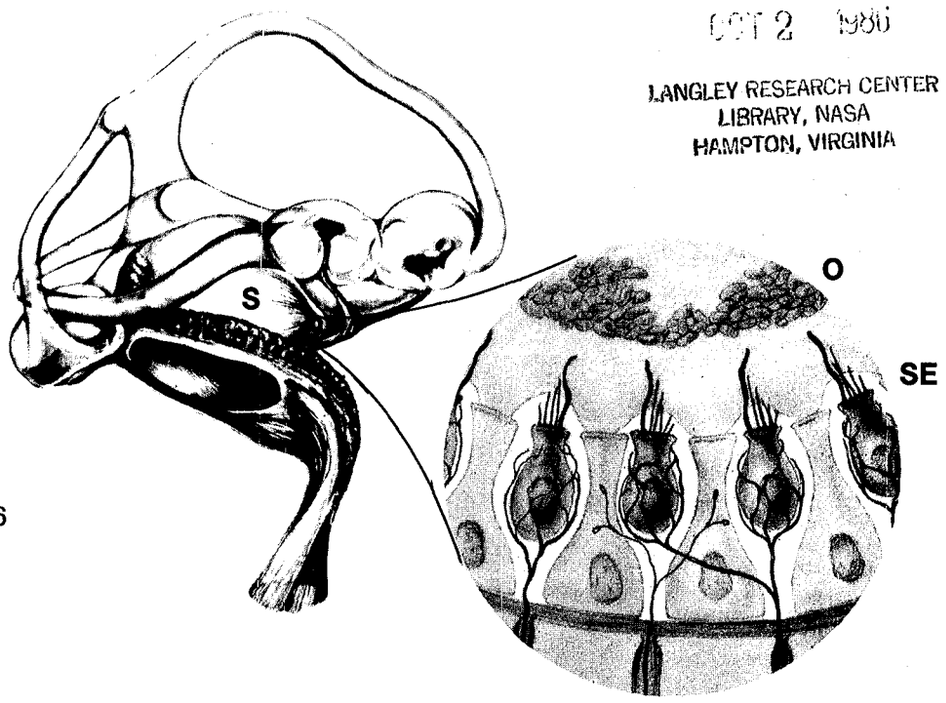
NASA Technical Memorandum 88249

NASA Workshop on Animal Gravity-Sensing Systems Pacific Grove, California February 1985



August 1986

National Aeronautics and Space Administration

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NASA Workshop on Animal Gravity-Sensing Systems Pacific Grove, California February 1985

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National Aeronautics and
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Ames Research Center
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Cover:
Drawing of the right membranous labyrinth
(lateral view) of the Caiman crocodilus
showing the location of the sacculus(S),
the sensory epithelium (SE), and otoconia (O).

PREFACE

The Life Science Division of NASA, as part of the continuing assessment and development of its research programs, convened a workshop on Animal Gravity-Sensing Systems in February 1985. The purpose of this workshop was to develop an integrated program of research on animal gravity-sensing systems and the role that gravity plays in the development and normal functioning of these systems. Scientists working on gravity receptors presented abstracts describing their research in the area and outlined their views of what the program should encompass. The abstracts are appended to this document. Overall research objectives, approaches, and fundamental issues that need to be considered in the early stages of a program designed to understand gravity receptor systems were established. Specific questions to be addressed, both on the ground and in space, and research strategies were identified.

The editor wishes to express her appreciation to Dr. Muriel Ross and Dr. Thora Halstead for serving as the workshop Chairpersons; to Dr. Nancy Daunton for helping to organize the workshop, and for her contributions to the program plan; and to Dr. Donald Beem and Ms. Patricia Russell of the American Institute of Biological Sciences for their assistance in the organization and conduct of the workshop.

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INTRODUCTION

The opportunity for space flight has brought about the need for well-planned research programs that recognize the significance of space flight as a scientific research tool for advancing knowledge of life on Earth, and that utilize each flight opportunity to its fullest. For the first time in history, gravity can be almost completely eliminated. Thus, studies can be undertaken that will help to elucidate the importance of gravity to the normal functioning of living organisms, and to determine the effects microgravity may have on an organism. This workshop was convened to organize a plan for space research on animal gravity-sensing systems and the role that gravity plays in the development and normal functioning of these systems. Scientists working in the field of animal gravity-sensing systems use a wide variety of organisms in their research. The workshop presentations dealt with topics which ranged from the indirect gravity receptor of the water flea, Daphnia (whose antennal setae apparently act as current-sensing receptors as the animal moves up and down in water), through specialized statocyst structures found in jellyfish and gastropods, to the more complex vestibular systems that are characteristic of amphibians, avians, and mammals.

The major purposes of the workshop were to 1) establish the rationale, goals, and objectives necessary for an integrated program of research on animal gravity-sensing systems, and 2) develop priorities and effective research strategies for achieving the goals. The material which follows reflects the recommendations of the workshop attendees.

PLAN FOR AN INTEGRATED RESEARCH PROGRAM ON

ANIMAL GRAVITY-SENSING SYSTEMS

Goals

This program is designed to increase our understanding of animal gravity-sensing systems, their evolution, and the role gravity plays in the development and normal functioning of these systems.

Objectives

This program is designed to determine

1. How animals sense gravity

2. How they process and use information about gravity

3. How both short- and long-term exposures to microgravity may affect the development, structure, physiology, and functioning of animal gravity-sensing systems

4. How gravity has influenced the evolution of gravity-sensing systems

Rationale

Life, since its first appearance on Earth, has existed in a 1-g environment. A number of different systems have evolved in the animal kingdom to sense gravity and thus facilitate the orientation and locomotion of organisms in three-dimensional space. However, the degree to which Earth's gravity has played a role in molding the structure and function of these systems is unknown. Now for the first time in biological history because of the Space Program, it is possible for organisms to live for prolonged periods of time without the influence of gravity. Space flight provides a unique tool for significantly advancing our understanding of animal gravity-sensing systems, their evolution, and the role gravity plays in the development and normal functioning of these systems.

General Strategies

To facilitate the understanding of complex organisms, it was a strong recommendation of the attendees that a variety of animals be studied that reflect different degrees of structural and behavioral complexity. Studies of the structure, chemistry, function, and development of representative gravity receptors in adult organisms, in developing organisms, and in multiple generations of organisms will be conducted in the normal 1-g environment and in the microgravity environment

(fig. 1). Ground-based data will be compared with data obtained in microgravity to assess the effects of exposure to microgravity, and to further our understanding of how animals sense and react to gravity. Data from these studies will provide a basis for understanding the role of gravity in the ontogenetic and evolutionary development of gravity receptors, as well as in the maintenance of gravity-sensing capabilities in the adult. A wide range of "state-of-the-art" techniques in morphology, neuroanatomy, biochemistry, neurophysiology, and neurobehavior will be used in establishing the database and assessing the effects of exposure to microgravity.

RESEARCH QUESTIONS

A series of fundamental questions were generated by the workshop participants. The questions cover five topic areas. Within each area a number of subquestions further define the major area and indicate the most important information to obtain.

Topic I. How Do Animals Sense Gravity?

1. What are the comparative anatomy and physiology of the "graviceptors?"[1]

2. How specialized are graviceptors (e.g., do graviceptors sense mechanical positions of such things as appendages and angular accelerations)?

3. How does graviceptive information flow through the nervous system? What are the specific neurotransmitters?

4. How is graviceptive information used (e.g., what reflexes and behaviors are controlled or modified by graviceptor inputs)?

Examples of studies that address questions in Topic I would be to:

1. Study the organization and innervation patterns of hair cells in graviceptors to gain a better understanding of the morphological basis for information processing (fig. 2),

2. Study the normal composition, development, size, and distribution of statoconia and otoconia and determine whether microgravity affects the size, number, and composition (fig. 3), and

[1]Although the term "graviceptor" is used in this paper, a more accurate term is "biological accelerometer".

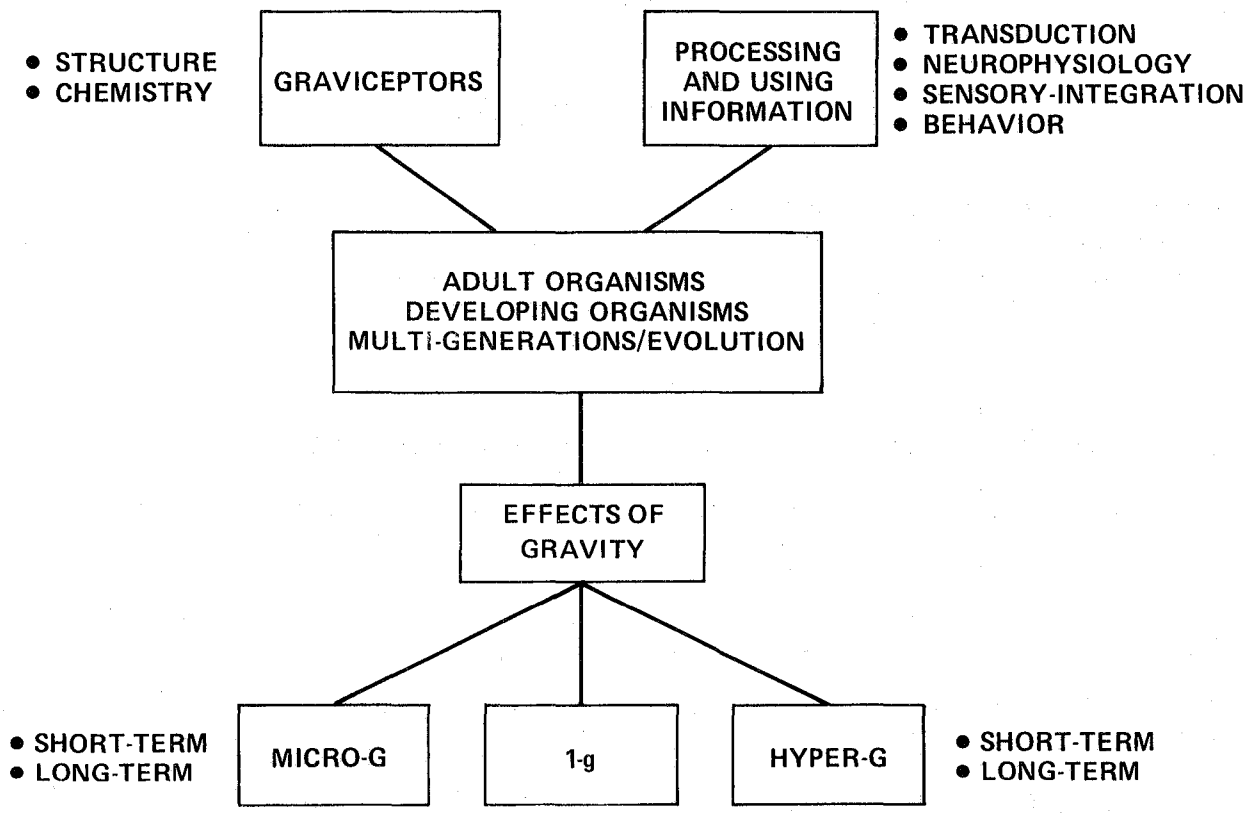

Figure 1.- Areas of research on the role of gravity in gravity-sensing mechanisms.

