communications, 7, (2016)), as well as to the fundamental frequency of continuous speech at a latency of about 40 ms (Kulasingham et al., High gamma cortical processing of continuous speech in younger and older listeners, NeuroImage 222, (2020)). However, subcortical contributions have not yet been observed in MEG measurements, while EEG recordings have not yet shown the cortical responses at the fundamental frequency.

Methods: In the present study, we detected and source-analyzed early MEG responses to continuous speech features. MEG recorded neural responses to the fundamental frequency were investigated using temporal response functions (TRFs) and subsequent source reconstruction. MEG data was recorded from 15 healthy, normal-hearing subjects listening to a continuous speech stimulus of a duration of 40 minutes. The data was subsequently analyzed in the range of the speaker's fundamental frequency.

Results: The measured neural response to the fundamental frequency of the male speaker showed a right lateralized, cortical origin at a delay of 44 ms. A second response to the modulation of the envelope of higher harmonics provided an even higher magnitude of cortical origin, at a time latency of 33 ms, also with a right hemisphere bias. Despite MEG being relatively insensitive to deeper subcortical structures, we were moreover able to identify a subcortical contribution to the neural response to the envelope modulation in continuous speech. This response emerged at a time delay of 9 ms, verifying previous studies on early subcortical contributions measured through EEG.

Conclusions: Using MEG, we were able to simultaneously record early subcortical and cortical contributions to the neural response to natural continuous speech. The detection of a short latency neural response at 9 ms verifies prior EEG studies on early subcortical neural responses to continuous speech that are assumed to be of subcortical origin.

TU219. A Comparison of Different Digits-In-Noise Test Implementations in Normal Hearing Individuals

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Category: Speech Perception

Background: The Digits-in-Noise (DIN) test is an adaptive, simplified speech-in-noise test, using digit triplets in a staircase procedure to measure speech reception threshold in noise. The DIN-test was originally

developed as a screening tool for the early identification of hearing loss. However, it is now also used for evaluating the effectiveness of hearing aids and cochlear implants, as well as for online screening, leading to a plethora of normative data for both normal-hearing and hearing-impaired populations across multiple languages. To meet the needs of these different populations, slightly modified versions of the DIN-test have been developed in previous studies. The purpose of the current study was to evaluate the effect of these variations on DIN scores in young normal-hearing adults.
Methods: We created various versions of the DIN-test in MATLAB using the original Dutch DIN materials (Smits et al. 2013). In this study, 15 normal-hearing individuals with hearing thresholds ≤20 dB HL across audiometric frequencies 250-8000 Hz participated. Based on the most common variations observed in the literature, our DIN-test implementations differed according to three variables: (1) Mixing Method determined whether the presentation level of the speech, of the noise, or of the mix was fixed at 65 dB SPL; (2) Starting signal-to-noise ratio (SNR) could be High (0 dB) or Low (-16 dB). (3) Finally, Sound Presentation could be diotic or dichotic (speech phase reversed in one ear). Otherwise, we used a typical implementation consisting of a fixed number of 24 trials, and where the DIN score is calculated by averaging the SNR over the last 21 trials.
Results: Results were analysed using a three-way repeated measures ANOVA. As expected, the DIN thresholds were significantly lower for dichotic compared to diotic Sound Presentation (-16.4 dB SNR vs -10.1 dB SNR, respectively). Unexpectedly, the results also showed a significant main effect for Mixing Methods (fixSp -12.9 dB SNR, fixNse -13.4 dB SNR, fixMix -13.5 dB SNR) and Starting SNR (High -12.9 dB SNR, Low -13.6 dB SNR), together with a significant three-way interaction. Post-hoc analysis revealed that Mixing Method, Starting SNR, and their interaction, were significant only in the dichotic condition.
Conclusions: The lower DIN score with dichotic listening may be related to the additional cue introduced by the speech phase reversal in one ear, which helps separating speech from noise. Ideally, DIN measurements should be immune to variations in Mixing Method and Starting SNR. We only saw a significant effect of these variables in the dichotic condition; however, the effects were small and limited to max of 1.3 dB SNR, and it seems that significance was only reached because the participant group was homogeneous, all young and had normal hearing.

TU220. Open Board

TU221. Differential Expression Profiles of Glutamatergic Receptors During Prolonged Salicylate Exposure in HEI-OC1 cells: Potential Role in Peripheral Tinnitus

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Category: Tinnitus
Background: Tinnitus is readily induced by high dose salicylate (SA) administration in both human and mice. Previous electrophysiological studies have implicated the role of glutamatergic neurotransmission in the generation of peripheral tinnitus caused by SA, including the potentiation of N-methyl-D-Aspartate (NMDA-R) receptors and inhibition of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA-R) receptors. However, it is unknown whether major glutaminergic receptor profiles are altered following prolonged SA administration. Here, we investigated RNA expression profiles of AMPA-R and NMDA-R following 7 days of SA administration in the House Ear Institute-Organ of Corti 1 (HEI-OC1) cells line.
Methods: We cultured HEI-OC1 cells in both permissive (33 degrees Celsius and 10% CO2) and non-permissive (39 degrees Celsius and 5% CO2) conditions in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum. Cells were then treated with saline control or SA (3 mM) for 7 days total. Cells were collected daily for total RNA extraction using commercially available RNA extraction kits (PureLink RNA mini kit, Invitrogen). RNA quantity and purity were then measured by Nanodrop Spectrophotometers. Only highly pure RNA with 260/280 ratios above 2.0 were used to further downstream assays. We performed two-step RT-qPCR using custom primers and SYBR Green florescent dyes to quantify the relative expression of mRNA for NMDA-R and AMPA-R against a house-keeping gene.
Results: In the permissive conditions (most analogous to immature, developing inner hair cells), relative expressions of NMDA-R peaked at 24 and 48 hours following SA exposure. AMPA-R also peaked at 48 hours following SA administration and return to baseline level at 72 hours. In the non-permissive conditions (most analogous to mature, non-dividing inner hair cells), relative expression of NMDA-R peaked at 72

hours and remained high thereafter following SA exposure, while expression of AMPA-R remained baseline and unchanged throughout the treatment session. We also investigated NMDA-R subunits (NR2A and NR2B) composition, which affects channel kinetic profiles of NMDA-R. We found that under both conditions, relative expression of NR2A predominates early in SA exposure and then subsequently switches to predominately NR2B following prolonged SA exposure.
Conclusions: SA treatment induced differential expression profiles of glutaminergic receptors in HEI-OC1 cells under both permissive and non-permissive conditions. Future investigation in animal models should be carried out to dissect the role of increased NMDA-R in the initiation and/or maintenance of SA induced tinnitus.

