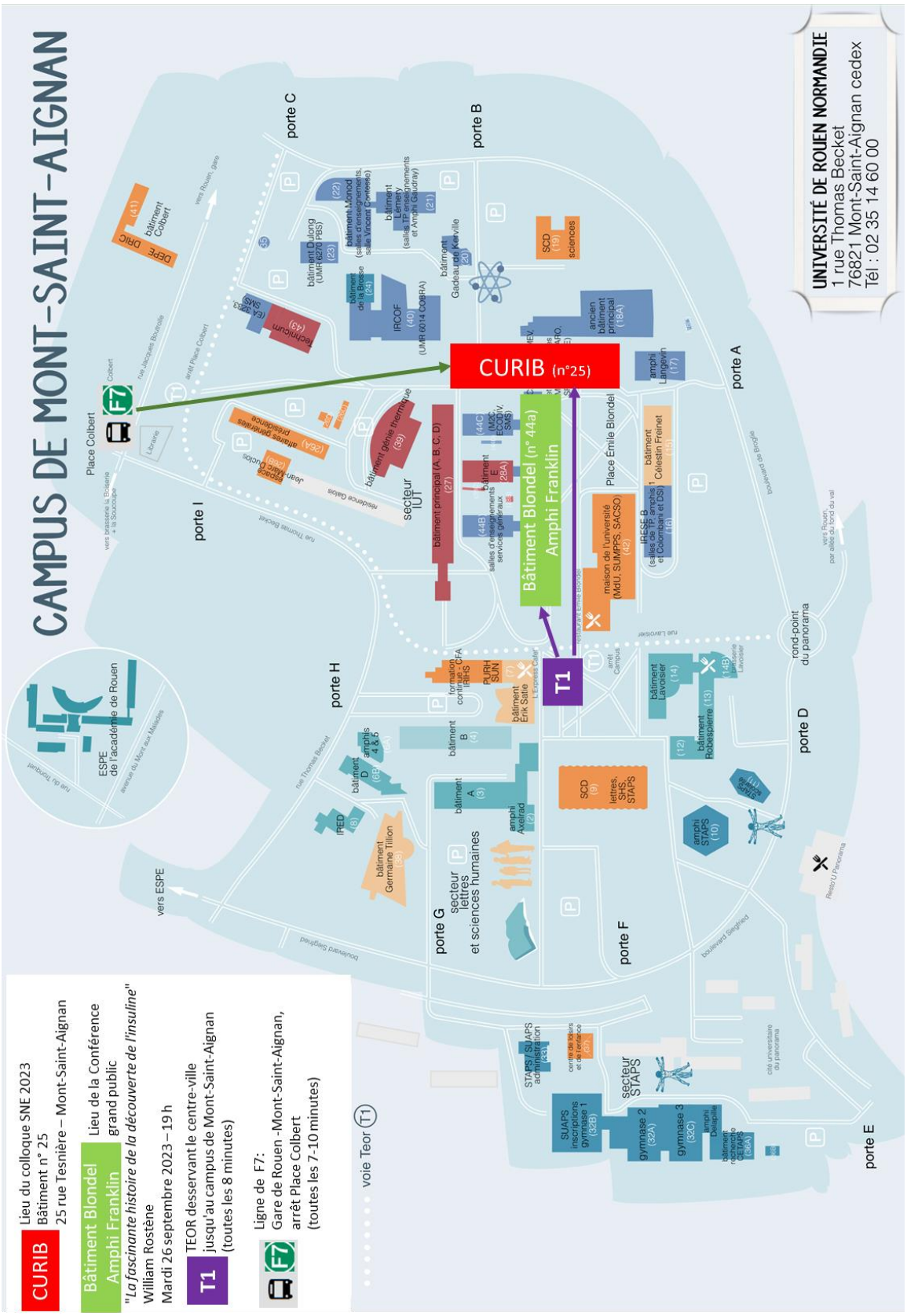
45ème Colloque de la Société de Neuroendocrinologie

Rouen

27-29 Septembre 2023

Plan de Rouen

Plan du Campus de Mont-Saint-Aignan



Partenaires

Cet événement a été labellisé COP21 par la Métropole Rouen Normandie

Bienvenue en Normandie pour ce 45ème colloque de la SNE

Cher(e)s Collègues,

Nous avons l'honneur au nom du Comité d'organisation rouennais et des membres du Conseil scientifique de la SNE de vous souhaiter la bienvenue au 45ème colloque de la SNE qui se tiendra du 27 au 29 septembre 2023 à Rouen.

Vingt-quatre ans après le dernier colloque de la SNE organisé à Rouen et treize ans après le *7th International Congress of Neuroendocrinology* également organisé à Rouen, nous serons ravis de vous accueillir à nouveau en Normandie pour ce premier colloque de la société post-épidémie COVID.

Ce colloque se déroulera sur 3 jours avec un programme scientifique qui fera place aux jeunes et aux moins jeunes pour faire le point sur les dernières découvertes et discuter les questions majeures auxquelles est confrontée la Neuroendocrinologie. Le programme scientifique élaboré par le Conseil scientifique de la SNE inclura des lectures plénières données par les meilleurs spécialistes sur des thèmes d'actualité en Neuroendocrinologie, des symposia sur les récentes avancées de notre discipline et des séances de communications orales et affichées dédiées principalement aux jeunes chercheurs. Un prix de la SNE, un prix de thèse et des bourses de voyage seront décernés lors de cet événement grâce au soutien de la Fondation Obélisque.

A l'occasion de cet événement scientifique, nous serons ravis d'accueillir la communauté neuroendocrinologiste tout en ayant la certitude que cette rencontre sera bénéfique aussi bien pour le rayonnement de la Neuroendocrinologie que pour les échanges scientifiques dans ce domaine entre spécialistes. La prestigieuse lecture Jacques Benoit sera donnée par le Pr Nicolas de Roux sur la génétique des pathologies affectant l'initiation de la puberté et la conférence « Grand Public » sera présentée par le Dr William Rostène sur « la fascinante histoire de la découverte de l'insuline ».