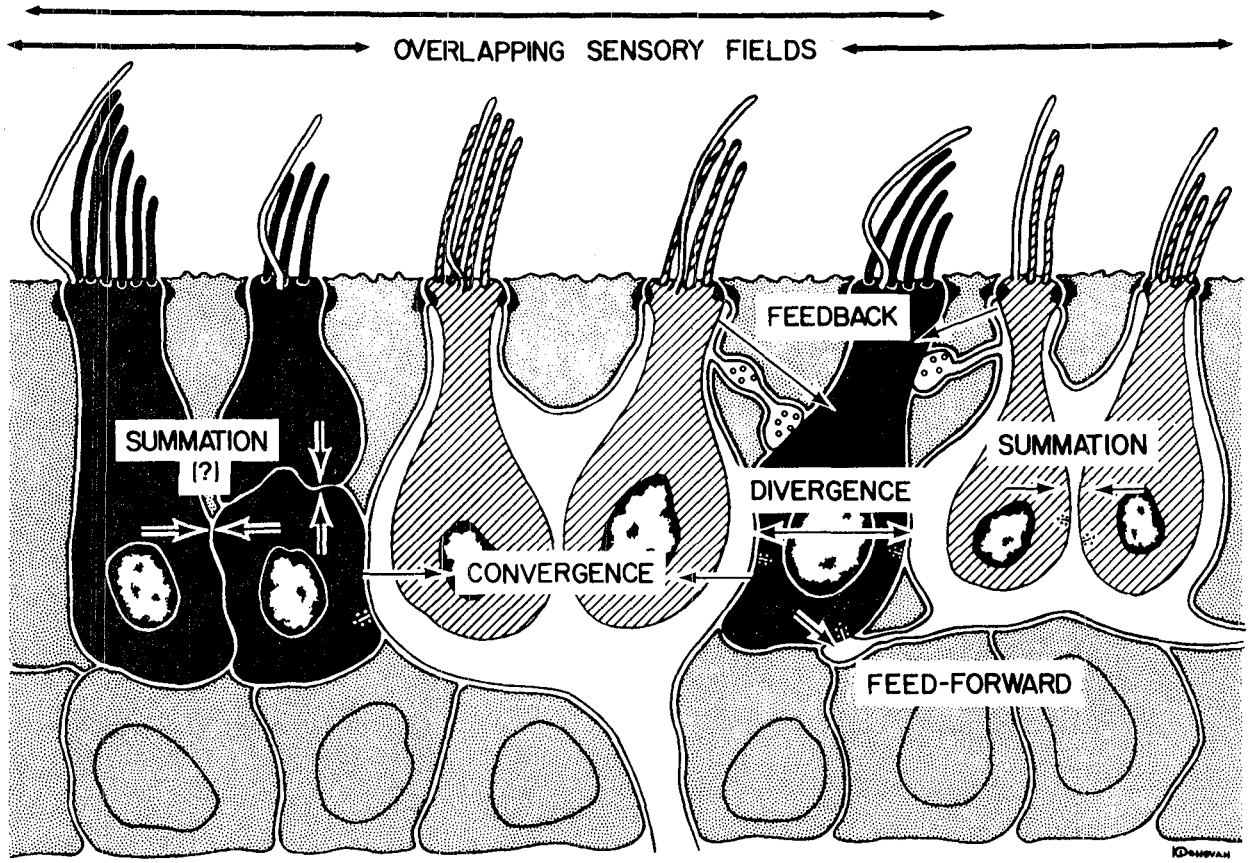

Figure 2.- Diagram of hair cells. How do animal sense gravity and how is this information processed and encoded? What is the significance of the organization and innervation patterns of hair cells in the graviceptors?

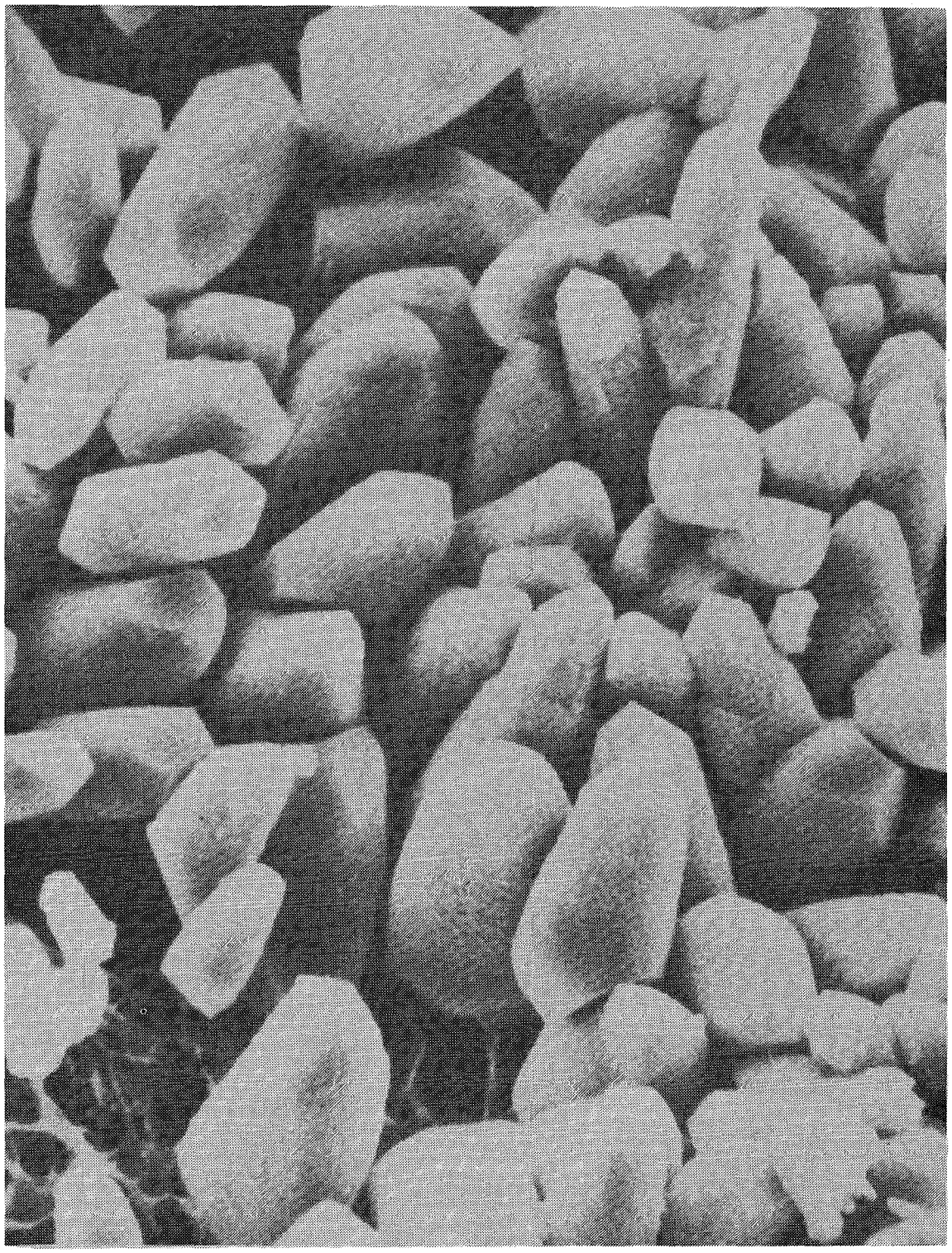

Figure 3.- Otoconia: Does altered gravity change their composition, size, number, or distribution? If it does, what are the physiological and behavioral conquences of the changes?

3. assess whether the semicircular canals are affected by gravity and/or linear acceleration by recording from the primary afferents in the semicircular canals in space.

Topic II. What Is The Role of Gravity in the Development and Maturation of Graviceptors?

1. To what extent is graviceptor development dependent upon genetic and environmental factors? Are there critical periods during early development?

2. What is the role of different levels of gravity on the physiology and anatomy of different components of graviceptors?

3. Will exposure to microgravity over multiple generations result in anatomical and physiological changes?

4. Does age at time of initial exposure to microgravity make a difference in the effects?

Examples of studies that address questions in Topic II would be to:

1. Expose a developing organism to microgravity during the critical stages in graviceptor development or during metamorphosis and assess alterations in the development of the graviceptor (figs. 4 and 5);

2. Determine if some level of gravity can prevent changes that may be found (fig. 6), and

3. Determine the consequences of complete deprivation of the Earth's gravitational input on the development and functioning of graviceptors by growing multiple generations of organisms in microgravity.

Topic III. What Is The Role of Electrolytes and Calcium in Transduction and Nerve Encoding in Graviceptors?

1. How might a systemic electrolyte imbalance (caused by microgravity) affect transduction and nerve encoding?

2. What is the role of calcium in graviceptor systems (transduction, transmission, and extracellular matrix)?

3. What is the distribution of calcium in graviceptor systems?

4. Is the distribution of calcium modified in altered gravity?

5. What level of gravity is required to maintain adequate electrolyte balance in graviceptors?

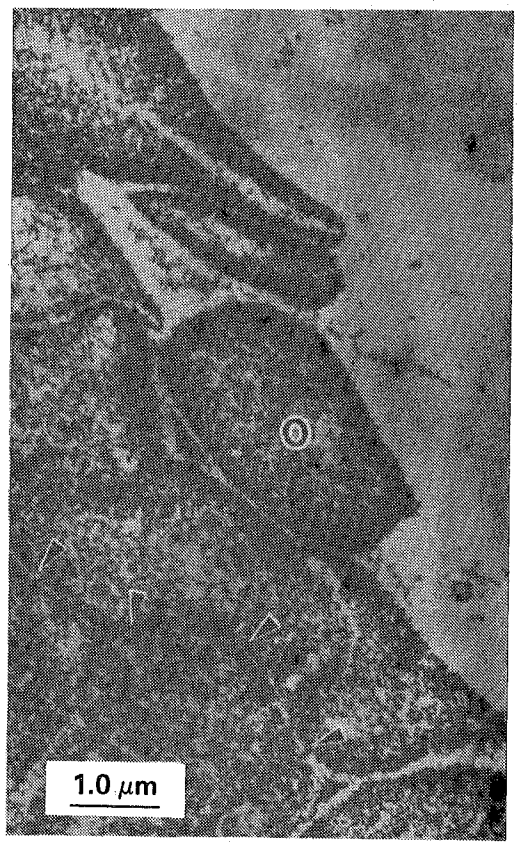

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Figure 4.- Noncalcified preotoconia (O) that seem to separate off from a mass of somewhat fibrilar material. Will microgravity affect this process?