TU222. Tinnitus-Related Changes in GABAB Receptor Expression in the Mouse Dorsal Cochlear Nucleus

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Category: Tinnitus
Background: Tinnitus, phantom auditory sensation, is one of the major audiological disorders. However, the mechanisms underlying this condition remain unclear. It has previously been shown that tinnitus is accompanied by hyperactivity of neurons in the dorsal cochlear nucleus (DCN), one of the first auditory processing stations in the brainstem. GABABRs are metabotropic receptors for the major inhibitory transmitter GABA and serve as important regulators of neuronal excitability.
Methods: Tinnitus was induced by acoustic trauma or deletion of the GABAB receptor modulatory subunit, the KCTD12 protein, and its presence was assessed by behavioural methods based on gap pre-pulse inhibition of acoustic startle reflex. Distribution of the GABAB receptor was described via quantitative immunohistochemistry and electrophysiology was used to study functional changes.
Results: We found that GABAB receptors are highly expressed throughout the DCN with a maximum density in the molecular layer and show a high degree of colocalization with KCTD12 proteins. Quantitative analysis of GABAB labelling in individual DCN cell types showed a decrease in GABAB density in KCTD12-positive inhibitory DCN interneurons, cartwheel cells, but not in KCTD12-negative DCN projection neurons, fusiform cells. The differential decrease in GABAB function in DCN neurons was subsequently confirmed by patch-clamp analysis of their pharmacological responses in live brainstem sections.
Conclusions: Our experiments revealed changes in GABAB receptor expression and function in the DCN of mice with tinnitus. Based on the data, we proposed a plausible model of the pathophysiological consequences of impaired GABAB function for the activity of neural circuits in this nucleus. Our results suggest that changes affecting GABAB receptors in the DCN may be part of the processes of maladaptive plasticity that lead to tinnitus.

TU223. Neural Correlates of Hyperacusis in Behaviorally-Assessed Guinea Pigs

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Category: Tinnitus
Background: Hyperacusis is characterized by enhanced loudness growth and reduced loudness tolerance. Though comorbid with tinnitus, hyperacusis can occur independently of tinnitus, suggesting a neural basis distinct from tinnitus. Following noise-overexposure, ventral cochlear nucleus (VCN) bushy cells demonstrate steeper suprathreshold firing-rates, consistent with the psychophysical characteristics of hyperacusis (Martel and Shore, Sci. Rep., 2020). Bushy cells also contribute to the auditory brainstem response (ABR), which shows increased wave amplitudes and shorter latencies in animals with hyperacusis behavior (Longnecker et al, Front. Neurosci, 2020). Increased bushy-cell excitability may underlie increased ABR amplitudes and reduced latencies in hyperacusis. Here, we examine bushy-cell firing patterns and ABR wave alterations in guinea pigs with hyperacusis.
Methods: Hyperacusis was assessed by measuring pinna-reflex responses to clicks (100us/phase) and rising chirps (100Hz-30kHz; 2.1ms duration). Tinnitus was assessed using gap/prepulse-inhibition of the acoustic

startle (GPIAS). For each session, carrier bands (3-6, 8-16kHz) were presented at 65 dB, while the ASR was activated using a broadband noise (BBN) pulse (2-20kHz; 20ms; 0.1ms rise/fall). Prepulse stimuli (gap/sound-pulse) were inserted into the background carrier (50ms; 5ms rise/fall; 50ms delay re startle). Trials (N=20/session) were randomly ordered for each frequency.
ABRs were measured under anesthesia (ketamine/xylazine) using tone-pips (8-20kHz, 4kHz-steps; 5ms; 0.5ms rise/fall), clicks (100us/phase) and rising chirps (0.1-30kHz; 2.1ms). After a baseline measurement, four weeks of baseline behavioral data were collected. Guinea pigs were exposed (N(exposed)=12/15) to unilateral narrowband noise (102 dB SPL; 7kHz centered, quarter-octave band). ABRs (every two weeks) and behavioral assessments (biweekly) continued for an additional twelve weeks. Single-unit electrophysiological data from bushy cells was collected (NeuroNexus electrodes) within a week of the final behavioral assessment. Receptive fields (4-30kHz) and rate-level-functions (RLFs) for tones at best-frequency (BF) and BBN were assessed (0 to 90 dB SPL; 50 ms duration).
Results: A single noise-overexposure elicited tinnitus (N=5/12) and hyperacusis (5/12; hyperacusis-alone: N=2/12). ABR amplitude-intensity functions were steeper with higher peak amplitudes compared to animals without hyperacusis nor tinnitus. Bushy-cells in animals with hyperacusis exhibited steeper BF-tone and BBN rate-level functions compared to animals with tinnitus and non-hyperacusis animals.
Conclusions: Tinnitus and hyperacusis were usually co-morbid. Moreover, steepened rate level functions in bushy cells within animals with hyperacusis were consistent with psychophysical characteristics of hyperacusis. Furthermore, ABR wave alterations reflected enhanced excitability of bushy cells, suggesting its use for future hyperacusis diagnoses in humans.

TU224. Blast Shockwaves via Bone Conduction Cause Central Myelin Defect and Synapse Degeneration, and Its Relevance to Tinnitus Development

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Category: Tinnitus
Background: Blast exposure often leads to tinnitus development and hearing impairment in complex auditory environments, known as “hidden hearing loss,” which is considered to be caused by cochlear synaptopathy and myelinopathy. However, the pathophysiology of blast-induced tinnitus (BIT) is not yet fully understood. We have previously reported that blast wave transmission, not only through the tympanic membrane but also through the temporal bone, could affect the central auditory system, indicating that blast-induced auditory damage might not be fully preventable with earplugs (EPs). In this study, we aimed to evaluate the auditory pathophysiology in BIT and the protective effects of EPs against BIT.
Methods: Male CBA/J mice with or without EPs inserted in both ears [Blast-EP(+) or Blast-EP(-)] were exposed to blast overpressure using a blast tube and allowed to survive for 2 months. Auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) were measured to confirm cochlear function. BIT was objectively measured using prepulse inhibition (PPI) and gap prepulse inhibition of acoustic startle reflex (GPIAS) tests. The cochleae and the brainstem, through the cochlear nucleus (CN) to the medial nucleus of the trapezoid body (MNTB), were studied for the expression of synaptic and myelination markers.
Results: At 2 months after exposure, although there were no significant differences between blast-exposed and unexposed ears in the ABR and DPOAE thresholds at all frequencies, a significant decrease in the ABR peak 1 amplitude and prolongation in the ABR peak 1 latency were observed in the Blast-EP(-) ears compared with the Blast-EP(+) and unexposed ears. However, the ABR latency from peak 2 to peak 5 was significantly longer in both Blast-EP(-) and Blast-EP(+) ears than in the unexposed ears. Regarding BIT, although blast exposure did not influence PPI scores, GPIAS significantly increased in blast-exposed mice, regardless of EP insertion. On histopathological assessment, the number of peripheral cochlear synapses and myelinated nerve fibers was significantly lower in the blast-EP(-) ears than in the blast-EP(+) and unexposed ears. In the CN, VGLUT-1 expression was significantly decreased in blast-exposed ears regardless of EP insertion. Furthermore, in the MNTB, apparent disruption of myelination was observed in blast-exposed ears, regardless of EP insertion.
Conclusions: Blast-EP(-) mice exhibited apparent cochlear synaptopathy and myelinopathy compared to Blast-EP(+) mice, suggesting that EPs are useful for peripheral auditory protection from blast exposure. However, blast-induced central auditory damage, including synaptic and myelin disruption in the brainstem, is not preventable by EPs due to blast shockwave transmission via bone conduction. Consistent with this,

blast-exposed mice, regardless of EP insertion, showed BIT development. Synaptic and myelin deterioration in both the peripheral and central auditory systems can potentially contribute to the promotion of BIT.