Nous espérons vous revoir nombreux en personne au prochain 45e Colloque de la SNE à Rouen au mois de septembre prochain.

Youssef Anouar et Maïté Montero pour le Comité d'organisation

Conseil scientifique

- Céline Cansell (Paris)
- Charlotte Cornil (Liège)
- Muriel Darnaudéry (Bordeaux)
- Rachida Guennoun (Paris)
- Fanny Langlet (Lausanne)
- Maïté Montero (Rouen)
- Amandine Stein (Lyon)
- Youssef Anouar (Rouen, **Président**)
- Sébastien Bouret (Lille, **vice-Président**)
- Vincent Hellier (Tours)
- Laurent Givalois (Montpellier)
- Agnès Martin (Montpellier)
- Sakina Mhaouty-Kodja (Paris)
- Patricia Parnet (Nantes)
- Ariane Sharif (Lille, **Trésorière**)
- Hervé Tostivint (Paris, **Secrétaire Général**)
- Nicolas Vitale (Strasbourg)
- Daniela Cota (Bordeaux)
- Cristina Miralpeix (Bordeaux)
- Carole Rovère (Nice)

Comité d'organisation

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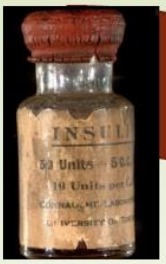
Sommaire



CONFERENCE GRAND PUBLIC

La fascinante histoire de la découverte de l'insuline

Par le **Dr William Rostène**

Mardi 26 septembre 2023 à 19h

Amphithéâtre Franklin, Bâtiment Blondel
UFR Sciences et Techniques,
Mont-Saint-Aignan

SCAN ME

Ligne TEOR T1 arrêt Campus

contact: maite.montero@univ-rouen.fr

Programme

Mardi 26 septembre 2023

13:00-18:00	Inscription	*Hall, Bât. CURIB*
14:00-17:00	Conseil scientifique SNE	*Salle 066, Bât. CURIB*
18:30	Cérémonie d'ouverture	*Amphithéâtre Franklin, Bât BLONDEL*
19:00	Conférence grand public « *La fascinante histoire de la découverte de l'insuline* » par William Rostène (Paris, France) Modérateur : Jean-Louis Nahon (Nice, France)	*Amphithéâtre Franklin, Bât. BLONDEL*
20:00-21:00	Cocktail de bienvenue	*Hall, Bât. CURIB*

Mercredi 27 septembre 2023

8:30-10:30		**Symposium 1** - ***Beyond neurons: role of glia in the central regulation of metabolism*** Modératrices : Daniela Cota (Bordeaux, France), Isabelle Denis (Paris-Saclay, France), Ariane Sharif (Lille, France)	*Amphithéâtre, Bât. CURIB*
8:30-9:00	**S1.1.**	Claire Martin (Paris, France) *Astrocytic response to obesity in the olfactory bulb*	
9:00-9:30	**S1.2.**	Jean-Denis Troadec (Marseille, France) *Glial modulation of energy balance: the dorsal vagal complex is no exception*	
9:30-10:00	**S1.3.**	Vincent Prévot (Lille, France) *Could midlife alterations of hypothalamic tanycytic shuttles lead to neurodegenerative disorders later in life?*	
10:00-10:30	**S1.4.**	Cristina Garcia-Caceres (Munich, Allemagne) *Astrocyte-neuron circuits in hypothalamic regulation of metabolism*	
10:30-11:00		Pause-café	*Hall, CURIB*

11:00-12:00		**Présentations « flash » des posters** Modératrices : Muriel Darnaudéry (Bordeaux, France), Rachida Guennoun (Paris, France)	*Amphithéâtre, Bât. CURIB*
11:00-11:05	**P1.**	Pierre-Yves Barelle (Lille, France) *Phenotypic characterization of a novel mouse model of Prader-Willi Syndrome with genetic invalidation of both Magel2 and Necdin*	
11:05-11:10	**P5.**	Lucile Butruille (Paris, France) *Long-term functional consequences of perinatal exposure to perfluoroalkyl substances*	
11:10-11:15	**P6.**	Marie-Stéphanie Clerget-Froidevaux (Paris, France) *Metabolic and neuroinflammatory consequences of hypothyroidism in two mouse strains with different metabolic adaptability capacities, the C57BL/6J and the WSB/EIJ strains*	
11:15-11:20	**P9.**	Mélodie Devère (Rouen, France) *The regulation of glucose and energy homeostasis by a hypothalamic subpopulation of neurons, expressing 26RFa and orexins*	
11:20-11:25	**P30.**	Marialetizia Rastelli (Lille, France) *The gut microbiota influences blood-CSF barrier structure and function in the hypothalamus*	
11:25-11:30	**P26.**	Cassandre Morel (Rouen, France) *Perturbation of maternal gut microbiota in mice during a critical perinatal window influences early neurobehavioral outcomes in offspring*	
11:30-11:35	**P32.**	Clara Sanchez (Nice, France) *Dietary fatty acid composition impacts obesity development, neuroinflammation and associated glial reactivity*	
11:35-11:40	**P10.**	Omayma Dlimi (Rouen, France) *Intranasal effects of an analogue of the gliopeptide ODN on mice food-intake and hypothalamic POMC expression*	
11:40-11:45	**P33.**	Alicia Sicardi (Lille, France) *GnRH, a fertile new pathway for the regulation of food intake*	
11:45-11:50	**P39.**	Jasmine Videlo (Lyon, France) *Gut-brain axis: neuronal characterization of portal glucose sensing*	
11:50-11:55	**P23.**	Marie-Anne Le Solliec (Valbonne, France) *Characterisation of metabolic profiles of knock-out MCH-receptor1 mice and humanized MCH-receptor2 knock-in mice*	
12:00-13:30		Déjeuner + Posters	*Hall, CURIB*