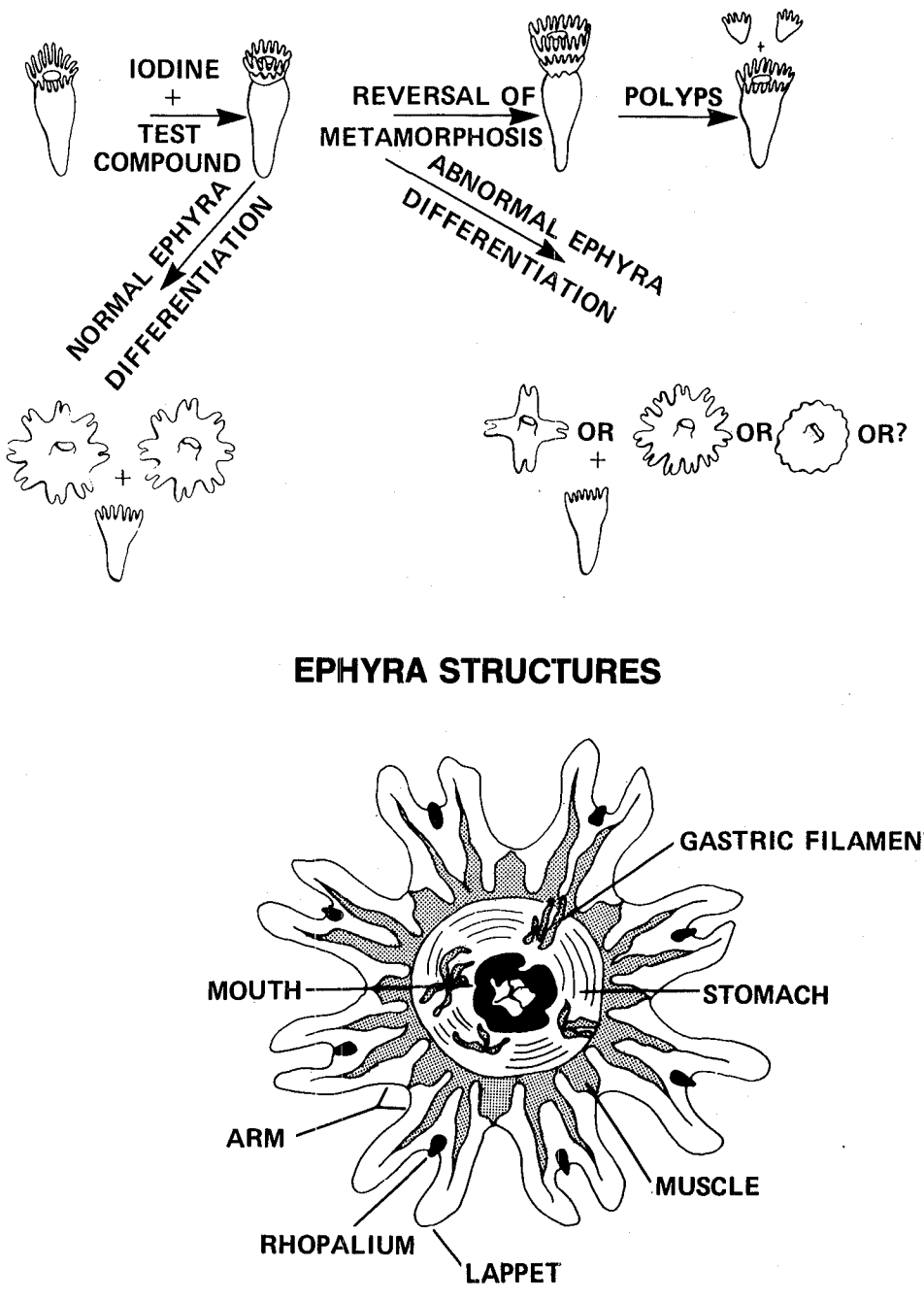

Figure 5.- Polyps metamorphose to produce ephyrae (immature jellyfish). Ephyrae have graviceptor structures (rhopalia). Will the rhopalia develop normally when polyps undergo metamosphosis in microgravity?

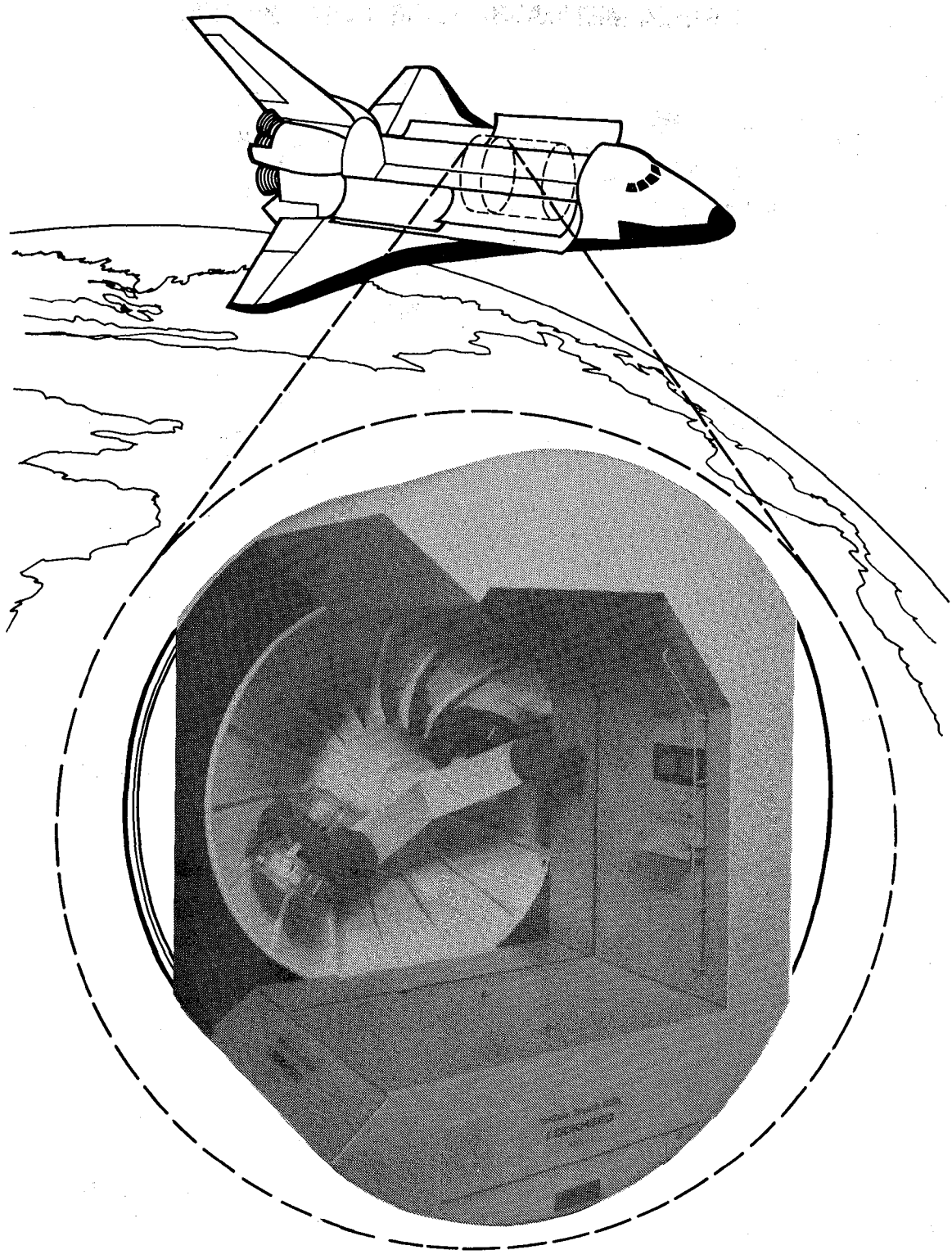

Figure 6.- Centrifuge in the Space Lab aboard the Space Shuttle (artist's conception). What is the role of different levels of gravity on the physiology and anatomy of different components of gravity receptors? Is there a "critical" level of gravity, below which changes will occur?

Examples of studies that address questions in Topic III would be to:

1. Determine whether altered gravity affects mechanosensory and/or voltage-dependent channels, and ultimately, hair-cell conductance and excitability, and

2. Determine whether altered gravity affects the chemical composition of otoconia.

Topic IV. How Do the Gravity Sensor and Related Effector Systems Adapt to Microgravity?

1. With respect to graviceptors, are there morphological, physiological, and neurochemical changes in hair cells and neural circuitry in microgravity? What is the onset and time course of such changes?

2. What are the behavioral correlations for graviception and its alteration?

3. When organisms are grown and adapted over multiple generations in space, can they readapt to 1-g atmosphere? What is the time course and nature of the adaptation process?

4. How are effectors and their neural circuits modified to adapt to microgravity?

5. How can adaptation to microgravity be facilitated?

An example of research that addresses questions in Topic IV would be to assess changes in graviceptor neural circuitry in single and multiple generations of organisms exposed to microgravity by using neuroanatomical tracing techniques (e.g., Lucifer Yellow).

Topic V. What Is the Hierarchical (Weighting) Processing of Multisensory Information Regarding Orientation?

1. What factors determine the weighting (experience/environment)?

2. Do neurons that receive multisensory convergence modify their morphological and physiological properties in microgravity?

3. What other stimuli can substitute for gravity in positional orientation?

4. What is the role of graviception in orientation, locomotion and navigation?

5. How is spatial information encoded in a microgravity environment?

An example of studies that address questions in Topic V would be to study the effects of microgravity on orientation, postural control, and locomotion of organisms to determine the relative importance of cues, other than gravity, that are used for orientation, postural control, and locomotion (e.g., dorsal-light reaction).

FACILITIES/TOOLS

A variety of research tools are available to provide ground-based and space-based exposures to altered gravity, including the Vestibular Research Facility, the KC-135 aircraft, the Space Shuttle, and the future Space Station. It is likely that all of these facilities (which are described later in more detail) will be used in this program.

Vestibular Research Facility

The Vestibular Research Facility (VRF), managed by the Life Science Division, Ames Research Center, provides a focus for ground-based research on vestibular-system physiology during adaptation to prolonged changes in background levels of gravito-inertial forces. (Examples of the forces are hypergravity on Earth and microgravity in space). The VRF was designed to characterize the effects of rotational and linear acceleration on physiological systems in Earth's gravity and under altered gravito-inertial loads. The VRF can accommodate laboratory hardware (including specialized equipment) and a support team to assist both Ames researchers and visiting scientists.

The scientific goals of the facility are to

1. Define critical questions about vestibular function which require the microgravity environment of space

2. Define methods to answer those questions

3. Provide an understanding of vestibular function in Earth's gravity

4. Provide a focal point for involvement of university-affiliated neurophysiologists in accomplishing NASA's goals

5. Facilitate planning of neuroscience flight experiments

6. Collect baseline data, in a systematic manner, for flight experiments on motion sickness, vestibular function, and other nervous-system functions affected by microgravity

The major stimulating devices of the VRF are a centrifuge (fig. 7) and a linear accelerator (fig. 8). The centrifuge is unique because it delivers rotational accelerations to small test animals around multiple axes in the presence of altered gravito-inertial loads. The linear accelerator is an air-bearing device that will provide pure, noise-free graviceptor stimulation. The VRF centrifuge can be modified or upgraded to meet specific needs of research tasks. An engineering support team is available to develop and integrate experiment-specific hardware.