TU225. Sound Measurements to Narrow the Indication for Cranial Angiography in Patients With Pulsatile Tinnitus

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Category: Tinnitus

Background: Pulsatile tinnitus can be indicative for a dural arteriovenous fistula (DAVF), which may be occult on non-invasive imaging techniques (MR, CT). A DAVF is a rare intracranial vascular malformation that can, if left untreated, lead to neurological sequelae and even death. To exclude the presence of a DAVF, patients with pulsatile tinnitus (PT-patients) without an evident diagnosis after non-invasive imaging are referred for a digital subtraction angiography (DSA). DSA is an invasive imaging procedure with a procedural risk of 1%-2% of neurological complications, of which 0.5% are permanent. In a tertiary setting approximately 75% of the PT-patients who undergo a DSA are unnecessarily exposed to these procedural risks. We studied whether an automated detection algorithm that analyzes sound measurements from PT-patients could narrow the indication for DSA.

Methods: Between 2015 and 2021, sound measurements of 41 PT-patients were collected via a sensitive microphone placed in the outer ear canal. The measurements were taken prior to a DSA. Measurements were analyzed by an automated detection algorithm that represents the presence of a pulsatile sound in several frequency bands as a Pulsatile-Tinnitus-Coherence-Index (PTCI).

Results: Our algorithm demonstrated that PT-patients with a DAVF have a significantly higher PTCI in frequency bands up to 4 kHz than PT-patients without DAVF. The PTCI in a frequency band centered at 1 kHz was the most discriminating band for predicting a DAVF on DSA, with a sensitivity of 100% (74-100) and specificity 83% (64-94) at a PTCI cut-off value of 0.75. At this cut-off value, the indication for DSA in this cohort would have been narrowed to 17 patients without missing a DAVF-diagnosis. A DSA would have been avoided for 24 PT-patients (59%).

Conclusions: Analysis of ear-canal measurements of PT-patients can narrow the indication for a DSA, thus avoiding unnecessary procedural risks. An automated algorithm that quantifies pulsatile tinnitus as an objective measure can be used in the diagnostic follow-up of PT-patients to help the clinician in the decision making on the necessity of a DSA.

TU226. Analysis of Cerebrospinal Fluid Provides Important Insight Into the Vestibular Schwannoma Secretome

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Category: Vestibular: Basic Research and Clinical

Background: Vestibular Schwannoma (VS) is a benign intracranial tumor arising from merlin-deficient Schwann cells of the vestibulocochlear nerve. VS can cause hearing loss, tinnitus, and dizziness. Recent studies suggest that VS can secrete pro-inflammatory factors that may contribute to tumor growth and hearing loss. However, the VS secretome is not well understood. We investigate the cytokine profiles in cerebrospinal fluid (CSF) from patients undergoing VS surgery with and without serviceable hearing.

Methods: We collected CSF from the cerebellopontine angle from 24 patients undergoing VS surgery and 1 patient undergoing surgery for intracochlear schwannoma (control). Pre-operative audiograms were analyzed, and patients were grouped into 2 cohorts (serviceable hearing versus unserviceable hearing loss). To analyze cytokine expression, cytokine arrays were incubated with CSF at 4°C overnight followed by detection antibody-conjugated to biotin at 4°C overnight. Subsequently, arrays were treated with streptavidin conjugated to horseradish peroxidase at room temperature for 2 hours. Images were taken using a chemiluminescence imager with 10 second exposure time. Protein expression was measured using ImageJ

software with microarray plug-in. Expression levels were normalized to positive controls. Data analysis was performed using 95% confidence intervals and two-way analysis of variance.
Results: When compared to intracochlear schwannoma, CSF from VS demonstrated a higher expression of angiogenin, RANTES, IL3, IL1-beta, PDGF-BB, and MIP3-beta and a lower expression of ArgP, CTACK, ARCP30, IGFBP6, IL6R, TIMP1, FGF9, sgp130, uPar, IL2R-alpha, and osteoprotegerin. CSF from VS patients with unserviceable hearing loss had higher expression of cytokines overall, when compared to VS patients with serviceable hearing. IL8 was expressed significantly higher for VS patients with unserviceable hearing loss.
Conclusions: When compared to intracochlear schwannoma, CSF from VS demonstrated a higher expression of angiogenin, RANTES, IL3, IL1-beta, PDGF-BB, and MIP3-beta and a lower expression of ArgP, CTACK, ARCP30, IGFBP6, IL6R, TIMP1, FGF9, sgp130, uPar, IL2R-alpha, and osteoprotegerin. CSF from VS patients with unserviceable hearing loss had higher expression of cytokines overall, when compared to VS patients with serviceable hearing. IL8 was expressed significantly higher for VS patients with unserviceable hearing loss.

TU227. C-Fos Activation and In-Vivo Calcium Imaging of Cholinergic Efferent Vestibular Nucleus Neurons in Response to Provocative Motion