13:30-15:30		**Symposium 2** - *New insights into the hypothalamic-pituitary control of fertility* Modérateurs : Lydie Naulé (Paris, France), David L'Hôte (Paris, France)	*Amphithéâtre, Bât. CURIB*
13:30-14:00	**S2.1.**	David L'Hôte (Paris, France) *Molecular mechanisms underlying the plasticity of gonadotrope cell activity*	
14:00-14:30	**S2.2.**	Elodie Desroziers (Paris, France) *Unusual suspect: role of microglia in the neuroendocrine disorder polycystic ovary syndrome*	
14:30-15:00	**S2.3.**	Ben Yamine Mallouki (Rouen, France) *Characterizing the neuroendocrine role of SELENOT in the regulation of reproduction*	
15:00-15:30	**S2.4.**	Vincent Hellier (Tours, France) *Development of a new strategy using kisseptin as a tool to manage reproduction in mammals*	
15:30-16:00		Pause-café	*Hall, CURIB*
16:00-17:00		Assemblée Générale SNE	*Amphithéâtre, Bât. CURIB*
17:00-18:00		**Table ronde « Sciences et Société »** Modératrices : Céline Cansell (Paris, France), Cristina Miralpeix (Bordeaux, France)	*Amphithéâtre, Bât. CURIB*
		Adèle B. Combes (Docteure en neurobiologie) *Santé mentale des jeunes chercheurs : constats et solutions*	

Jeudi 28 septembre 2023

8:30-10:30		**Symposium 3** - *Interest of non-conventional animal models to understand adaptation of neuroendocrine rhythms to environmental constraints* Modérateurs : Sakina Mhaouty-Kodja (Paris, France), Etienne Challet (Strasbourg, France)	*Amphithéâtre, Bât. CURIB*
8:30-9:00	**S3.1.**	Jérémy Terrien (Brunoy, France) *Life under the tropics: effect of sex in the phenology of seasonal rhythms in a Malagasy primate*	
9:00-9:30	**S3.2.**	Valérie Simonneaux (Strasbourg, France)	

Neuroendocrine mechanisms driving reproductive cycles in the female camel

9:30-10:00 **S3.3.** Khalid El Allali (Rabat, Maroc)
Ambient temperature cycles synchronize circadian rhythms in the desert goat

10:00-10:30 **S3.4.** Sakina Mhaouty-Kodja (Paris, France)
Risk of a plasticizer-enriched environment on the neuroendocrine control of reproduction and cognitive behaviors

10:30-11:00 Pause-café *Hall, CURIB*

11:00-12:00 **Communications orales** *Amphithéâtre, Bât. CURIB*
Modératrices : Maïté Montero (Rouen, France),
Carole Rovère (Nice, France)

11:00-11:15 **OC.1.** Juliette Salvi (Dijon, France)
Microglial inflammasome is involved in the regulation of food intake

11:15-11:30 **OC.2.** Mélanie Chester (Paris, France)
Mini-puberty could participate to the modulation of reproductive lifespan in female mice

11:30-11:45 **OC.3.** Adeline Coursan (Bordeaux, France)
Moody-Fructose: Effect of fructose consumption on mood disorders

11:45-12:00 **OC.4.** Laurine Decoster (Lille, France)
An olfactory bulb GnRH neuronal population translates social relevant odors into reproductive behavior in male mice

12:00-13:30 Déjeuner + Posters *Hall, CURIB*

13:30-14:30 **Lecture Jacques Benoit soutenue par Karger** *Amphithéâtre, Bât. CURIB*
Nicolas De Roux (Paris, France)
Genetics of puberby onset diseases: Where do we stand?
Modérateur : Youssef Anouar (Rouen, France)

14:30-15:30 **Communications orales** *Amphithéâtre, Bât. CURIB*
Modérateurs : Patricia Parnet (Nantes, France), Laurent Givalois (Montpellier, France)

14:30-14:45 **OC.5.** Ho Yin Thomas Lee (Bordeaux, France)
Unmasking the role of hypothalamic Tbx3 in the pathophysiology of maternally inherited diabetes

14:45-15:00 **OC.6.** Cassandra Malleret (Rennes, France)
Consequences of brain aromatase knock-out on cell proliferation, differentiation and behavior in zebrafish

15:00-15:15 **OC.7.** Clémentine Pajot (Lausanne, Suisse)
EphrinB3 in POMC neurons and control of energy and glucose homeostasis

15:15-15:30 **OC.8.** Hervé Tostivint (Paris, France)
Ontogeny of the caudal neurosecretory system in zebrafish revealed by a transgenic (uts2a: gfp) line

15:30-16:00 Pause-café *Hall, CURIB*

16:00-17:00 **Symposium Jeunes Chercheurs** - *Amphithéâtre, Bât. CURIB*
Recherche translationnelle en Neuroendocrinologie"
Modératrices : Céline Cansell (Paris, France), Rachel Ginieis (Bordeaux, France), Cristina Miralpeix (Bordeaux, France)

16:00-16:10 **SJC.1.** Ludovica Cotellesa (Lille, France)
High Anti-Müllerian Hormone (AMH) at mini-puberty induces PCOS-like traits in the offspring

16:10-16:20 **SJC.2.** Chloé Tezenas Du Montcel (Paris, France)
The role of dysregulated ghrelin/LEAP-2 balance in eating disorder: a translational study in anorexia nervosa

16:20-16:30 **SJC.3.** Emilie Lahaye (Rouen, France)
Functional validation of oxytocin-like bacterial proteins produced by Lactobacillus species

16:30-16:40 **SJC.4.** Rachel Ginieis (Bordeaux, France)
Beneficial effects of chrononutrition on high fat diet-related memory impairments - A translational study

16:40-16:50 **SJC.5.** Inès Drissa (Rouen, France)
Exocytosis is regulated by a subset of miRNAs in pheochromocytoma, a neuroendocrine tumor

16:50-17:00 **SJC.6.** Marine Simonneaux (Strasbourg, France)
Chronic shifts impact the female mice reproductive axis, fertility and offspring development

17:00-18:00 **Session Posters** — *Hall, CURIB*

18:30-19:00 Concert Carillons — *Cathédrale de Rouen*

20:30 Dîner de gala — *Restaurant « Au Bureau »*

Vendredi 29 septembre 2023

8:30-10:30 **Symposium 4 - *Nutrients and microbiota: regulators of emotional and cognitive functions*** — *Amphithéâtre, Bât. CURIB*
Modérateurs : Amandine Gautier-Stein (Lyon), Xavier Fioramonti (Bordeaux, France)