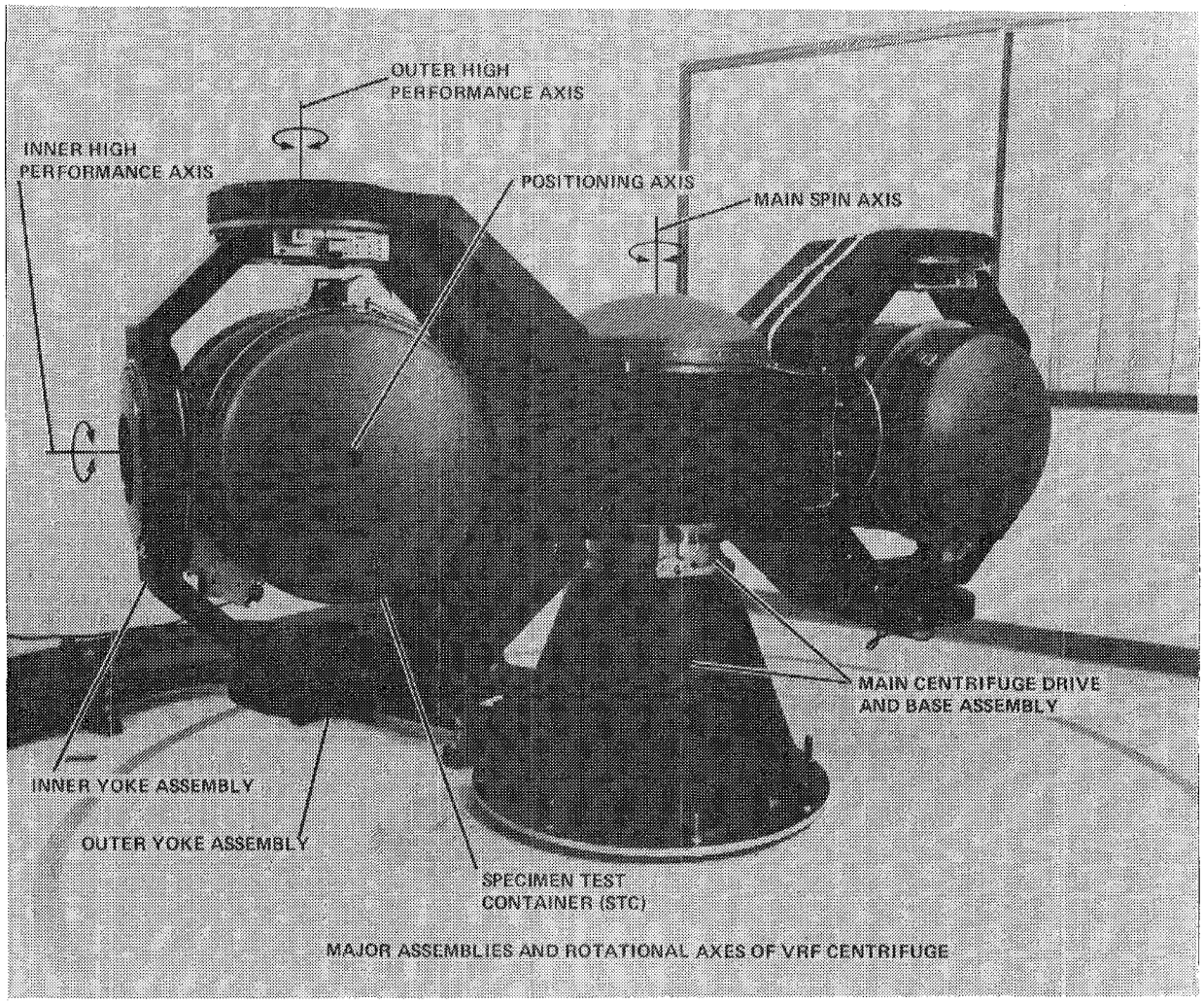

Figure 7.- Centrifuge: one of the major stimulating devices in the VRF.

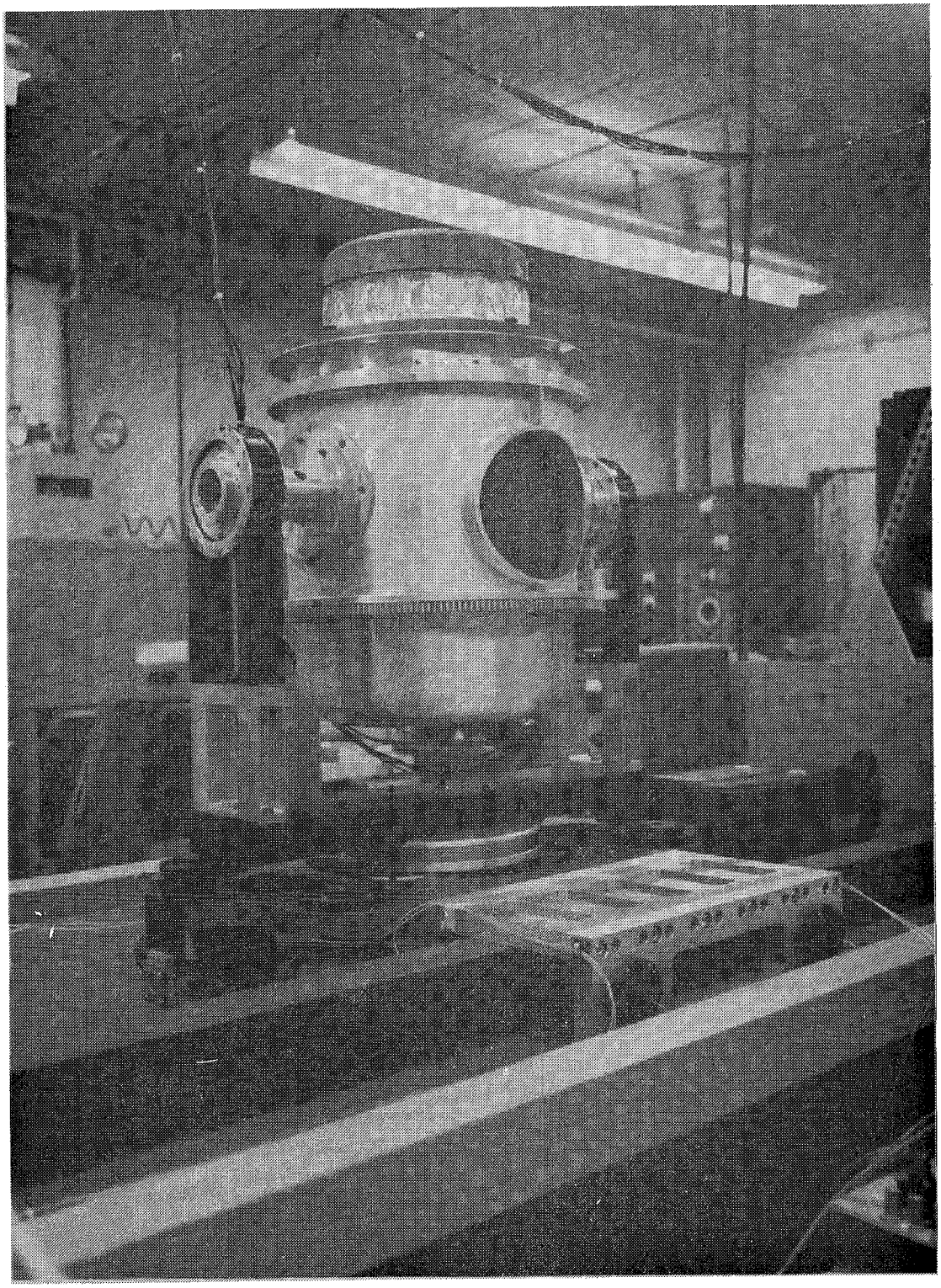

Figure 8.- Linear accelerator: one of the major stimulating devices in VRF.

Science projects will be selected from peer-review-approved proposals. Some examples of types of experiments which are possible using this unique facility follow:

1. A determination of the effect of altered gravito-inertial forces on
   a. Interactions between linear (e.g., gravity) and rotational acceleration sensors during pitching and rolling head movements
   b. Brain-cells which sense linear and rotational accelerations
   c. Nervous system adaptation mechanisms
   d. Normal and abnormal eye movements
   e. Reflexive activity of postural muscles
   f. Susceptibility and adaptation to terrestrial motion sickness

2. A determination of linear acceleration sensitivity of physiological systems sensitive to motion by measuring
   a. Absolute values of response thresholds and how they can be experimentally altered
   b. Effects of pure linear acceleration on functions which normally depend on both linear and rotational acceleration inputs

KC-135 Aircraft

The KC-135 aircraft (fig. 9), operated by the NASA Johnson Space Center, Flight Operations, Houston, Texas, provides a "weightless" environment similar to the environment of spaceflight. The reduced gravity environment is obtained with a specially modified KC-135A jet transport flown over a parabolic arc to produce short periods of less than 1-g acceleration force. The parabolic maneuver is initiated and terminated with a pullup and pullout, of 1.8 to 2.0 g (fig. 10). The length of these reduced-gravity periods depends on the gravity level required for the specific test. Typical lengths of various maneuvers are as follows:

1.	Negative gravity	(-0.1 g)	15 sec
2.	Zero gravity	(0 g)	23 sec
3.	Lunar gravity	(0.167 g)	30 sec
4.	Martian gravity	(0.333 g)	40 sec

These maneuvers may be flown consecutively (roller-coaster fashion), or separated by enough time to alter the test setup. A normal mission out of Ellington Air Force Base lasts 2- to 3-hr and includes 30 to 40 maneuvers.

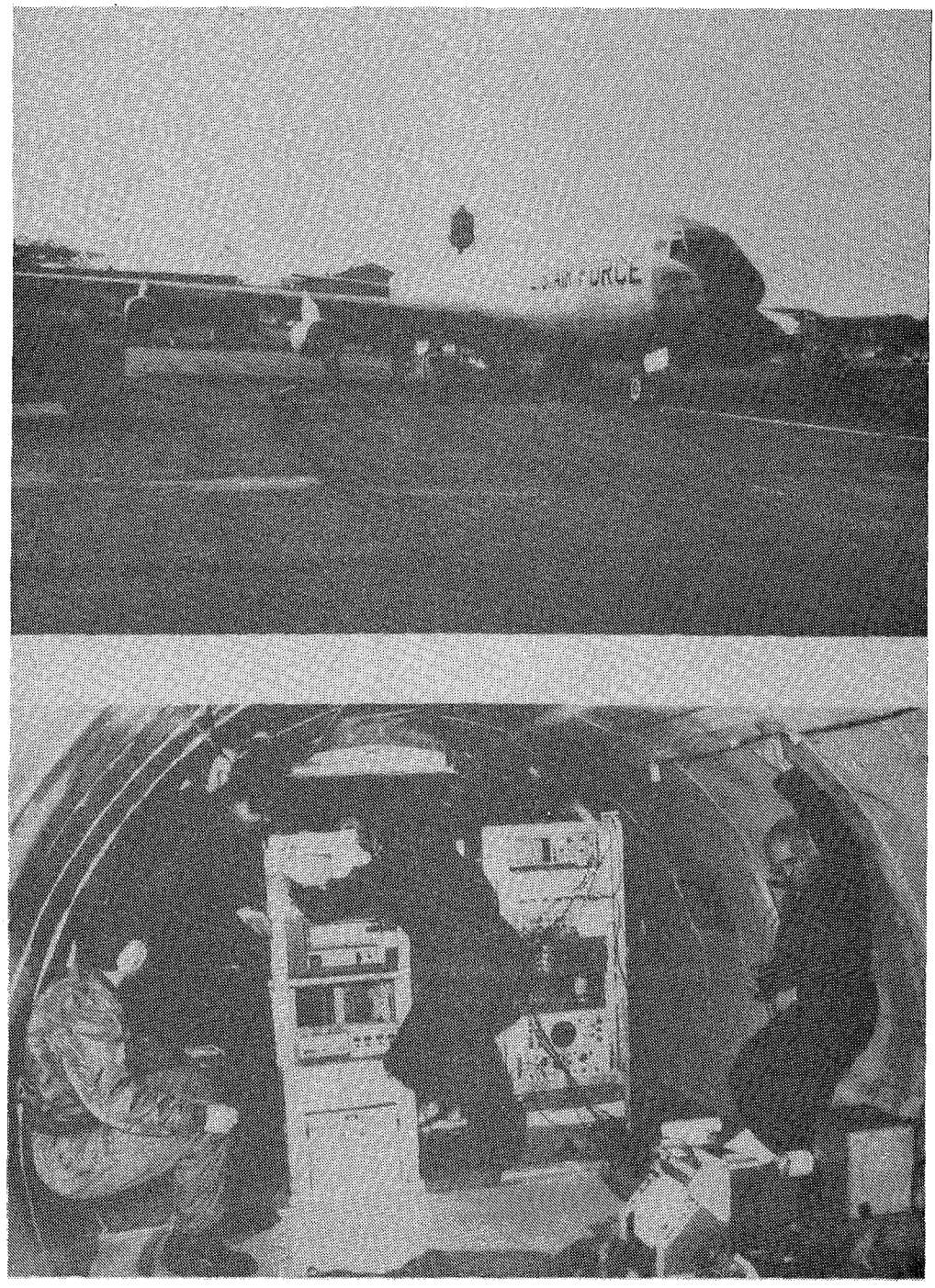

Figure 9.- KC-135 Aircraft: through parabolic maneuvers the aircraft is capable of simulating, 0 g for short periods.