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Category: Vestibular: Basic Research and Clinical
Background: Our recent anatomical study in mice, showed brainstem Efferent Vestibular Nucleus (EVN) provides extensive cholinergic innervation of both ipsilateral and contralateral peripheral vestibular organs. However, despite being a rich source of bouton contacts in the periphery, we do not know how, when, or why the EVN is activated. Recent studies have shown the mouse EVN can modify afferent vestibular output and may contribute to motion sickness symptoms. To better understand the function of cholinergic EVN neurons and their potential role in motion sickness, we examined the effects of provocative motion (PM) on EVN neurons. We used two methods 1) c-Fos protein expression and 2) in vivo calcium imaging using head-mounted miniscopes.
Methods: c-Fos is an immediate-early gene product and considered an indirect marker of neuronal activation. To study the effects of PM on EVN, adult Wild Type (WT) (C57/BL6; 3-10 months), aged WT (C57/BL6; > 2 years), and adult transgenic Chat-gCaMP6f mice were exposed to 15 minutes of orbital rotary motion in the horizontal plane. After 90 min recovery, mice were perfused for c-Fos immunolabelling. To examine real-time effects of EVN activity, we used calcium imaging captured by head-mounted miniscopes in freely moving animals before, during, and after PM. The abducens nucleus (AbdN), another brainstem cholinergic nucleus, served as a positive control. We used Chat-gCaMP6f-Ai148D transgenic mice, which express calcium indicator, GCaMP6f, in all cholinergic cells, including EVN and AbdN. A miniscope lens was implanted into the brainstem, dorsal to either EVN or AbdN, to monitor fluorescence changes in GCaMP6 due to neuronal activity. Precise locations of the miniscope lens-track in relation to EVN and AbdN, were confirmed post-mortem, using immunolabelling and confocal microscopy.
Results: VN neurons showed increased expression of c-Fos protein after PM in adult WT (n=5) and Chat-gCaMP6f (n=5) mice, but not aged WT mouse group (n=5). These data were consistent with PM-induced behavioral responses (tail warming, and crouching) which occurred in adult WT and transgenic mice but not in aged WT. Miniscope calcium imaging in GCaMP6 mice suggests EVN activity occurs during self-generated movements but becomes less active during PM when the mouse become stationary due to motion sickness. In contrast, AbdN showed regular and predictable calcium signals during PM.
Conclusions: c-Fos expression in EVN neurons only occurs in mice that exhibit motion sickness symptoms (tail warming and crouching) in response to PM. This suggests that EVN activity is related to motion sickness as has been previously reported. Real-time calcium imaging of adult EVN neurons showed increases in fluorescence that correlated with self-generated head movements. However, fluorescence diminished markedly during PM when mice became stationary. This apparent discrepancy between c-Fos activation and reduced calcium signaling during PM has yet to be resolved.

TU228. Open Board

TU229. The Role of Endotoxemia in Aminoglycoside-Induced Auditory and Vestibular Toxicities in a Clinically Relevant Model for Cystic Fibrosis

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Category: Vestibular: Basic Research and Clinical

Background: Patients diagnosed with Cystic Fibrosis (CF) are at a significantly increased risk of developing hearing and vestibular dysfunction throughout their lives. This has largely been associated with their increased exposure to aminoglycosides and in particular Tobramycin. Tobramycin is often used in patients with CF because of its activity against a common pathogen in this population; Pseudomonas aeruginosa. Previously, endotoxemia has been shown to increase the potentiation of the negative effects of kanamycin on the cochlea, however, it is not known if this finding is also consistent with tobramycin. In addition, whether endotoxemia potentiates vestibular ototoxicity as it does in the cochlea is unknown. The current animal models for aminoglycoside ototoxicity do not fully recapitulate the clinical findings of cochlear and vestibular dysfunctions seen in patients with CF. Here we present the characterization of a mouse model using Tobramycin that produces significant vestibular and cochlear deficits like that observed in some patients with CF.

Methods: Cdh23 (ahl) allele corrected B6N(Cg)-Cdh23tm2.1Kjn mice were treated with Lipopolysaccharide (LPS) at 2.5 mg/kg I.P. 3 times over a 14-day period alone or in combination with subcutaneous injections of Tobramycin (TM) at 200 mg/kg two times a day, 4.5 hours apart for 14 days. Assessments of auditory and vestibular functions were performed using auditory brainstem response (ABR), distortion product otoacoustic emissions (DPOAE), and Vestibular-sensory Evoked Potentials (VsEPs) on days 1, 10, and 21 post-treatment.

Results: We found that vestibular function, measured via VsEP, was impaired after 14 days of twice-a-day tobramycin treatment. In those same animals, there was no difference in pre- and post-treatment ABRs or DPOAEs and no changes when compared to age-matched controls. In our animals treated with LPS and Tobramycin significant changes in auditory function were observed. Interestingly, no additional decrement in vestibular function was seen. In addition, no ABR, DPOAE, or VsEP changes were observed in animals exposed to LPS alone when compared to age-matched controls.

Conclusions: We report for the first time, tobramycin-induced vestibular impairment in B6N(Cg)-Cdh23tm2.1Kjn mouse strain. Tobramycin without adjuvant LPS administration only produced vestibular dysfunction without auditory dysfunction. In addition, our data suggest that LPS and subsequent inflammation are required for tobramycin-induced cochleotoxicity. These findings are consistent with what is seen clinically in patients with Cystic Fibrosis and suggest that the developed mouse model can be utilized for further studying tobramycin-induced ototoxicity in this clinical population.

TU230. Otolithic But Not Semicircular Canal Loss is Associated With Decreased Cognition in Adults

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Category: Vestibular: Basic Research and Clinical

Background: Emerging research demonstrates that cognitive impairment is greater among people with vestibular dysfunction, but the specific vestibular deficits driving these associations are unclear. This study leveraged a large cohort with comprehensive physiologic testing to examine associations between cognition and vestibular function. We hypothesized that otolithic function would have more associations with cognition than semicircular canal function.

Methods: Records from the University of Washington Dizziness and Balance Center Data Repository were retrospectively reviewed for people undergoing comprehensive vestibular testing between 2018-2020. Cognition was measured using the Montreal Cognitive Assessment (MoCA). Each vestibular test was scored on an ordinal scale from 0 (normal) to a maximum score of 1-4. Three composite scores were calculated as the sum of ordinal scores divided by the number of tests included in that composite score. Canal loss scores were calculated from video nystagmography (during head shake, positioning/al, and caloric testing); video head impulse test; rotational chair testing, and dynamic visual acuity. Otolithic loss scores were calculated

from cervical and ocular vestibular evoked myogenic potentials and subjective visual vertical. A total vestibulopathy score was calculated from all tests completed. Maximum possible scores were 1.78 for canal loss, 1.80 for otolitic loss, and 1.76 for total vestibulopathy, with higher scores indicating greater vestibular impairment. Spearman correlations were used to examine associations between vestibular composite scores and cognition (MoCA sub-scores and total score).
Results: A total of 252 records were included, with 153 (60.7%) female participants. Mean age (standard deviation) was 55.6 (15.5) years, and total MoCA score was 25.5 (3.4). Mean scores were 0.28 (0.18) for total vestibulopathy, 0.21 (0.21) for canal loss, and 0.23 (0.25) for otolithic loss. Higher vestibulopathy scores were correlated with worse visuospatial/executive function (r=-0.17, p=0.007). Canal loss scores were not associated with MoCA total or sub-scores. Greater otolithic loss scores were associated with worse visuospatial/executive function (r=-0.21, p=0.001), attention/vigilance (r=-0.14, p=0.004), language/repetition (r=-0.14, p=0.028), and total MoCA scores (r=-0.18, p=0.006).
Conclusions: This study used composite scores to summarize vestibular function across various physiologic tests. Otolithic involvement was associated with worse global cognition and worse function across multiple cognitive domains. Higher levels of vestibulopathy scores were correlated more specifically with visuospatial/executive function, consistent with prior research and potentially reflecting shared pathways in hippocampal and basal ganglia areas. These findings suggest the importance of screening cognition in people referred for dizziness and balance concerns. Associations between cognition and vestibular function also indicate potential shared neurologic pathways that could be targeted in novel therapeutic interventions.