8:30-9:00 **S4.1.** Serguei Fetissov (Rouen, France)
Oxytocin-binding immunoglobulins as modulators of oxytocin signaling and motivated behavior-link to gut microbiota

9:00-9:30 **S4.2.** Rochellys Diaz Heijtz (Stockholm, Suède)
Early-life gut microbiome and neurodevelopmental outcomes

9:30-10:00 **S4.3.** Justine Vily-Petit (Lyon, France)
Nutrients, microbiota and intestine, a triumvirate that controls cognitive brain functions

10:00-10:30 **S4.4.** Quentin Leyrolle (Bordeaux, France)
Gut-derived metabolites as modulators of brain inflammation and behaviour

10:30-11:00 Pause-café — *Hall, CURIB*

11:00-12:00 **Prix SNE et Prix de Thèse** — *Amphithéâtre, Bât. CURIB*
Modérateurs : Sébastien Bouret (Lille, France), Hervé Tostivint (Paris, France)
Soutenu par Karger Publishing

11:00-11:30 Pieter Vancamp (Nantes, France)
Building a brain: The need for thyroid hormone and protein during foetal development

11:30-12:00 Nolwen Adam (Paris, France)
Exposure to environmental plasticizers disturbs the neural control of reproductive behaviors in female mice and their descendants

12:00-12:30 Cérémonie de clôture — *Amphithéâtre, Bât. CURIB*

Résumés

Conférence Grand Public

La fascinante histoire de la découverte de l'insuline

Rostene W[1,2]

[1] *UMR 968 Sorbonne-Université, Inserm, CNRS, Paris, France ;* [2] *Directeur de recherche émérite INSERM. Institut de la Vision, Paris, France*

Si les symptômes du diabète ont été décrits depuis l'Antiquité et caractérisés par la présence de sucre dans les urines et une soif intense, ce n'est qu'à la fin du 19ème siècle que plusieurs équipes s'efforcent de trouver la substance active de la sécrétion interne du pancréas afin de produire des extraits susceptibles de diminuer le glucose dans le sang et les urines chez le chien diabétique. Rarement une découverte telle que celle de l'insuline a été tant attendue. Avant cette découverte, il n'y avait aucun espoir de survie pour les patients atteints de diabète de type 1. C'est à l'Université de Toronto, au Canada, il y a 100 ans, entre 1921 et 1922, que Frederick Banting, Charles Best et James Collip, travaillant dans le département de physiologie dirigé par John MacLeod obtiennent des extraits pancréatiques suffisamment purifiés qui permettent de traiter de jeunes patients diabétiques. Rarement, sauf peut-être pour les vaccins ARN contre la Covid-19, une recherche a abouti si rapidement à un produit disponible pour la société. Jamais le prix Nobel de Physiologie ou Médecine n'a été décerné si rapidement après une découverte comme c'est le cas pour l'insuline en 1923. Fréderick Banting, reste toujours le plus jeune récipiendaire de ce prix prestigieux. Jamais avant Fréderick Banting et John Macleod, un lauréat n'avait renoncé à recevoir son prix à Stockholm comme c'est la tradition le jour anniversaire d'Alfred Nobel le 10 décembre. L'insuline a été la source de nombreuses innovations et autres découvertes. C'est la fascinante histoire de cette découverte centenaire, aux multiples retombées scientifiques, industrielles et cliniques, toujours d'actualité, qui est contée dans cette conférence grand public.

Lecture Jacques Benoit

Genetics of puberty onset diseases: where do we stand?

De Roux N

INSERM U1141/Université Paris Cité, Paris, France

Since the discovery of the first Kallmann syndrome gene in 1991, many genes have been linked to isolated or syndromic hypogonadotropic hypogonadism. These studies have revealed the very high complexity of the genetic determinism of the gonadotropic deficiency with several modes of transmission. This strategy helped to make major advance in the understanding of puberty onset diseases but also paved the way for fundamental research to understand the development of the gonadotropic axis as well as in its neuroendocrine regulation. At the opposite side of the spectrum, the genetic of central precocious puberty represents a more complex question. Recent epidemiological studies revealed monogenic, polygenic but also multifactorial modes of transmission. In addition, the development of an in-vivo model of central precocious puberty in mice looks more challenging than previously thought which impedes the validation of candidate genes.

In this lecture, I will present the present and the future of the genetic of pubertal onset diseases and its very close interactions with fundamental research.

Prix de la SNE

Building a brain: The need for thyroid hormone and protein during foetal development

Vancamp P, Parnet P and Amarger V

NU, INRAE, UMR 1280, PhAN, IMAD, 44000 Nantes, France

Thyroid hormone (TH) is a multifaceted hormone, promoting neurodevelopment, metabolism and mental wellbeing. The discovery of the TH transporter MCT8, whose mutations were linked to a rare psychomotor disability syndrome, raised questions on how its functional absence contributes to the physiopathology of these patients. In Leuven, Belgium, we used the chicken embryo to knock down MCT8 and reduce TH action in specific neural stem cell (NSC) populations that give rise to key cell types such as the Purkinje cells and GABAergic cells, and observed cell cycle perturbations, hampered migration and impaired differentiation.

At the Natural History Museum in Paris, we then investigated how TH and disruptors of TH action affected NSCs of the mouse brain. We found how exposure to the endocrine disruptor Bisphenol F interfered with gene expression and adult neurogenesis, resulting in altered olfactory behaviour. In another study, we revealed that exposure to the TH disruptor pyriproxyfen worsened outcomes of ZIKA virus infection in NSCs, identifying a potential mode of action explaining why more cases of microcephaly were diagnosed in North-Eastern Brazil regions. Lastly, we found that the capacity of adult NSCs to differentiate into neurons and oligodendrocytes, the latter responsible for generating myelin, had changed due to impaired TH action during development. Building forth on these data in Germany, we applied proteomics together with conditional knockout mouse models, and identified the striatal enzyme PDE10A as a novel target underlying locomotor problems in patients.