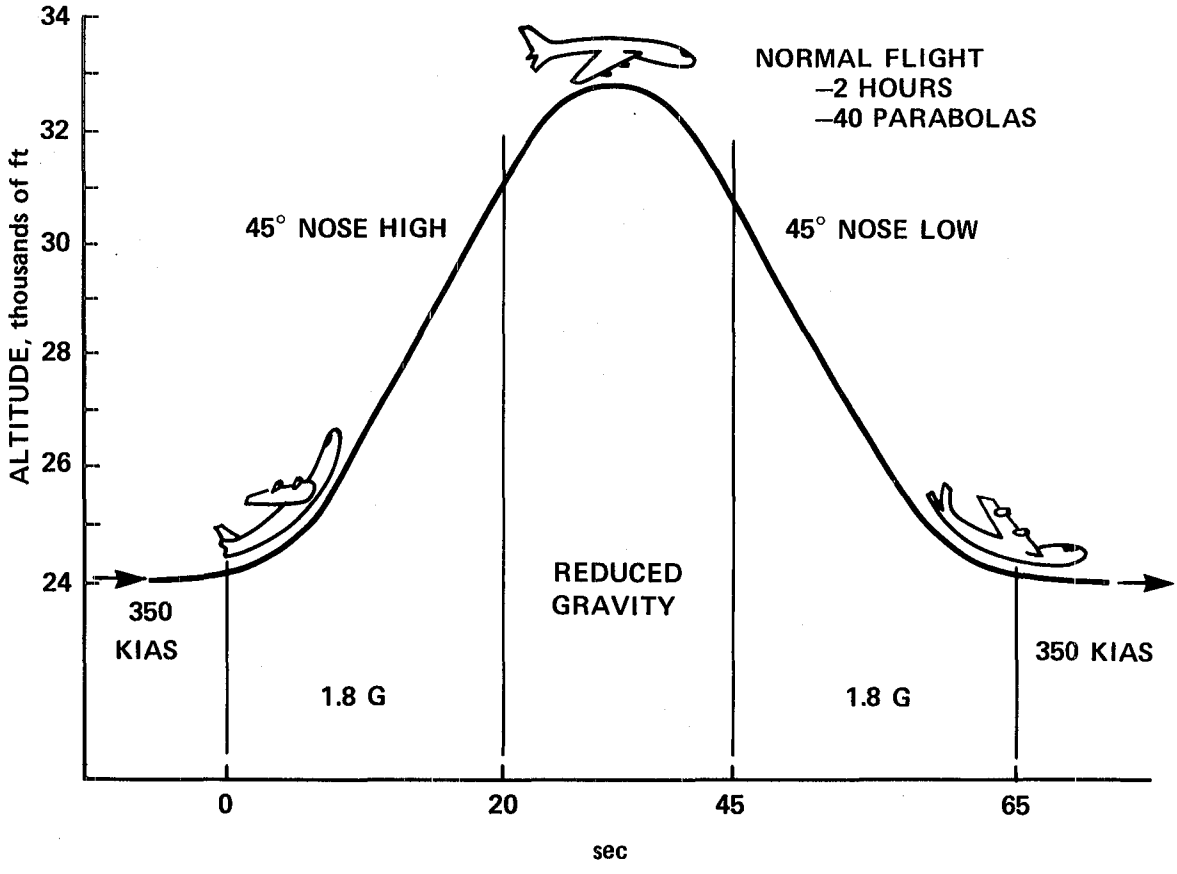

Figure 10.- Reduced-gravity maneuver.

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Space Shuttle

The Space Shuttle (fig. 11) has been developed into a transportation system capable of carrying people and equipment into low Earth orbit for short periods of time; i.e., 7-10 days. Space Shuttle flights provide the opportunity for biological experiments to be flown in the Shuttle Orbiter's middeck in a pressurized laboratory module, Spacelab, carried in the orbitor payload bay on a space-available basis. Thus, the Space Shuttle is the current means of providing exposure to the microgravity environment.

Space Station[3]

The Space Station (fig. 12) will provide an orbiting, low-gravity, permanently manned facility for scientific research, starting in the 1990s. The facilities for life sciences research are being designed to allow scientific investigators to perform research in space biology in order to study the consequences of long-term exposure to microgravity. An important segment of the initial Space Station configuration will be a science laboratory module, which will include life-sciences research facilities to conduct research. For additional information on life sciences on the Space Station, contact

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CONCLUSION

NASA has continuing opportunities for research in Space Biology. The focus and goals of a new program in Space Biology on Animal Gravity-Sensing Systems are described herein. The investigations of the basic mechanical, physiological, and

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[2]Users' Guide JSC-17385, May 1981.

[3]For additional information, see NASA Technical Memorandum 86836, Sept. 1985. (A copy of the article may be obtained by contacting the Chief, Advance Programs office.)

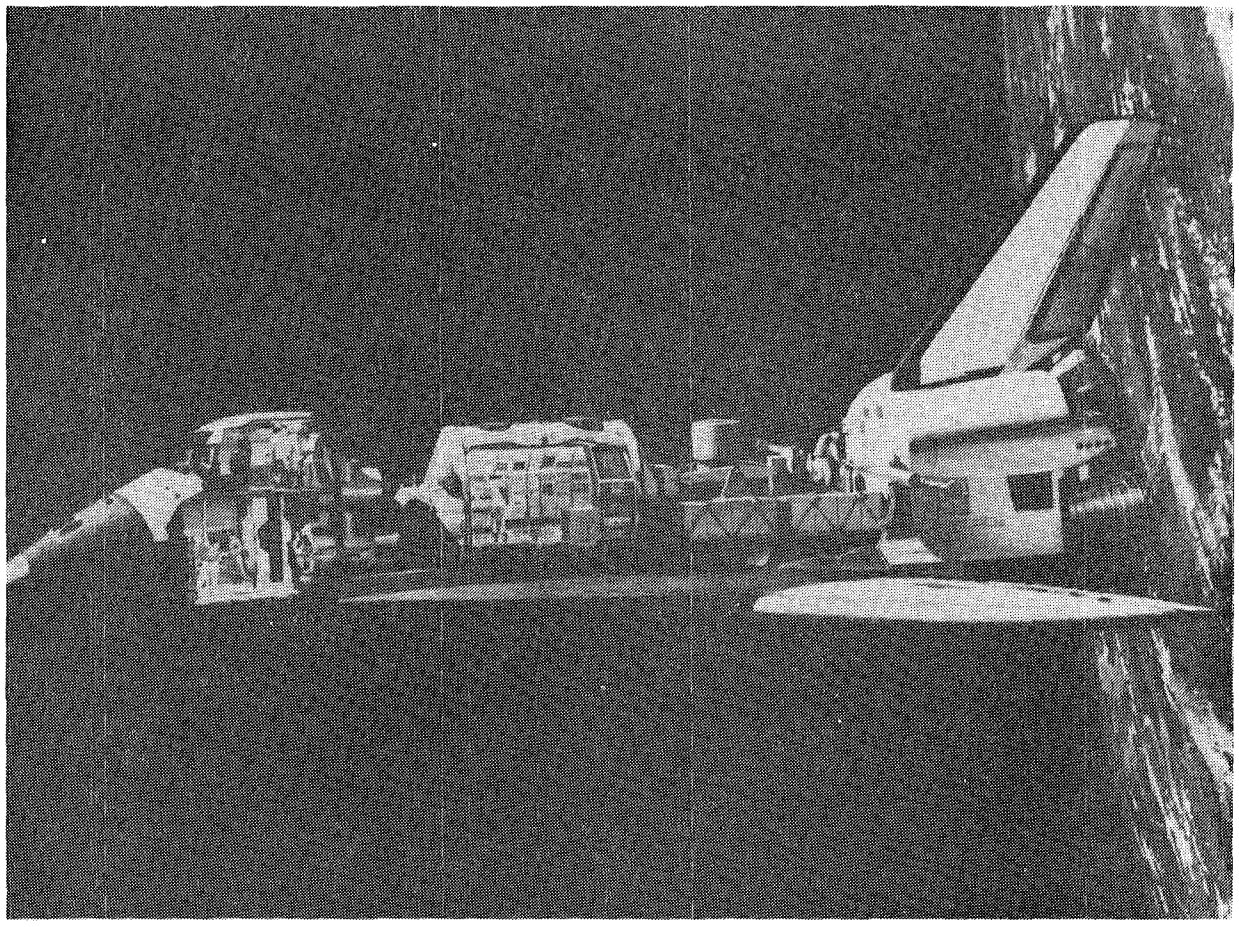

Figure 11.- Space Shuttle: provides short-term access to the microgravity environment.