TU231. CUDC907 Promotes Cell Death via Caspase-3/7 Pathway in Primary Vestibular Schwannoma Cells

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Category: Vestibular: Basic Research and Clinical
Background: Neurofibromatosis type 2 (NF2) is a genetic disorder caused by mutations in the NF2 gene, which lead to inactivation of the tumor suppressor protein, merlin. NF2 patients develop multiple tumors around the brain and spine. The hallmark of NF2 is bilateral vestibular schwannomas (VS), which cause progressive hearing loss and deafness. There are no approved drug therapies for NF2. Previous work identified phosphoinositide-3 kinase (PI3K) as a potential target for NF2-associated schwannomas. In this study, we investigated the effect of CUDC907, a dual histone deacetylase (HDAC)/PI3K inhibitor, on viability, cleaved caspase-3/7, and annexin for five primary VS cell lines. We also studied the mechanistic activity through which CUDC907 acts on primary VS cells.
Methods: We harvested fresh tumor chunks from patients undergoing VS surgery. Subsequently, primary VS cell lines were cultured and treated with vehicle (0.0005% DMSO) or CUDC907 (0.1nM, 1nM, 10nM, 100nM) for up to 72 hours. We performed cell-based assays for viability at 72 hours, cleaved caspase-3/7 at 36 and 48 hours, and annexin from 0 to 48 hours. Total YAP, HDAC2 and pHDAC2 expression levels were measured using Simple Western. We compared the expression levels of p21 and total YAP in VS cells at 24 and 48 hours with immunocytochemistry. NF2 mutations were identified using whole exome sequencing.
Results: CUDC907 (100nM) caused a dose-dependent reduction in viability at 72 hours in VS cells, promoted significant increases in cleaved caspase-3/7 at 36 and 48 hours, and induced annexin V expression at 16 hours. Simple western blot was performed on protein extract from naïve tumor chunks. All VS expressed YAP, HDAC2, and pHDAC2 protein. Regression analysis was performed on normalized target protein levels and viability. We found a moderate inverse relationship between pHDAC2 and viability and a moderate positive relationship between totalYAP and viability. On immunocytochemistry, CUDC907 reduced cytoplasmic YAP and increased nuclear p21 in VS cells at 24 and 48 hours, compared to vehicle (0.0005% DMSO). NF2 mutations were identified in all VS.
Conclusions: CUDC907 initiated apoptosis of primary VS cells, as demonstrated by reductions in viability, increased cleaved caspase-3/7 activity, and upregulation of annexin. CUDC907 acts by inhibiting pHDAC2, reducing YAP expression, and increasing p21. Further investigations in preclinical models are warranted to determine whether CUDC907 will be an potentially effective for VS in NF2 patients.

TU232. Advancing Bioinformatically Informed Targets to Achieve In Vivo Vestibular Regeneration

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Category: Vestibular: Basic Research and Clinical
Background: Vestibular hair cells are the sensory receptors in the inner ear that are essential for balance and spatial orientation. Loss of hair cells in the vestibular system can result from certain medicines or natural aging. Extensive hair cell loss can lead to chronic balance problems, increased risk of falls, and result in significant life impairment or incapacitation. The National Institutes of Health estimates that up to eight million U.S. adults suffer from a chronic balance disorder. A subset of these individuals suffer from a profound bilateral loss of vestibular sensation in both ears called bilateral vestibulopathy, or BVP. Regeneration of vestibular hair cells, and their neuronal connections, is an attractive strategy to restore function to the many patients suffering from vestibulopathy. Because there are two physiologically distinct vestibular hair cell subtypes (Type I and Type II) with unique contributions to function, a successful regeneration strategy will restore the correct proportion of both subtypes. Here we detail identification and in vivo testing of a bioinformatically-informed target that drives hair cell identity and maturation.
Methods: We performed a systematic characterization of transcriptional changes leading to the specification of each type of hair cell in mouse utricles using single cell RNA-seq, with a focus on identifying targets required for Type I formation. Mouse vestibular organs were collected at 18 developmental time-points between E11.5 and adulthood. With ~10,000 cells collected at each stage we find excellent representation of both hair cells and supporting cells. We isolated hair cells from the dataset and inferred the order in which they differentiate using trajectory analysis. We applied topic modeling and gene regulatory analyses as orthogonal approaches to add confidence in our selection of regulatory targets.
Results: These analyses identified temporally expressed genes and molecular pathways during Type I hair cell specification and maturation. To obtain targets that are potential drivers of Type I specification and maturation, we identified transcriptional factors with master regulatory profiles and downstream modulatory partners. Here we demonstrate the effect of one candidate factor on Type I differentiation and maturation in vivo.
Conclusions: This data demonstrates in vivo validation of a bioinformatically selected target predicted to regulate Type I hair cell differentiation.

TU233. A Simple Model for Mechanical Activation and Compound Action Potential Generation by the Utricle in Response to Sound and Vibration

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Category: Vestibular: Basic Research and Clinical
Background: Air-conducted sound (ACS) and bone-conducted vibration (BCV) are routinely used in the clinic and research to excite the otolith organs and evoke compensatory vestibular compound action potentials (vCAPs) and vestibular evoked myogenic potentials (VEMPs). Otolith afferents making calyceal synaptic contacts with type I hair cells respond to the onset of phasic ACS or BCV stimuli with short-latency action potentials and respond to high-frequency sinusoidal stimuli with precisely timed phase-locked action potentials. In guinea pigs, the most sensitive otolith afferents phase-lock with vector strength greater than auditory spiral ganglion neurons. Although neural responses are well documented, precisely how sound and vibration lead to short-latency action potentials is not well understood. Here, we present and validate a simple model for mechanical activation of the utricle, action potential generation, and vCAPs in response to ACS and BCV.
Methods: We developed a 2-degree-of-freedom model of utricular mechanics to quantify vibration of the epithelium relative to the temporal bone and vibration of the otoconial layer relative to the epithelium in response to ACS and BCV. The relative motion between the epithelium and the otoconial layer was used to determine hair bundle shear and the mechano-transduction current. The current was used to drive a simple integrate-and-fire (IAF) model of the afferent neuron to predict the mean action potential time of the population of sensitive neurons. A saturating nonlinearity was used to describe the number of units recruited, and a Gaussian distribution was used to describe the population variance in timing. vCAPs were simulated by convolving the distribution of action potentials with an extracellular voltage kernel. We

validated the model by comparing predictions to experimentally measured vibrations of the epithelium (laser doppler vibrometry) and to vCAP measurements for ACS and BCV. All procedures were approved by the University of Sydney Animal Ethics Committee. Once validated, the model was used to predict the utricular vibration and hair bundle shear in response to acoustic blast exposure.
Results: The magnitude and latency of the short-latency vCAPs in response to transient ACS and BCV scale with the hair bundle shear rate. For pulse BCV with durations shorter than 0.8 ms, the magnitude of vCAPs scales with linear acceleration, while for durations longer than 0.9 ms, it scales with the linear jerk. Blast-induced hair bundle angular deflection is predicted to be two orders of magnitude larger than physiological stimulation, indicating severe damage to hair bundles immediately after acute blast exposure.
Conclusions: Our model can be used to predict utricular hair bundle shear, short-latency action potential generation in calyx-bearing afferents, and vCAPs for ACS or BCV stimulus. Results are relevant to the design of ACS and BCV stimuli and to the interpretation of evoked responses for both clinical and research applications.