At the PhAN unit of the INRAE in Nantes, we are currently investigating how protein restriction (PR) interferes with early brain development, more specifically the hypothalamus, the neuroendocrine control centre of an organism's metabolism. We thereby focus on hypothalamic neurogenesis in rats at gestational day 15 (G15), and at G17 when neuronal differentiation takes place. We feed dams an 8% isocaloric PR diet for four weeks prior to mating, followed by a 4% PR diet until either of both time points. The control group receives a diet containing 20% of proteins. Preliminary data have shown that both foetus and placenta are negatively impacted by the diet, and that hypothalamic cell proliferation is impaired. We plan to perform single-cell RNA-Seq on cultured cells grown from dissected hypothalami at G15 to identify which cell types are the most vulnerable to perinatal PR, and which associated molecular pathways are dysregulated. These data ultimately offer insights into the origins of metabolic disease in adult life.

Prix de Thèse

Exposure to environmental plasticizers disturbs the neural control of reproductive behaviors in female mice and their descendants

Adam N, Lachayze MA, Brusamonti L, Hanine R, Bascarane K, Parmentier C, Hardin-Pouzet H, Naulé L, Mhaouty-Kodja S

Sorbonne Université, CNRS UMR 8246, INSERM U1130, Neuroscience Paris Seine – Institut de Biologie Paris Seine, 75005 Paris, France

Phthalates are organic pollutants widely detected in the environment due to their massive use in plastic production. Di-2-ethylexyl phthalate (DEHP), the most abundant, and phthalates have been extensively studied for their reproductive effects. However, whether they affect the neuroendocrine systems controlling reproduction still needs to be investigated, in particular for environmentally relevant doses of these substances. In a previous study, the team showed that adult exposure of male mice to phthalates severely disrupted their sexual behavior and the underlying neuroendocrine regulation (1). During my PhD, I investigated the effects of phthalate exposure on the expression of sexual and maternal behaviors in females.

Adult female mice were chronically exposed to low doses of DEHP alone (5 or 50 µg/kg of body weight/d), or in an environmental phthalate mixture.

Behavioral analyses showed that phthalate exposure severely altered multiple aspects of female sexual behavior. These behavioral changes were associated with a lower number of neurons expressing the progesterone receptor (PR) in the circuitry underlying sexual behavior, including the ventromedial hypothalamus, a key region for sex behavioral processing (2). A proteomic study of this region also revealed alterations of proteins involved in neurotransmission, neuroplasticity, neuroinflammation, and neuroprotection. Further investigations showed a reduction of dendritic spine density in phthalate-exposed females and modifications in the expression of several neurotransmission components. Immunohistochemical analyses confirmed an astroglial activation in phthalate-exposed females.

Maternal behavior analysis revealed that chronic phthalate exposure disrupted several aspects of spontaneous maternal behavior, with a lower number of pup-directed behaviors. However, in the pup-retrieval test, dams exposed to phthalates retrieved their pups more rapidly. This can be explained by the increased number of ultrasonic vocalizations emitted by their pups. The oxytocin and estrogen signaling pathways, both crucial for the initiation of maternal behavior, are disrupted in dams exposed to phthalates.

Female offspring born from these phthalate-exposed dams presented a delayed pubertal. In adulthood, they exhibited sexual deficiencies either comparable or even more important to those reported for adult exposure. This was also associated with a downregulation of the neural PR. However, in contrast to the adult exposure, these perturbations were maintained even after the exposure at weaning, indicating long-term effects of developmental exposure to phthalates.

Altogether, the data show that the adult and developing neural circuitries underlying female reproductive behaviors are highly vulnerable to environmentally relevant doses of phthalates. The relevance of these results with respect to human health and environment, and to the current reference doses established by regulatory agencies will be discussed.

(1) Dombret C, et al., *Environ Health Perspect* (2017) 125 :097001 ; (2) Adam N, et al S., *Environ Health Perspect* (2021) 129:017008 ; (3) Adam N, et al., *Environ Pollut* (2022) 120487.

Symposium 1

Beyond neurons: role of glia in the central regulation of metabolism

S1.1. Astrocytic response to obesity in the olfactory bulb

Martin C

Université Paris Cité, CNRS, Unité de Biologie Fonctionnelle et Adaptative, Paris, France.

Dysregulation in the control of food intake is a major contributor to the rising obesity rates in both adults and children. It is likely that sensory perception of food plays a role in this process. Smell is one of the most relevant sensory cues predicting food and is likely to play a key role in food choice and food consumption. Several studies in human individuals with obesity have provided evidence of significant alterations in olfactory perception, characterized by elevated detection thresholds and distinct variations in hedonic perception.

Astrocytes play a pivotal role in the functional network of the olfactory bulb at different steps of odor processing and have been shown to exhibit plasticity in response to the nutritional status. The brain of high fat diet-fed animals is characterized by inflammatory processes. While several studies have focused on the effect of high fat diets on astrocyte's reactivity within the hypothalamus, this has not been studied in the olfactory system.

We assessed the impact of obesity on the olfactory system in mice. Olfactory capacities and the activity of the olfactory bulb were compared between mice fed with high-fat, high-sucrose diet and controls under standard diet. We next compared the plasticity of astrocytes in the different layers of the olfactory bulb and in the piriform cortex. Our findings highlight astrocyte heterogeneity within the olfactory bulb and suggest a potential role for astrocyte in olfactory deficits observed in obese individuals.

S1.2. Glial modulation of energy balance: the dorsal vagal complex is no exception

Troadec JD

Aix Marseille Université, UMR CNRS 7291, Marseille, France

The avoidance of being overweight or obese is a daily challenge for a growing number of people. The growing proportion of people suffering from a nutritional imbalance in many parts of the world exemplifies this challenge and emphasizes the need for a better understanding of the mechanisms that regulate nutritional balance. Over the last few decades, our understanding of glial cells has changed dramatically. These cells are increasingly regarded as important neuronal partners, contributing not just to cerebral homeostasis, but also to cerebral signaling. Our understanding of the central regulation of energy balance is part of this evolution. Evidence is accumulating that glial cells play a dynamic role in the modulation of energy balance. In this talk, I will summarize recent data indicating that the multifaceted glial compartment of the brainstem dorsal vagal complex (DVC) should be considered in research aimed at identifying feeding-related processes operating at this level. The DVC is a frequently underappreciated and understudied area of the brain that integrates peripheral cues from metabolic status and relays them to the forebrain to control and maintain energy balance. In light of recently published data, it has become evident that glial cells of the DVC must be taken into account in order to better understand the mechanisms regulating the energy balance and to develop truly effective strategies against obesity and associated metabolic disorders.