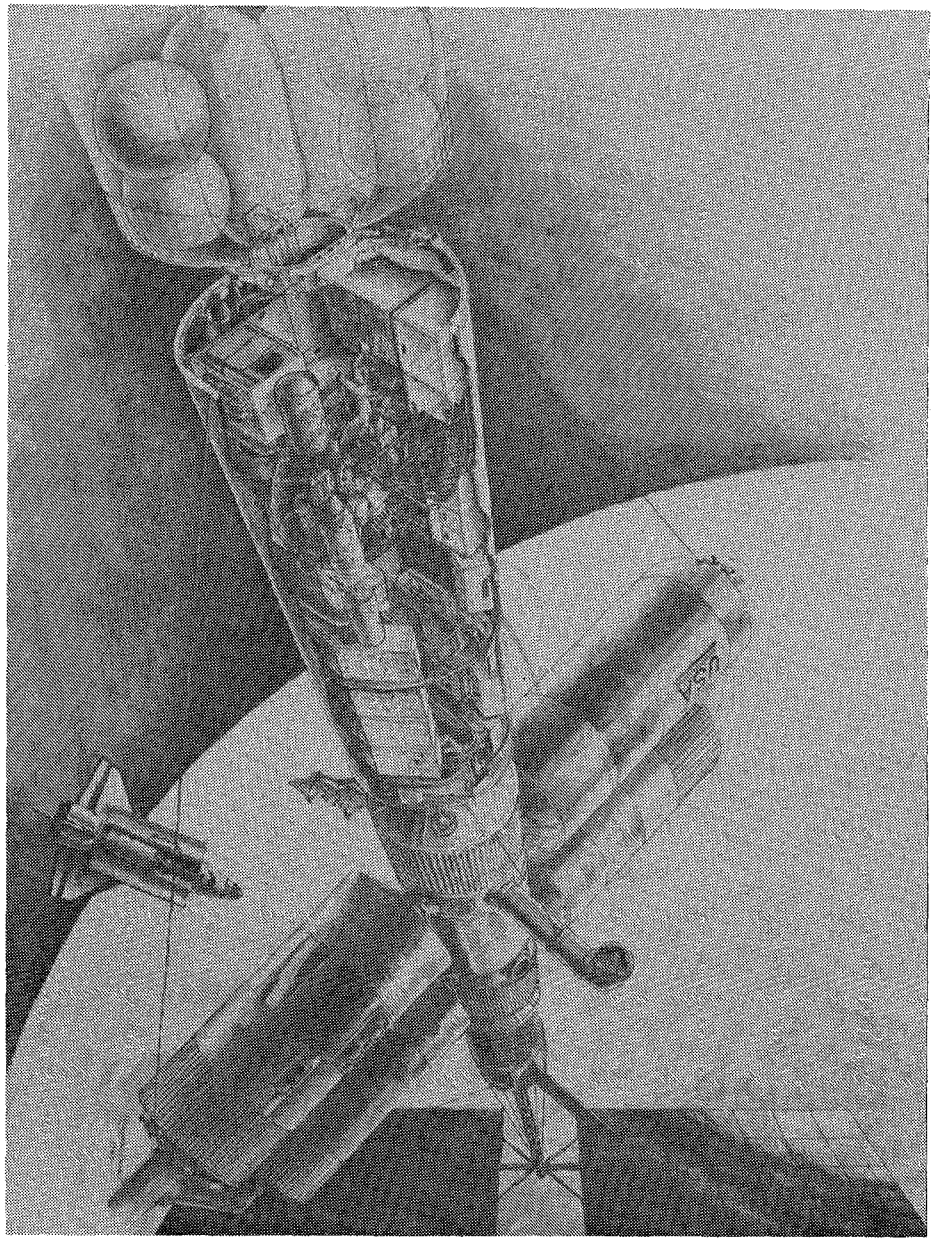

Figure 12.- Space Station: will provide long-term access to the microgravity environment.

neurological processes involved in gravity-sensing mechanisms are supported under the Space Biology Program from NASA Headquarters, and are managed at Ames Research Center. All proposals are reviewed for scientific merit by a peer-review group coordinated by the American Institute of Biological Sciences. Projects which rate highly on scientific merit and relevance to the Space Biology Program will have top priority in assignment of available funds.

For further information about the workshop or the NASA Gravity-Sensing Systems Program, including procedures to become involved within it, contact:

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APPENDIX A

EFFECTS OF LONG-TERM EXPOSURE TO MICROGRAVITY ON THE DEVELOPING MAMMALIAN GRAVITY-SENSING SYSTEMS:

Proposed Experiments For NASA Space Biology Program

Robert H. I. Blanks, Ph.D.

Proposal

We propose that several experiments be performed on the effects of long-term exposure to microgravity of the developing mammalian gravity-sensing systems.

Background

Over the past 14 years, we have been particularly interested in the functional interaction between otolith, semicircular canal, and visual afferents in the normal functioning of the vestibular-locomotory and vestibular-autonomic reflex pathways. Our approach has been to examine the response properties of single units (first- and second-order vestibular afferents; trochlear motor neurons) or compound action potentials (gastro-intestional tract recordings) during controlled stimulation of otolith, semicircular canal, and visual receptors. Parallel neuroanatomical experiments using tritiated amino acid, light autoradiography, and retrograde horseradish peroxidase have been employed to examine the anatomical basis of visual-vestibular interaction. These studies have involved a variety of mammalian (cats, monkeys, rats, and rabbits) and nonmammalian (frogs) vertebrates.

The microgravity environment of space offers the unique opportunity to examine the developmental and long-term effects of functional deafferentation of the gravity-sensing systems, and to examine the mechanisms underlying adaptation to this new gravitational environment. Our proposed studies on the receptor function and central pathways would expose neonatal animals (rats or suitable mammals) to critical periods of microgravity. Upon the immediate return to the 1-g environment, three types of experimental data would be obtained.

First, eye-movement recordings would be obtained to assess abnormalities in ocular counter-rolling. Ocular counter-rolling would be examined in the frequency range from 0 to 0.75 Hz and would be measured using currently available infrared or modified magnetic search coil techniques. Abnormalities in otolith function would show up well by testing the output of the oblique muscles (i.e., those involved to a great degree in counter-rolling) given that trochlear and inferior oblique motoneurons have a well-defined convergence between canal and otolith receptors.

Second, recordings would be obtained from first-order afferents innervating the otolith receptors in the same, or related, groups of animals. Parameters such as sensitivity, resting rate, and response class (phasic, phasic-tonic, and tonic) would be assessed and correlated with Earth-bound control groups and with animals exposed for varying durations to microgravity or to various forms of artificial gravity in space. Abnormalities in the transduction mechanism or otoconia size and/or total mass could affect any or all of the response parameters.

Third, following the recording sessions, the labyrinths of all animals would be microdissected and analysed as to the size of the otolith crystalline structure and the estimated total otoconial mass on each receptor. This aspect of the proposal could be carried out in collaboration with local experts in crystallography, scanning electron microscopy, and the newly developed technique of nondestructive acoustical microscopy.

These studies will require significant ground-based experimental testing and will require that animals be reared during, or at least be exposed to, spaceflights of significant duration. However, experiments of this type would provide a wealth of data related to the central and peripheral effects of microgravity on the otolith system.

APPENDIX B

PHYSIOLOGY AND PHARMACOLOGY OF SYNAPTIC TRANSMISSION IN THE VESTIBULAR SYSTEM OF THE FROG

S. L. Cochran, Ph.D.

Proposal

We propose that studies be performed on the developing frog's vestibular system to study changes that may be caused by variations in gravity during development.

Background

Investigations have been initiated to determine the nature of the transmitter substances within the frog's vestibular system, which has basic elements that are common to all vertebrates. In addition, the frog brain will survive in vitro for many days. These traits make the frog nervous system amenable for electrophysiological investigation. While various postsynaptic elements were being recorded, assessments were made as to the specific agonists and antagonists capable of acting at given synaptic regions. These findings indicate that the transmitter released from hair cells within the labyrinth is glutamate, or a related compound. Acetylcholine is the principal transmitter of the centrally arising efferent system. The eighth cranial nerve's afferents transmit through electrical and "glutamatergic" chemical excitatory synapses. Excitatory synapses within the cerebellum also use glutamate or something similar from the climbing fibers, parallel fibers, and probably mossy fibers. The principal second-order vestibular neurons also appear to release glutamate. In accordance with the goals set forward in the Program Plan, we propose that similar investigations be undertaken in the tadpole, where it is known that vestibular pathways are present and functioning. These studies would establish a basis for providing or withholding gravitational stimulation at various stages of development. Any alterations in the vestibular pathways could then be assessed by combined electrophysiological and anatomical techniques with these synapses, as well as others, serving as "test points" for normal or altered vestibular function and development.

APPENDIX C

FACTORS AFFECTING GRAVITY RECEPTION IN BIRDS

M. J. Correia, Ph.D., T. J. Anastasio, Ph.D., and D. G. Lang, Ph.D.

Proposal

We propose that studies be performed on birds' vestibular apparatus to determine: 1) what role each hair cell in a multiple calyx plays in determining a macular afferent's polarization vector for gravity reception; 2) what role the marginal fiber mass (MFM) plays in the static and dynamic response of the utricle to changes in linear acceleration; and 3) what information, if any, semicircular canal sensitivity to tilt provides the central nervous system about the position of the head in space.

Background

There are three morphological and physiological features of the bird's vestibular apparatus which may modify or augment the otolith organ's normal mode of response to changes in linear acceleration. These are MFM, tilt sensitivity of semicircular canal afferents, and multiple hair cells in a single type-1 calyx.

We (Young, et al., 1974) have suggested that the MFM is a marginal extension of the cupular zone of the statoconial membrane which has folded back over on itself and in the process has sandwiched the statoconial zone between itself and the cupular zone. The basal end of the MFM terminates as a foot-like structure on the macula utriculi while the apical end attaches the statoconial membrane securely to the utricular wall. The size of the MFM in the pigeon utricle suggests that it must be considered as a functional component in mechanical models of statoconial membrane displacement.

Analysis of electrophysiological recordings of extracellular action potentials on semicircular canal primary afferents in the unanesthetized pigen (Anastasio, T. J.; Correia, M. J.; Perachio, A. A.; J. Neurophysiol., submitted 1985) revealed that 8/15 (53%) of horizontal canal afferents and 5/11 (46%) of anterior canal afferents tested showed tilt sensitivity to head pitch tilts of ±10°. Canal afferents were considered tilt sensitive if their mean discharge rate during tilt differed by more than 10% from their discharge rate in the 0° tilt position. When tilt was increased to ±30°, a greater percentage of horizontal (6/9, 67%) and anterior (4/4, 100%) canal afferents tested showed tilt sensitivity.

Using light and electron microscopy, we (Correia et al., 1985) counted the number of single and multiple hair cells in one type-1 calyx in the pigeon anterior crista. We found that 64% of single type-1 calyxes contained two to five hair cells (multiple calyxes) while 28% contained a single hair cell (single calyx). Eight

percent of the single type-1 calyxes contained 6-12 hair cells. We have confirmed that multiple and single calyx hair cells also exist in the utricular macula. We (D. G. Lang and M. J. Correia, unpublished observations) have injected Lucifer yellow into preganglionic axons of primary afferents innervating the utricle and the horizontal semicircular canal (membrane potential-$\bar{X}$ = 44.2 mV, SEM = 1.03, N = 83). Both multiple and single calyx hair cells have been labeled in the utricular macula.

Supported in part by NASA grants NAG2-293 and NGT 44-088-800.

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APPENDIX D

PERSISTENT MODULATIONS OF VISUALLY AND GRAVITATIONALLY EVOKED LOCOMOTOR BEHAVIOR IN HERMISSENDA: BIOPHYSICAL BASES OF SENSORY RECEPTOR CHANGES

Joseph Farley, Ph.D.

Proposal

We propose that studies be performed to identify and characterize current changes in the somatic membrane of statocyst hair cells, produced by associative training and microgravity environments by means of voltage-clamp studies, current noise analysis, and single-channel patch-clamp analysis. Incorporation of mechano-transducing ion channels into planar lipid bilayers should also permit analysis of changes in channel function in a well-defined biochemical context.

We also propose to assess the consequences of extended exposure to microgravity environments upon both gravitationally initiated and visually guided locomotor behavior, as well as reduced gravity effects, upon the capacity for associative learning. We are particularly interested in the possibility that exposure to microgravity environments may have debilitative effects upon visual and vestibular sensory integration, which extend transsynaptically beyond hair cells.

Background

The nudibranch Hermissenda exhibits a simple form of associative learning. When repeatedly exposed to pairings of light and strong stimulation (2.2 g) of graviceptor/vestibular pathways, Hermissenda exhibit reduced phototaxic and gravitationally evoked behavior for days following training.

Three sites within the visual and vestibular neural systems have been identified which undergo long-term changes in neural excitability, and contribute to reduced locomotor behavior. Type-A photoreceptors, which provide polysynaptic excitatory input to locomotor circuitry, exhibit reductions in their light-evoked, depolarizing, generator potentials. Type-B photoreceptors, which inhibit Type-A cells, evidence a training-produced enhancement of their light response. Statocyst hair cells, which transduce gravitational/vestibular information and ultimately excite identified motoneurons, undergo a persistent hyperpolrization and reduction in tonic spontaneous levels of impulse activity.