TU234. Vestibular Ganglion Organoid Development Using Progenitor Cells: Comparison of 2D and 3D Method

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Background: Vestibular neuronitis is one of the common causes of dizziness. It affects many people but neither cure nor medical / surgical therapeutic option exist. There are candidate mechanism for this recovery or regeneration of vestibular neuronal structures but the exact mechanism is still unclear. One way to confirm the exact process would be the generation of organoids using vestibular ganglion progenitor cells. Organoids is an in vitro model similar to cell lines, but have the advantages of long-term culture and cryopreservation, easy manipulation and observation. Organoids can reproduce the cell organization of specific organs in vivo, thus it has been actively used as a model for drug development and toxicity. Two dimensional (2D) and three dimensional (3D) in vitro cell culture environments are very different. Cell densities and cell-to-cell interaction differs in these two conditions could alter the final differentiation outcome of vestibular organoids.
The aims of this study were to develop a protocol for producing vestibular organoids by utilizing neurotrophic factors and compare the effect of 2D and 3D conditions on differentiation.
Methods: Vestibular ganglions from P1-2 of SD rats were dissected and cultured in 2D and 3D conditions for proliferation. Upon reaching cell confluency at 80%, cells were used as is for 2D or formed into neurospheres for 3D culture. Media was changed to differentiation media which includes BDNF and NT-3 (10 ng/ml). Cell conditions were compared through immunocytochemistry throughout the duration of differentiation.
Results: There was no significant difference in Nestin (progenitor marker) and NeuN (neuronal marker) expression between before and right after differentiation in both 2D and 3D. At D21, Nestin was significantly decreased in both conditions suggesting that the organoids were losing pluripotency. In both conditions, NeuN positive cells were observed and were comparably increased at D21 than in early stages, possibly indicating neuronal differentiation. Consequently, 3D cultured organoids also generated glial cells including microglia (expressing ionized calcium-binding adaptor molecule 1 protein, Iba1) and astrocytes (expressing the glial fibrillary acidic protein, GFAP). Whereas in 2D culture, only GFAP+ cells were found, but Iba1+ cells could not be confirmed.
Conclusions: It was confirmed through immunocytochemistry that cells with stem cell characteristics were differentiated into neurons, as well as non-neuronal cells in a specific media composition and time. In 3D culture, pronounced cell population was differentiated into neurons, microglia and astrocytes, whereas in 2D culture, most of cells were differentiated into neurons and astrocytes.

TU235. Increased Head-Turning Activity and Low-Penetrance Circling Behaviour in Mice Lacking ZPLD1, a Protein That Scaffolds the Cupula to the Roof of the Ampulla

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Category: Vestibular: Basic Research and Clinical
Background: ZPLD1 (zona pellucida-like domain 1) is a major component of the cupula, the extracellular matrix that sits atop of the ampullae in the inner ear (Dernedde et al., 2014). Two spontaneous, recessive missense mutations have been reported in Zpld1, circler with hearing (cwh) and spiral (sprl). Both mutations were found to cause circling behaviour in mice. The penetrance of this trait was, however, lower in the sprl/sprl mice, and Zpld1-/- mice homozygous for a null mutation (Zpld1(em1/IMPC)) did not circle (Vijayakumar et al., 2019). The aim of this study was to determine if the loss of ZPLD1 affects the structure of the cupula and how this influences the behaviour of Zpld1-/- mice.
Methods: Zpld1(em1/IMPC) mice were purchased from the Jackson laboratory. To study the structure of the cupula, inner-ear cryosections were stained with anti-otogelin, anti-ZPLD1 and wheat germ agglutinin. To study behaviour, litters comprising Zpld1+/- and Zpld1-/- mice were raised under a normal light-dark cycle and individuals were video-recorded for 5 minutes in a small sawdust-covered arena at postnatal day (P) 20, 23, 27, 34 and 46. DeepLabCut was trained to track the position of mouse body parts to allow quantitation of head-turning activity. To determine whether visual cues and/or age compensate for loss of ZPLD1, additional litters were raised in darkness for a period of 8 days, from P12 to P20, before return to normal lighting conditions and subsequent testing.
Results: Immunofluorescence microscopy shows ZPLD1 is present throughout the cupula in Zpld1+/- mice but is, unlike otogelin, more concentrated at the upper end of the cupula. ZPLD1 cannot be detected in Zpld1-/- mice and the cupula appears reduced in size, no longer reaching the roof of the ampulla. Seven litters of mice were reared under normal lighting conditions, and 9 litters were dark-reared; a total of 109 mice. On testing, and irrespective of rearing conditions, 42% of the Zpld1-/- mice (21/55) exhibited circling behaviour. None of the 54 Zpld1+/- mice circled when tested. The average percentage of circling Zpld1-/- mutants in the dark-reared litters (49.1%) and those reared under normal conditions (35.7%) was not significantly different ($p=0.783$, Fisher's exact test). Head-turning activity in the two groups of mice, assessed using a repeated measures ANOVA model, revealed statistically significant main effects of genotype ($p<0.001$) and age ($p=0.030$); head-turning activity decreases with age but is considerably greater in Zpld1-/- mice at all stages tested. Dark rearing, however, does not cause a significant change in head-turning activity ($p=0.123$).
Conclusions: Zpld1-/- mice exhibit an increase in head-turning activity, and the cupula fails to reach to the roof of the ampulla in the absence of ZPLD1. Further studies are required to determine why only a subset of Zpld1-/- mice circle.