S1.3. Could midlife alterations of hypothalamic tanycytic shuttles lead to neurodegenerative disorders later in life?

Prevot V[1]

[1]*Univ. Lille, Inserm, CHU Lille, Laboratory of Development and Plasticity of the Neuroendocrine Brain, Lille Neuroscience and Cognition, UMR-S 1172, Lille, France.*

Some regions of the brain lack the conventional blood-brain barrier (BBB), the circumventricular organs, and represent areas of extensive blood to brain communication thanks to fenestrated blood capillaries. One of them is the median eminence where the BBB is formed by specialized ependymoglial cells, the tanycytes. Tanycytes have been described as a privileged brain to blood interface especially in the context of hypothalamic-pituitary hormonal axes and metabolic regulation. Interestingly, diet-induced obesity impairs the ability of hypothalamic tanycytes to shuttle blood-borne hormones such as leptin into the brain. Because obesity at mid-life is a known risk factor for Alzheimer's disease, we hypothesized that alteration of tanycytic shuttles may play a role in the pathophysiology of the disease. Alzheimer's Disease is characterized by the aggregation of amyloid-ß (Aß) into plaques and hyperphosphorylated Tau protein (Tau) into tangles. Both proteins are known to be able to cross the blood brain barrier (BBB) but while Aß transport from brain to periphery has been extensively described, Tau excretion remains enigmatic. To determine whether tanycytes could act as a Tau conduit and transport it from CSF to blood. Using fluorophore-coupled 2N4R Tau (Tau-565) we tested Tau transport by tanycyte *in vitro* using primary cultures and *in vivo* by injecting Tau-565 in the lateral ventricle of wild-type and transgenic animals selectively expressing Botulinum toxin in tanycytes blocking vesicular trafficking. Altogether, our result show that tanycytes transport Tau from the CSF to the blood and raise the intriguing possibility that they may be involved in the pathophysiology of Alzheimer disease.

S1.4. Astrocyte-neuron circuits in hypothalamic regulation of metabolism

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The hypothalamus serves as a crucial metabolic center responsible for regulating systemic metabolism and body weight. Recent years have brought about a growing body of evidence that highlights the significant role of astrocytes and other glial cells in the regulation of hypothalamic circuits responsible for food intake and energy metabolism. Astrocytes play a key role in integrating food-related cues and internal signals, which in turn influence behavior and metabolism based on the body's energy status. Our research focuses on investigating the specific contribution of astrocytes in the regulation of hypothalamic circuits related to satiety. By doing so, we aim to understand how dysfunction in the hypothalamus is linked to obesity. This research deepens our understanding of the intricate mechanisms underlying energy balance through astroglia-neuron communication and provides valuable insights for addressing issues associated with obesity.

Symposium 2

New insights into the hypothalamic-pituitary control of fertility

S2.1. Molecular mechanisms underlying the plasticity of gonadotrope cell activity

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Human fertility in both sexes depends on the tight regulation of gonadal gametogenesis and steroidogenesis by the two pituitary gonadotropins LH (Luteinizing hormone) and FSH (Follicle-Stimulating hormone). In all vertebrates, gonadotropins are produced by gonadotrope cells, one of the six endocrine cell populations of the pituitary gland. During the sexual cycle in adult females, gonadotrope cells exhibit a remarkable endocrine plasticity that orchestrates cyclic ovarian activity, and, hence, the coordinated activity of all reproductive organs. During each estrus cycle, this plasticity culminates to generate a massive release of LH and FSH, i.e. the preovulatory surge, which is essential for female fertility as it triggers ovulation. Although it is considered that gonadotrope cell activity is mainly regulated by the hypothalamic neurohormone GnRH, regulation is far more complex as gonadotrope cells are also targeted by numerous endocrine and paracrine signals. The molecular mechanisms underlying the integration of all these signals by gonadotrope cells *in vivo* are far from being elucidated, mostly because of the structural complexity of the pituitary gland.

The past ten years have seen an exciting revolution with the development of single-cell technologies that have removed major obstacles preventing molecular analyses of highly heterogeneous tissues *in vivo*. We have thus performed single-cell analyses of rat pituitaries at different moments of the estrus cycle, in order to precisely characterize *in vivo* the molecular mechanisms underlying gonadotrope plasticity. Significant transcriptional variations were detected not only in gonadotrope cells but also in most endocrine and non-endocrine cell types, suggesting that the whole pituitary gland is implicated in the regulation of the estrus cycle. Focusing our attention on gonadotrope cells, we observed that transcriptional plasticity mainly takes place during pro-estrus day. We detected massive changes in the expression of unexpected gene families. For instance, the expression of several genes encoding extracellular matrix proteins such as Tnc, Col17a1 or Dmp1 was dramatically increased in gonadotrope cells during the late afternoon of pro-estrus, suggesting that these cells dramatically reshape their proximal environment around the time of the gonadotropin surge. We also discovered that the gonadotrope cell response relies on the activation of numerous transcription factors. Among them, we have identified a gonadotrope-specific transcription factor, FOXP2, which is known to be crucial for neurogenesis, but whose role in in the pituitary was still elusive. Interestingly, our *in vitro* data suggest that FOXP2 might be implicated in gonadotrope cell motility and network remodeling through the regulation of some of the aforementioned extracellular matrix genes.