A key theme in our present understanding of neural plasticity in Hermissenda is the long-term modulation of ionic conductances, and how these are triggered by both phasic (e.g., stimulus pairings) and tonic (e.g., reduced or increased ambient illumination, microgravity environments) environmental perturbations. For example, voltage- (and more recently patch-clamp) analysis of the light-produced depolarizing

response of Type-B cells reveals seven functionally distinct conductance systems: two light-gated currents ($I_{Na\text{-}light}$; $I_{SI\text{-}light}$), two voltage-dependent K^+ currents (I_A, I_K), a voltage-dependent calcium current (I_{Ca}), a chloride current (I_{Cl}), and one or more calcium-activated potassium currents ($I_{K\text{-}Ca}$). Associative training results in persistent reductions in $I_{K\text{-}Ca}$ I_A, and enhancement of I_{Ca}. Two classes of mechanisms have been identified to reduce Type-B-cell K^+ currents. The first is a training-produced increase in intracellular calcium levels, leading to a presumed phosphorylation of K^+ channels. The second involves a reduction of K^+ currents, and enhancement of I_{Ca}, by activation of a calcium-independent protein kinase (C kinase), initiated by 5-HT-stimulated phospholipid metabolism.

Despite the fact that the transduction portion of Hermissenda photoreceptors and hair cells are derived from quite different membrane types (rhabdomeric and ciliary, respectively), the methods of sensory encoding, integration, and transmission of electrical signals are quite similar. Motile cilia transduce the effects of gravity through active interaction with statoconia, resulting in mechanical deformation of the hair-cell membrane at the basal-insertion region of the axoneme. Such stimulation produces increased voltage noise and a depolarizing generator potential if sufficiently intense. Preliminary to an analysis of the changes produced by training or microgravity environments, we have studied the processes of integration and amplification of these sensory signals in the hair cell's somatic membrane through current-noise analysis of resting potential conductances, and voltage-clamp studies of the voltage-dependent conductances.

Current-noise amplitude is typically one to two orders of magnitude greater for loaded (1 g) versus unloaded hair cells, and progressively increases with holding potentials more negative than -40 mV. Removal of extracellular Na^+ from the bath results in reversible decreases in noise amplitude. These observations indicate that Na^+ ions contribute greatly to the depolarizing voltage noise in the unclamped cell.

We have also identified two voltage-dependent K^+ currents in the hair cells and a calcium-activated K^+ current. The fast, rapidly inactivating current (I_A) is elicited at -30 mV, is TEA-resistant, is abolished by 4-aminopyridine (4-AP) and is inactivated at potentials more positive than -40 mV. The slower, sustained K^+ current (I_K) is selectively reduced by TEA, but not 4-AP. Both currents are also calcium regulated. Removal of extracellular Ca^{++} increases I_A by 10-35% while I_K is affected to a much smaller degree.

Because statocyst hair cells and photoreceptors share the same complement of voltage-dependent ionic currents, we are encouraged to believe that the mechanisms identified as underlying learning-produced changes in B photoreceptor K^+ currents may also provide a framework for the analysis of training-produced and low-gravity, environment-induced changes in statocyst hair cells.

APPENDIX E

MORPHOGENESIS OF THE CHICK'S OTOCONIA

Cesar D. Fermin, Ph.D.

Proposal

We propose that studies be performed to investigate the vestibular function of birds, small mammals, and primates (including humans) along the evolutionary scale, specifically observing the incorporation of calcium into the organic matrix.

Background

One of the aims of the studies in our Otological Research Laboratory is to comparatively investigate the vestibular function of birds, small mammals, and primates along the evolutionary scale. The chick's otoconia are essentially similar to those of higher mammals. The incubation period of the chick is short (21 days), and the developmental gradients of the auditory organ have been well established; also, eggs do not require special handling and/or maintenance during incubation. Initial ultrastructural data obtained in this laboratory indicate that:

1. During otoconia morphogenesis the otolithic membrane is secreted by the supporting cells of the macula, in the same manner that the supporting cells in the basilar papilla secrete part of the tectorial membrane.

2. Preotoconia separate off (segment) from the upper part of this membrane.

3. Electron-dense granules (20-150 nm diam) attach to the preotoconia.

4. X-ray microanalysis of the granules shows strong calcium peaks.

5. Histochemical staining indicates that the granules are dissociated in older embryos, in which calcium is deposited in fibrilar form instead of in granular form.

The incorporation of calcium into the organic matrix provides a good opportunity for understanding not only the mineralization of the otoconia, but also other aspects of calcium metabolism that are probably common to many biochemical pathways in living organisms.

APPENDIX F

GRAVITATIONAL EFFECTS ON THE BIOLOGY AND BEHAVIOR OF THE HOLOMETABOLUS INSECT

Alfred Finck, Ph.D.

Proposal

We propose that studies be performed to investigate the manipulation of the weight of the larvae of holometabolus insects by suprathreshold gravito-inertial forces (centrifugation) to reduce the number of instars. We hypothesize that hypergravity decreases the time course of the developmental aging process. An alternative hypothesis is that larvae in the microgravity of space would remain in that juvenile state significantly longer than Earth-gravity controls.

Background

Among the insects, gravity and pressure receptors are interrelated and behaviors related to equilibrium seem particularly spared by metamorphosis. It is proposed that learning with gravity as a conditioning stimulus is effectively transferred from the larva to the adult and that removal of gravity during metamorphosis would not cause the extinction of gravity-relevant behaviors learned during an earlier developmental stage.

Remarkable changes occur in the postembryonic development of holometabolus insects (e.g., Coleoptera, Lepidoptera, Diptera and Hymenoptera). The sequence of events are larval periods (primarily growth by molting), a final metamorphic molt leading to differentiation, and adulthood. The insect hormones underlying this developmental process are known. In the beetle (Tenebrio molitor) and similar insects the number of instars (molts) can be reduced by feeding, and in the case of Rhodnius the onset of the metamorphic process is associated with the engorgement of blood and/or food. This process appears to indicate that a proprioceptive weight or stretch receptor is involved in triggering the inhibition of the juvenile hormone, thus permitting a metamorphic molt to occur.

The consequence of metamorphosis is massive changes in the morphology, physiology, and behavior of the insect. A small literature reports that behaviors, conditioned in larvae (Tenebrio), are "saved" during complete metamorphosis; however, it is recognized that the nervous system is largely replaced. Perhaps the "memory" engram is retained as a chemical rather than a neural organization?

APPENDIX G

MECHANO-ELECTRICAL TRANSDUCTION BY VESTIBULAR HAIR CELLS

A. J. Hudspeth, Ph.D.

Proposal

We propose that studies be performed to determine how transduction occurs in the bullfrog (Rana catesbeiana) and to study the micromechanical properties of hair bundles from the saccule. The questions we'd like to answer are:

1. What is the most effective mode of stimulation to a hair bundle?

2. Is shear uniquely effective or are cells also excited by vertical loading (e.g., with otoconia)?

3. Is it possible to describe the internal motions of the hair bundle (e.g., by measuring bundle dimensions in detail with high-resolution light microscopy and fitting the results with a computer model)?

4. Can one infer from data on the two previous issues how displacement acts at a molecular level to open transduction channels?

5. Do the data suggest how adaptation of the receptor potential to static stimuli (ref. 8) comes about?

Background

Throughout the vertebrates, the detection of accelerational stimuli within the internal ear is conducted by hair cells. While there is considerable structural diversity among these receptors, the available evidence suggests that all receptors operate in a fundamentally similar manner (ref. 2). Accelerations produce forces that move mechanical accessory structures -- the cupulae of the semicircular canals or the otolithic membranes of the utricle and saccule -- which in turn displace the distal tips of hair bundles. Displacement of stereocilia (ref. 3) produces a very rapid (refs. 4 and 5), relatively nonselective (ref. 6) increase in ionic conductance (ref. 7) near their distal tips (ref. 8).

Our research group employs simple, in vitro preparations from the internal ears of lower vertebrates to investigate how transduction occurs.

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APPENDIX H

EFFECTS OF MICROGRAVITY AND VESTIBULAR DEPRIVATION ON DEVELOPMENT OF AVIAN EMBRYOS

Robert V. Kenyon, Ph.D. and Russell Kerschmann, Ph.D.

Proposal

We propose that studies be performed to determine how vestibular morphology and behavior are affected by decreased vestibular stimulation during development.

Background

To make this determination, we propose to subject a group of developing chick embryos to microgravity on the Space Shuttle, and simultaneously produce defined periods of embryonic quiescence through the administration of certain drugs while monitoring behavior. We hypothesize that stimulus deprivation will lead to changes in the vestibular receptor neuroepithelium and otoliths, reductions in the appearance, size, and/or number of neurons in the vestibular brain-stem nuclei, and a retardation of the normal sequence of eighth nerve mylenization. Furthermore, these and other anatomical changes will manifest themselves as modified vestibular responses to acceleration stimuli as well as altered behavior in ovo during and after the flight. By examining postflight the morphology of the vestibular nervous system (brainstem nuclei, eighth nerve, and end organ), together with testing of vestibular reflexes and visual vestibular adaptation (eye and head movements), and monitoring the level of embryonic activity during development (in flight and postflight), we hope to identify the effects of sensory deprivation on the developing vestibular system and associated neural structures.

APPENDIX I

ASSOCIATIVE LEARNING AND BEHAVIORAL CHANGES IN HERMISSENDA

Izja Lederhendler, Ph.D.

Proposal

We propose that studies be performed to provide a framework for understanding how learning-induced biophysical and biochemical changes in a single cell can have a cascade of effects through identified cells leading to behavioral changes. These studies can be made through neural-network and detailed-behavioral analysis.

Background

Associative learning in Hermissenda has been measured as a reduction in positive phototaxis following repeated pairings of light and rotation stimuli. A detailed knowledge of the neural network mediating visual and graviceptive stimulation has enabled identification of a cellular locus for learning and information storage. This locus is the medial type-B photoreceptor. An inverse relationship exists between this photoreceptor and visually mediated behavior such that an enhanced photoresponse after training leads to reduced phototactic performance. One specific visual input/output pathway has been identified and it can account, at least in part, for the inverse relationship. The enhanced photoresponse after training results from calcium-dependent inactivation of two K^+ currents, I_A and I_c, which leads to a persistent increase in input resistance and photoreceptor excitability.

The phototactic pattern is complex. The nature of the learning has, therefore, been elusive. Recent work, however, suggests that light takes on characteristics of rotation in a Pavlovian fashion. Rotation (2.0 g) causes rapid contraction of the foot. Light, on the other hand, causes lengthening. Following associative training, light now elicits a new response shortening similar to that produced by rotation.

A second line of investigation has sought to reduce the complexity of the phototaxis to a response to simple differences in light intensity. Associative modification of such shadow withdrawal responses are predicted by, and correspond closely to, known electrophysiological processes in the photoreceptor.

APPENDIX J

ACCELERATION SENSITIVITY IN THE AMPHIBIAN SACCULE

Edwin R. Lewis, Ph.D.

Proposal

We propose that studies be performed to determine acceleration sensitivity in saccular-afferent axons.