TU236. Image Based Analysis of the Otoliths Macula in the Rat

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Background: In the vestibular system, the sensory epithelia of the otolith endorgans present a distinctive cellular organization. Different zones of the macula exhibit regional diversity in the number and type of hair cells and afferent innervations and morphology. Our lab utilizes a rat model to detail the sensitivity of the vestibular epithelia to various stimuli, such as infrared radiation or sound, and the role of different cell types and synaptic inputs. It is pivotal to characterize the morphology of the sensory epithelium accurately. A detailed spatial characterization of the maculae could reveal new information on spatiotemporal properties of head movement encoded by the otoliths. It can also improve preclinical models of vestibular hypofunction following noise or blast impulse trauma and clinical translation. Vestibular morphological analysis has been typically performed through labor-intensive manual processing of confocal images. Here, we present a semi-automated approach to detail the morphological map of the otolith endorgans in a rat model that can be extended to other preclinical models.
Methods: The vestibular endorgans were collected from rats, including those exposed to different levels of noise and blast trauma. In whole mount preparations of utricular and saccular specimens, the hair cell bundles were labeled using antibodies against phalloidin. High-resolution images were obtained and analyzed via a custom MATLAB script. Image segmentation and thresholding were employed with a semi-automated parameter space for the determination of vestibular hair cell quantity, distribution, and

stereociliary bundle orientation. Image analysis was extended to complete a map of the rat maculae, including morphometric data and hair cell distribution.
Results: In all the samples analyzed, vestibular hair cells present in the neuroepithelia were successfully identified using custom thresholds with image filters based on size, cell boundaries, and fluorescence intensity. The epithelial disruption observed in blast-exposed samples did not affect the accuracy of the code. Details of macular differentiation, including the localization of the line of polarity reversal, were also determined by analysis of cell orientation measured as the direction of the vector from the center of the cuticular plate to the center of the kinocilium.
Conclusions: The MATLAB-based approach represents an efficient tool for analyzing regional specializations in otoconial maculae and significantly reduces the time required, especially when compared with manual image quantification. Detailing the cellular and synaptic architecture of the neuroepithelium is important for studies of various vestibular pathologies and for understanding the efficacy of potential therapeutic targets. Semi-automated tools such as those presented here could provide higher throughput for such work.

TU237. Gender Differences in Blast-Induced Vestibular Deficits in Rats

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Category: Vestibular: Basic Research and Clinical
Background: As air-filled structures, the ears are among the most frequently damaged sites following exposure to blast overpressure waves, such as that produced by explosive devices. Blast victims often report vestibular symptoms in the days or weeks after blast exposure that persist for months or even years. Our previous studies showed that blast waves delivered into the external ear canal of rats produce injuries of the peripheral and central pathways (Sandlin et al., 2018; Yu et al., 2020). In this study, we further examined gender differences in the blast-induced vestibular deficits in rats.
Methods: VOR responses to sinusoidal head rotation (rVOR, 0.2~4Hz) and translation (tVOR, 0.2-2 Hz) were measured in adult male and female Long-Evans rats. After establishing VOR baseline, anesthetized rats were exposed to a single blast of 138kPa (20 PSI, 40% of lethal dose determined in previous studies) to the left ear. VOR responses were measured at day 1, 3, 7, 14, 28 and 56 after blast exposure. A second group of rats were sacrificed for histology of vestibular hair cells and the vestibular nuclei at the same time points. In a third group of rats, single unit recordings of vestibular afferents were performed to measure blast-induced changes in afferent spontaneous firing rate, discharge regularity and sensitivities to head rotation and translation.
Results: Exposure to a single blast of 20PSI induced larger increases in respiratory rate in female rats (N=12) than male rats (N=20) (33.7% vs 17.7%, $P<0.008$). In male rats, steady state VOR gains exhibited little changes over the 8 weeks after blast exposure. In female rats, however, VOR gains exhibited no change at day 1 post exposure, an increase at day 3 post exposure, and progressive declines up to 8 weeks post exposure. In particular, 2 out of the 7 female rats exhibited right beat nystagmus at 2 weeks post blast exposure, indicating progressive vestibular deficits in the left vestibular end organs. Preliminary analysis in male rats showed that a single blast of medium intensity resulted in small changes in spontaneous firing rates, but significantly changes in regularity of spontaneous discharge in the irregular afferent neurons. The blasted rats had fewer vestibular afferents that were responsive to head rotation and translation than the control rats [82% (sham) vs. 56% (1day post blast) vs. 46% (14 days post blast)]. The medium blast also resulted in activation of microglia and astrocytes in the central vestibular nuclei. Some ED1+ macrophages were found in the brainstem parenchyma, indicating compromise in the blood-brain barrier following ear blast exposure.
Conclusions: These results showed the effects of gender on blast induced-vestibular deficits in rats. Ongoing studies are to further characterize these effects and investigate the underlying mechanisms.
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TU238. Comparison of the Schellong Test for Patients Complaining of Dizziness/Vertigo

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Background: The causes of vestibular disorders are diverse and we often find cases with multiple causes rather than a single cause. Vestibular disorders are often complicated by Orthostatic Intolerance (OI), and in 2019 the Barany Society also presented diagnostic criteria for Hemodynamic orthostatic dizziness/vertigo. We perform the Schellong test on patients with complaints of dizziness/vertigo whenever possible. In this study, we report a retrospective study of the association between vestibular disorders and classification of patients complaining of dizziness/vertigo who visited our hospital on the results of the Schellong test.
Methods: Between March 2018 and March 2021, 1072 patients with patients complaining of dizziness/vertigo presented to our hospital, of whom 683 underwent the Schellong test. The Schellong test was performed by measuring blood pressure and pulse rate immediately after standing up and 5 minutes after standing up, and at least 20 mmHg reduction in systolic blood pressure or 10 mmHg reducton in diastolic blood pressure was diagnosed as Orthostatic Hypotension (OH). Postural orthostatic tachycardia syndrome (POTS) is characterized by a sustained heart rate increase of at least 30 beats per minute or a heart rate of 120 beats per minute during the Schellong test in the absence of OH. Comparisons were made for OI, i.e., OH and POTS, with respect to age, vestibular disorders, and medications.
Results: OI was seen in 114 cases (17%), of which 65 cases (10%) of OH were diagnosed immediately after standing, 21 cases (3%) after 5 minutes of standing, and 28 cases (4%) of POTS. Younger patients were more likely to have POTS and more likely to have an elevated pulse rate on standing. On the other hand, the proportion of OH diagnosed after 5 minutes of standing increased with older age. Benign paroxysmal positional vertigo (BPPV) and unilateral peripheral vestibulopathy were also more frequently associated with OH diagnosed immediately after standing, while Meniere's disease, Delayed endolymphatic hydrops and Vestibular migraine were more frequently associated with POTS. In our study of medications, patients with OH diagnosed immediately after standing were more likely to take calcium antagonist, angiotensin II receptor blocker, and angiotensin-converting-enzyme inhibitor, then patients with POTS were more likely to take SSRI/SNRI.
Conclusions: By performing the Schellong test, a comparison was made regarding age, vestibular disorders, and medications, with the results shown above. Although we do not perform the head-up tilt test at our hospital, the conventional Schellong test is a simple and useful method. The American College of Cardiology/American Heart Association (ACC/AHA), in collaboration with the Heart Rhythm Society (HRS), published the first published syncope guidelines in 2017, which includes a detailed classification of OI. We believe that detailed classification of OI will lead to more appropriate medication therapy.