To conclude, the role of gonadotrope cells has long been restricted to the transmission of hypothalamic signals to the body. Our single-cell data by showing that gonadotrope cells also exhibit complex molecular and cellular changes throughout the estrus cycle and are part of a coordinated pituitary response, suggest that the role of these cells is far more sophisticated. Because gonadotropin imbalances are associated with reproductive diseases such as polycystic ovary syndrome and with ovarian cancers, a better understanding of gonadotrope cell activity is an issue of crucial importance.

S2.2. Unusual suspect: role of microglia in the neuroendocrine disorder polycystic ovary syndrome

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Infertility disorders currently affect 1 in 6 couples worldwide. Polycystic Ovary Syndrome (PCOS) is the most common infertility disorder in women of reproductive age worldwide. PCOS is characterised by an elevated blood level of androgens, menstrual dysfunction and multiple cyst-like follicles in the ovary. Although commonly considered an ovarian disorder, the brain is now a prime suspect in both the development and maintenance of PCOS. Recent animal-based studies demonstrate that androgen excess in early life and adulthood contribute to the pathological neuronal wiring associated with infertility. To date, the mechanisms underlying this altered brain wiring remain unknown. Microglia, the immune cells of the brain, are active sculptors of neuronal wiring across development, mediating both the formation and removal of neuronal inputs. *Therefore, we hypothesized that microglia may contribute to the PCOS phenotype responsible for infertility*. To this aim, we assessed whether microglia phenotype and function are altered in the brain of the PNA mouse model of PCOS across development. In PNA mice, changes in the number and morphology of microglia have been only observed in the vicinity of the GnRH neurons where the neuronal wiring is detected and in time-specific manner. In addition, an altered refinement of GABA inputs onto the GnRH neurons has been observed prior to the remodelling of the circuitry in PCOS. To conclude, this study is the first to characterize microglia in a mouse model of PCOS and suggest a role of microglia in the brain wiring abnormalities associated with PCOS.

S2.3. Characterizing the neuroendocrine role of SELENOT in the regulation of reproduction

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Reproductive disorders are associated with neuroendocrine disturbances of the hypothalamic-hypophysis-gonadal (HHG) axis, which can result from a defective production and action of the gonadotropin-releasing hormone (GnRH). We have recently shown that SELENOT, a new thioredoxin highly expressed in neuroendocrine and endocrine cells, plays a crucial role in hormone secretion by controlling ER stress. Interestingly, the conditional knockout of the SELENOT gene in the central nervous system resulted in a very strong decline in fertility and impairment of sexual behavior in mice. Therefore, we sought to characterize the relationship between SELENOT gene expression and the function of the HHG axis. To this end, we performed mating tests and sexual behaviour recordings to probe the degree of fertility in KO mice. This behavioral analysis revealed a lack of sexual motivation in the KO mice. We also carried out histological studies, immunohistochemistry and hormonal assays to examine the morphology of the gonads and to assess the levels of hormones and neurohormones produced in mice. The results showed that female animals exhibit altered estrous cycle and a phenotype similar to polycystic ovary syndrome (PCOS), with an increase in LH and testosterone levels and a decrease in estradiol levels. In male mice, a marked increase in LH, testosterone and estradiol levels were observed. In the hypothalamus, SELENOT KO mice showed increased density of GnRH neurons in the preoptic area, and in their terminals in the median eminence. In order to determine whether the hypofertility observed in KO mice was due to GnRH hypersecretion, we administered a GnRH antagonist (Cetrorelix) to potentially revert the phenotype observed. Interestingly, we found that an intermittent administration of the GnRH antagonist to female SELENOT KO mice is able to restore ovarian cyclicity and normal LH secretion. Additional experiments are ongoing to decipher the molecular mechanisms involved. Altogether, these results demonstrate the instrumental role of central SELENOT expression in the function of the HHG axis.

S2.4. Development of a new strategy using kisspeptin as a tool to manage reproduction in mammals

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In mammals, the neuropeptide kisspeptin (Kp) and its receptor, KiSS1R, are well known to modulate the reproductive function by triggering puberty onset and ovulation via the stimulation of gonadotrophin-releasing hormone (GnRH) secretion. In this context, the Kp hold an interesting clinical potential in reproductive health. To overcome the drawback of kisspeptin short half-life we designed a Kp analog called C6, combining original modifications, triazole peptidomimetic and albumin binding motif, to reduce proteolytic degradation and to slow down renal clearance, respectively. In cells expressing the Kiss1r, the C6 showed improved potency and dramatically enhanced pharmacodynamics compared to the endogenous Kp. In vivo studies showed that injections (in mice, goats or ewes) are advancing puberty and inducing fertile ovulations. In addition, preliminary studies performed in animal models of human diseases reveal the ability of C6 to stimulate LH release and ovulations in case of reproductive dysfunction. Thus, our results are demonstrating that our Kp analog may find application in the management of livestock reproduction and opens new possibilities for the treatment of reproductive disorders in humans.

Symposium 3

Interest of non-conventional animal models to understand adaptation of neuroendocrine rhythms to environmental constraints

S3.1. Life under the tropics: effect of sex in the phenology of seasonal rhythms in a Malagasy primate

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Seasonal species use the photoperiodic signal as a crucial clue to synchronize their behavioral and physiological rhythms to annual variations of their environment. The grey mouse lemur, a nocturnal primate endemic from Madagascar, shows unique physiological features in a sex-dependent manner, related to the life-history traits of this species. Adaptation to season, which is centrally controlled by the hypothalamus, is associated with deep remodeling of metabolic capacities, involving for example the liver, and is observable at the cellular level. During this seminar, I will provide insights on the mechanisms, both at the peripheral and centrals levels, involved in the seasonality of the mouse lemurs and the difference between males and females, as well as perspectives on the adaptive value of being seasonal in the context of global change.