Background

We have taken extensive measurements of spike activity in saccular-afferent axons of the American bullfrog (Rana catesbeiana) under conditions of steady-state sinusoidal acceleration. Among other things, we found that the peak spike rate of individual afferents (determined from cycle histograms) is very nearly directly proportional to the peak acceleration over ranges of at least 60 dB. Our abilities to probe the actual extent of the range are limited by sensor noise (at low accelerations) and maintenance of electrode penetration (at high accelerations). All axons appear to exhibit either low-pass or band-pass frequency characteristics, with band centers ranging from less than 20 Hz to approximately 100 Hz. Midband sensor gains range approximately from 50 to 3000 spikes/sec/cm/sec^2 (50,000 to 3,000,000 spikes/sec/g). In the most sensitive axons, the noise equivalent input is of the order of 0.01 cm/sec^2 (10^{-5} g). In another frog species (Leptodactylus albilabris), Narins and I found noise equivalent inputs as low as 0.001 cm/sec^2 (10^{-6} g).

APPENDIX K

GRAVITY RECEPTION AND ORIENTATION IN A LOWER-ORDER CRUSTACEAN: RHEOCEPTIVE ANTENNAL-SOCKET SETAE OF DAPHNIA MAGNA

D. Meyers, Ph.D.

Proposal

We propose that studies be performed to determine the effects of gravity variations on lower-order crustaceans, which apparently have a multidirectional sensitivity that is gravity dependent.

Background

Higher-order crustaceans, members of the subclass Malacostraca, are docuemnted as having specific gravity responses and internal gravity-detection organs known as statocysts. No similar reactions or mechanisms have been reported for any of the lower-order crustacea. Continuously swimming Daphnia magna of the lower-order cladocera are known to orient to light during the day, but, at night in the absence of visual cues, are suspected of maintaining orientation through gravity perception. An analysis of swimming path and antennal beat frequency revealed erratic swimming behavior when D. magna were exposed to a neutrally buoyant medium in the dark, and, thus, indicated an apparent lack of internal gravity-sensing organs. Surgical removal of paired setae on the basal sockets of the two swimming antennae elicited a similar disoriented behavior of the negatively buoyant daphnids in an unilluminated water medium. Antennal-socket setae appear essential to night orientation, apparently functioning as gravity-induced current detectors, or rheoceptors, stimulated by the rush of water past the daphnid as it sinks between upward swimming strokes. Sensitivity of this gravitationally mediated, rheoception mechanism was tested by subjecting daphnids to a series of five decreasingly dense aqueous solutions in darkness. Three-dimensional, video analysis of body position and swimming path revealed a gradual threshold near a density difference between the animal and its environment of less than 0.25%, and a probable multidirectional sensitivity. Ongoing anatomical studies suggest that the setae differ from reports of similar mechanoreceptive structures in higher-order crustacea by containing dendritic connections through to the distal ends of the shafts that may contribute to their apparent multidirectional sensitivity.

APPENDIX L

THE LAGENA OF GOLDFISH AS A MODEL OF THE OTOLITH ORGAN SYSTEM

Christopher Platt, Ph.D.

Proposal

We propose that studies be performed to explore the gravistatic and acoustic function of the lagena of goldfish.

Background

The lagena of goldfish is an unusual otolith organ. Its anatomy and ultrastructure show the sensory epithelium forms two large regions that remain distinct throughout postembryonic and adult growth. Physiololgy and behavior suggest the lagena in teleost fish may have both gravistatic and acoustic function.

The proposed work explores the duality further by anatomy and physiology. Central projections of the entire lagenar nerve are known to overlap in brainstem nuclei that are separable for presumed gravistatic (utricle and canals) and acoustic (saccule) inputs. Dye filling (HRP) of separate branches of the lagenar nerve should clarify whether the otolith-laden, caudal-region projects exclusively to overlap with utricule and canal input. Microelectrode recording should establish whether gravistatic responses are present in the goldfish lagena, whether they are of the "into-position" type as in rays and frogs, and whether caudal responses are exclusively gravistatic. If feasible, development of the lagena under microgravity conditions for a period 1-2 mo. should allow estimates of growth changes, if present, of the sensory epithelium under otolith-unladen and otolith-laden regions. The possible duality of this lagena has important developmental and evolutionary implications.

APPENDIX M

INNERVATION PATTERNS IN THE UTRICLE AND SACCULE: AN EVOLUTIONARY VIEWPOINT

Muriel D. Ross, Ph.D.

Proposal

We propose that studies be performed to determine the innervation patterns in the utricle and saccule from an evlutionary viewpoint.

Background

A total of 96 horizontal and 91 vertical sections through the anterior part of the utricular macula and 117 vertical sections through the superior part of the saccular macula were photographed in a Philips 400 transmission electron microscope and reconstructed as montages. Study of these series demonstrated that Type-II hair cells were integrated into the neural circuitry of Type-I hair cells. An independent afferent innervation of Type-II hair cells was not apparent. Type-II cells were closely applied to calyces, with which they formed ribbon junctions, or they received calyceal collaterals. Such collaterals often were vesiculated, whether they were of afferent or of efferent type according to synapses formed. Some collaterals were beaded and vesiculated. These provided only efferent-type endings on neighboring Type-II cells. Although this basic description applies to both maculas, all indications are that saccular and utricular macular innervation patterns are not identical, nor is the innervation similar across a given macula. For example, within the saccular series there were three discrete patterns in the 0.5 mm width of the sections, starting from the more medial border. These were, in order: long, unmyelinated nerve fibers ending in complex calyces having numerous collaterals; myelinated nerve fibers that lose their myelin at the base of the neuroepithelium and provide a short, unmyelinated segment ending in a less complex calyx; and fibers that remained myelinated to the base of an apparently noncomplex calyx. In all cases, the nerve was eccentric to the calyx and efferents were located on nerve fibers, calyces, and Type-II cells with great specificity. These innervation patterns have physiological consequences that require investigation and interpretation.

Aside from the basic conclusion that a morphological basis for complex sensory processing has been established, these and other of our results raise numerous questions of possible evolutionary interest. Some of these are:

1. What is the significance of the evolution of the calyx, which is poised to function as a filter with different pass characteristics for Type-I and Type-II cells?

2. What is the physiological significance, in terms of total macular function, of localized complex calyces that can modulate Type-II cells (feed-forward system or efferent-type collaterals) and be modulated by them (feedback system or afferent junctions)?

3. Is there foreshadowing of these complex neural patterns in submammalian species?

4. Do mammalian species differ in details of innervation patterns, and does this have behavioral consequences?

5. Is one macula more advanced in an evolutionary sense than the other?

6. What is the role of calcium in a system that has:
a) a kinociliary complex feeding into a striated nework in the cuticular plate
b) striated connections to the borders of hair cells
c) hypolemmal cisterns of transitional ER
d) ribbon junctions
e) smooth ER spanning the calyx?
f) What is the evolutionary history of the these structures?

7. Are there two efferent systems, intra- and extramacular, and if so, what is their physiological significance and their evolutionary history?

8. What is it that keeps the receptor activated so that it can sense gravity, a constant stimulus, (e.g. is it the kinocilium)?

9. Are otoconia and otoliths piezoelectric, (e.g., what, if any, are the morphological and physiological consequences of short and long-term exposure of maculas to microgravity?

APPENDIX N

EFFECTS OF WEIGHTLESSNESS OF *AURELIA* EPHYRA DIFFERENTIATION AND STATOLITH SYNTHESIS

Dorothy B. Spangenberg, Ph.D.

Proposal

We propose that studies be performed to determine the effects of microgravity on ephyra formation, including the development of graviceptor structures (rhopalia), using the Aurelia Metamorphosis Test System.

Background

Ephyrae make graviceptor structures during their development on strobilae and use them for orientation when swimming. Horizontal clinostat rotation of developing ephyrae at 0.25 rpm causes the synthesis of significantly fewer statoliths in rhopalia than controls.

Rhopalia of ephyrae which had developed during clinostat rotation and in space will be compared with controls with regard to histological and cytological organization. High-voltage electron microscopy will be used to explore the integration between graviceptor cells, neurons, and statocysts. Mineralization and demineralization of these rhopalia will be studied by counting statolith numbers and by determining total calcium content of the rhopalia.

Ultimately, these structural and mineralization studies of graviceptors from *Aurelia* ephyrae will be compared with graviceptor studies of higher organisms to uncover the role(s) of gravity in the evolution of the graviceptor structures. Further, these *Aurelia* studies will be correlated with behavioral responses (swimming and pulsing) of ephyrae on Earth and in space to uncover the specific role(s) of gravity in graviceptor development and function.

APPENDIX O

EFFECTS OF MICROGRAVITY ENVIRONMENT ON STATOCONIA FORMATION IN APLYSIA

Michael L. Wiederhold, Ph.D.

Proposal

We propose that studies be performed to determine the effects that microgravity has on statoconia formation in the aplysia and how the formation is affected when back in Earth's gravity.

Background

The experiment which we propose is to take young aplysia on several Shuttle missions so that the preparation can be exposed to microgravity for varying times. After the marine mollusc, aplysia are brought back to Earth, they will be studied both physiologically, using intracellular recording to look for changes in function, and anatomically, using light and electron microscopy, to determine if the number, size, structure, and elemental composition of the statoconia is altered. The sensory cells will also be examined for changes in their gross structure, the appearance of the cilia, and for evidence of either increased or decreased exocytosis of statoconia.

The statocyst of the marine mollusc, aplysia, is a simple gravity-sensing organ. The paired statocysts are small spheres, 200 μm diam, composed primarily of 13 mechanoreceptor cells whose ciliated (sensory) surface faces the lumen of the cyst. The lumen is filled with fluid ("statolymph") and approximately 1,000 dense stones ("Statoconia") which are free from one another and, in a normal 1-g environment, fall to fill the bottom one-third of the cyst. When a statocyst is tilted, receptor cells exhibit increased membrane potential fluctuations and become depolarized as they approach the "down" position, where all of their cilia are loaded by statoconia. The cell's axon relays inforamtion about the direction of gravity or linear acceleration to the central nervous system. This preparation lends itself easily to intracellular recording and its physiology has been well characterized. The cell was the first model system in which a "hair cell"-like receptor was shown to increase its membrane conductance during stimulation, consistant with Davis' models dating back to the early 1950's.

The aplysia statoconia are comprised of a crystalline array (probably calcium carbonate) laid down on a layered, double-membrane protein structure, similar to mammalian otoconia. The statoconia are ellipsoidal, ranging from 5 to 20 μm diam. In some sections of the statocyst, small statoconia were seen in narrow invaginations within the ciliated surface of the receptor cells. This suggests that the statoconia, or "seeds" upon which they develop, might actually be produced within the receptor cells and that this production could be influenced by sensory

stimulation. Thus, if the receptor cells do not receive sufficient stimulation in a microgravity environment, they might produce more stones. Conversely, since the sensory cilia are also motile and "beat" the stones into continuous motion, all 13 cells could be excited simultaneously in space and the number of stones could be reduced.

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16. Abstract

The opportunity for space flight has brought about the need for well-planned research programs that recognize the significance of space flight as a scientific research tool for advancing knowledge of life on Earth, and that utilize each flight opportunity to its fullest. For the first time in history, gravity can be almost completely eliminated. Thus, studies can be undertaken that will help to elucidate the importance of gravity to the normal functioning of living organisms, and to determine the effects microgravity may have on an organism. This workshop was convened to organize a plan for space research on animal gravity-sensing systems and the role that gravity plays in the development and normal functioning of these systems. Scientists working in the field of animal gravity-sensing systems use a wide variety of organisms in their research. The workshop presentations dealt with topics which ranged from the indirect gravity receptor of the water flea, Daphnia (whose antennal setae apparently act as current-sensing receptors as the animal moves up and down in water), through specialized statocyst structures found in jellyfish and gastropods, to the more complex vestibular systems that are characteristic of amphibians, avians, and mammals. ...

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