TU239. The Significant Difference in Vestibulo-Ocular Reflexes Between Vestibular Neuritis and Labyrinthitis as Assessed by the Video Head-Impulse Test

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Category: Vestibular: Basic Research and Clinical
Background: To expand understanding of the pathophysiologic mechanism by elucidating whether there is a difference in vestibulo-ocular reflex (VOR) for each semicircular canal between vestibular neuritis (VN) and labyrinthitis
Methods: 23 patients with VN and 27 patients with labyrinthitis who had been hospitalized were included in this retrospective analysis. Pure-tone audiometry (PTA), the bithermal caloric test, and the video head-impulse test (vHIT) were performed within 5 days from symptom onset.
Results: In the VN group, mean VOR gains were decreased on the ipsilesional horizontal canal (iHC, 0.51) and anterior canal (iAC, 0.55), leading to marked asymmetry, while the gain of ipsilesional posterior canal (iPC, 0.85) was relatively preserved. For the labyrinthitis group, the mean VOR gain was 0.72 in the iHC, 0.73 in the iAC, and 0.55 in the iPC. We observed statistical differences in the VOR gain and incidence of corrective saccade on the ipsilesional side in three semicircular canals between groups ($p=0.002$ in HC, $p=0.003$ in AC, and $p<0.001$ in PC). The receiver operating characteristic (ROC) curve showed that PTA, iPC gain, and gain asymmetry of PC (Gs in PC) are excellent parameters to distinguish labyrinthitis from VN.

Conclusions: The current study found that patients with VN and labyrinthitis had a different degree and patterns of vHIT involvement in three semicircular canals, suggesting that the two disorders may have distinct etiologies.

TU240. Vestibular Compound Action Potentials and Macular Velocity Evoked by Sound and Vibration in the Guinea Pig

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Category: Vestibular: Basic Research and Clinical
Background: Calyx-bearing afferents in the utricle are remarkably sensitive to auditory frequency sound and vibration, and fire action potentials at short latencies and precise times relative to the stimulus waveform, known as phase locking[1]. This feature of otolith function is commonly tested in the clinic using transient air-conducted sound (ACS) or bone-conducted vibration (BCV) to evoke reflexive cervical or ocular myogenic potentials (VEMPs)[2]. However, precisely how high-frequency transient stimuli lead to mechano-transduction and neural responses in mammalian otolith organs is not well understood. In this report, we examine the stimulation sensitivity of the calyx-bearing afferents in the utricular nerve of guinea pigs, via the vestibular compound action potential (vCAP), with corresponding measures of epithelial macular vibration and MET current output relative to input drives.
Methods: This study was approved by the University of Sydney Animal Ethics Committee (Approval #: 2019/1533). Experiments were performed on 28 adult guinea pigs (Cavia porcellus) weighing between 300-500g of either sex. Guinea pigs were anaesthetized and mounted in earbar frames for surgery, electrode implantation, and response recording. Stimuli and responses were generated and acquired using custom-developed LabVIEW programs. BCV was delivered via an electrodynamic mini-shaker (Type-4810, Brüel and Kjær, Denmark) attached to the earbar in the inter-aural plane via a 50mm steel rod. ACS was delivered via a Beyerdynamic speaker (Heilbronn, Germany) in the earbar, with a flat frequency response. vCAPs, vestibular microphonics (VMs), macular and stapes velocity (acquired using vibrometry), and associated input drives (such as, bone acceleration) were recorded and analysed to examine how transient sound and vibration generate neural responses in the mammalian otolith organ.
Results: vCAP generation and sensitivity is similar for pulsatile BCV and ACS, with equivalent vCAP Input-Output functions across stimuli. vCAPs evoked by transient stimuli scale with macular velocity and temporal bone acceleration for brief drive rise-times (<1ms). For longer duration rise-times, vCAP scaling displays a modal switch to linear jerk, consistent with previous findings in the field. Comparisons of the vCAP and VM frequency tuning curves to tone-burst stimuli reveal that the vCAP scales with macular velocity, whereas the extracellular MET current (microphonic) scales with displacement, highlighting key differences in signal processing between type-I vestibular hair cells and their irregular afferents.
Conclusions: These findings provide new insight into the stimulation sensitivity of irregular striolar afferents at the level of the macular neuroepithelium, with clinical relevance for understanding the activation and tuning of neurons responsible for driving rapid compensatory reflex responses. Results also provide a framework to help explain the response characteristics of the vCAP and VsEP, which are commonly used to screen otolith function in the laboratory in animal models of health and disease.
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TU241. Evidence and Main Site of ATP Secretion in the Vestibular System by Mechanical Stimulation

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Category: Vestibular: Basic Research and Clinical
Background: The vestibular system is important in maintaining balance during acceleration stimulation in daily life. The cochlear perilymph and endolymph have long been known to contain nanomolar concentrations of ATP that can mediate cochlear and hearing functions through various purinergic receptors. Similarly, ATP is thought to exist in vestibular organs to modulate the function of balance system; however,

direct functional evidence for the ATP release in the vestibular labyrinth is lacking. The goal of the present study was to investigate the evidence and location of ATP release in vestibular organs.
Methods: The membranous vestibular labyrinths of C57BL/6 mice were harvested and the utricle, saccule, ampulla, and common crus were separated by microdissection. To measure the ATP secretion rate per area of each tissue, the surface area of each tissue was calculated by the geometric method with a confocal microscope. Rotational mechanical stimulation was applied with a three-dimensional multi-shaker. The amount of ATP secretion was measured by a bioluminescent-based method using an EDTA-containing luciferin-luciferase assay kit. In selected samples, bafilomycin A1 and carbenoxolone (CBX) were applied to inhibit ATP secretion by vestibular dark cells and connexin hemichannels. ATP-enriched vesicles were stained with quinacrine for the localization of ATP secretion. We investigated the distribution of connexin 26 (CX26) in mouse vestibular dark cell areas by immunohistochemical staining.
Results: The ATP secretion per surface was significantly increased during the stimulation (0.11 ± 0.03 nM/mm2, 0.06 ± 0.02 nM/mm2, and 0.05 ± 0.01 nM/mm2 in utricle with ampulla, saccule, and common crus, respectively). The amount of ATP secretion in utricle with ampulla was significantly higher than common crus ($p < 0.05$). ATP secretion was partially or completely blocked by bafilomycin A1 (100 nM) but was almost completely blocked by CBX (10 nM) in all tissues. Expression of the ATP containing vesicle was detected in the dark cell area in the utricular roof epithelium, utricular macula, around the ampullary crest, and in the saccular roof epithelium, saccular macula and in the common crus. CX26 was identified to be distributed within the vestibular dark cell area and in the roof epithelium of utricle and saccule.
Conclusions: Paracrine ATP Secretion after mechanical stimulation is accomplished through connexin hemichannel in vestibular dark cell and nonsensory epithelium. The results of this study justify the role of ATP mediated ion transport via purinergic receptors for various physiological responses in the vestibular system during mechanical stimulation.