S3.2. Neuroendocrine mechanisms driving reproductive cycles in the female camel

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The dromedary camel (Camelus dromedarius) is a short-day desert breeder in which female ovulation is induced by mating. Current data indicate that male-induced ovulation is triggered by its seminal plasma nerve growth factor beta (β-NGF), but the exact mechanisms involved in the induction of ovulation are still unknown. In this study, we report that an intramuscular injection of β-NGF in sexually active short dayadapted female camels induces an ovulation attested by a surge of circulating LH (2h to 6h after treatment) followed by an oocyte release with its cumulus oophorus (confirmed by ultrasonography 72 h after treatment) and a large and progressive increase in circulating progesterone (significant from the 2nd to the 10th days after β-NGF injection). Additionally, this β-NGF treatment induces a broad nuclear c-Fos activation in cells located in various hypothalamic areas, notably the preoptic area, the arcuate nucleus, the dorso- and ventro-medial hypothalamus, the paraventricular nucleus, and the supraoptic nucleus. A double immunostaining with neuropeptides known to be involved in the central control of reproduction indicates that approximately 28% kisspeptin neurons and 43% GnRH neurons in the proptic area, and about 10% RFRP-3 neurons in the dorso- and ventro-medial hypothalamus are activated following β-NGF injection. In conclusion, our study demonstrates that systemic β-NGF induces ovulation in the female dromedary camel and indicates that this effect involves the central activation of hypothalamic neurons, notably the Kp neurons.

S.3.3. Ambient temperature cycles synchronize circadian rhythms in the desert goat

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Mammals living in the desert environment such as camel and goats have to cope and adapt themselves to these harsh conditions. We have previously shown that in the dromedary camel, the ambient temperature (Ta) cycles synchronize the circadian clock. In the desert goat, we tested if as in the camel, Ta cycles would be strong enough to entrain the central clock. Three well-known robust outputs of the circadian clock, the rhythms of body temperature (Tb), locomotor activity (LA), and melatonin (Mel) were studied under different Ta cycles conditions. Ten bucks were subjected first to constant conditions of total darkness (DD) and constant Ta, then transferred to DD with a Ta cycle (heating during the subjective day and cooling during the subjective night). Finally, in the last stage of the experiment, Ta cycles were reversed with heating during the subjective night and cooling during the subjective day. Results showed that when bucks were subjected to constant conditions, all the studied circadian rhythms persisted, and free runs with a circadian period was different from 24.0. Thereafter, when the Ta cycle was applied, it entrained the rhythms of Tb and LA to an exact period of 24.0 hours. However, when Ta was reversed, it induced an inversion of Tb and LA rhythms. Likewise, Mel rhythm was entrained by Ta cycles, by coinciding its peak secretion with the cooling period of Ta cycle. These findings demonstrate clearly that as in the camel, the Ta cycles entrain the master circadian clock in the desert goat.

S3.4. Risk of a plasticizer-enriched environment on the neuroendocrine control of reproductive and cognitive behaviors

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Phthalates are among the most frequently detected organ pollutants in the environment. They are used in the manufacture and processing of polyvinyl chloride plastic, cosmetic and personal care products, cleaning products, etc. Di-2-ethylhexyle phthalate, dibutyl phthalate, benzyl butyl phthalate and other substances of this family were identified as endocrine disruptors due to their ability to reduce fetal testosterone production and impair reproductive male function (European Chemical Agency, European Food Safety agency). In this context, the effects of phthalates on the neuroendocrine control of reproduction and cognition in males and females, in particular at environmentally relevant doses, received little attention.

This presentation aims to give an overview of the most recent data from the team, describing the effects and neuroendocrine mode of action of exposure to environmentally relevant doses of phthalates on reproductive and cognitive behaviors. The obtained results will be presented and discussed in the context of risk assessment of these substances for human health and environment.

Symposium 4

Nutrients and microbiota:
regulators of emotional and cognitive functions

S4.1. Oxytocin-binding immunoglobulins as modulators of oxytocin signaling and motivated behavior - link to gut microbiota

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Oxytocin is a neuropeptide hormone produced mainly in the hypothalamus and secreted in the CNS and the blood. In the brain, it regulates motivated behavior and plays a major role in promoting social interactions. In the blood, oxytocin is bound to plasma proteins but their nature and functional role were not characterized. In this talk I will present recent data revealing that in humans the majority of plasma oxytocin is naturally bound to immunoglobulin (Ig) G. Moreover, results of *in vitro* experiments show that oxytocin-binding IgG modulate oxytocin receptor signaling affecting the dynamics of intracellular Ca^{2+} mobilization and oxytocin receptor internalization. Furthermore, the IgG properties to bind oxytocin may be relevant to altered social behavior including increased hostility and aggression in humans. Animal models support the central targets of oxytocin-reactive IgG. Indeed, peripheral administration of oxytocin together with human oxytocin-reactive IgG to resident mice in a resident-intruder test was accompanied by reduction of c-fos activation in several brain regions involved in the regulation of aggressive behavior correlating with the attack number and duration. The origin of oxytocin-reactive IgG may involve antigenic stimulation by bacterial proteins displaying conformational molecular mimicry with oxytocin. Experimental examples validating this possibility will be presented. Oxytocin-reactive IgG, hence, play a role of oxytocin carrier protein and appear as a molecular link in the gut microbiota-brain axis involved in regulation of motivated behavior.

S4.2. Early-life gut microbiome and neurodevelopmental outcomes

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It is now widely recognized that the gut microbiota exerts a broad range of effects on host physiology and development beyond the gastrointestinal tract, including the modulation of brain development and behavior. However, the mechanisms mediating the interactions between the gut microbiota and the developing brain are still poorly understood. Germline-encoded pattern-recognition receptors (PRRs) that recognize conserved microbial molecular signatures such as bacterial surface molecules (e.g., peptidoglycans, PGNs) have emerged as potential key regulators of gut microbiota-brain interactions (Gonzalez-Santana and Diaz Heijtz, Trends Mol. Med, 2020). The gut microbiota contains trillions of commensal bacteria producing a diverse peptidoglycome that can disseminate systemically and reach peripheral organs such as the brain. We have previously shown that PGN fragments from gut microbiota can be translocated into the developing brain (under normal physiological conditions) and sensed by specific PRRs of the innate immune system. Using expression-profiling techniques, we have previously shown that two families of pattern recognition receptors of the innate immune system that specifically detect PGN (i.e., PGN-recognition proteins and NOD-like receptors) and the PGN transporter PepT1 are highly expressed in the developing brain during specific windows of postnatal development, in both males and females. In this talk, I will present new evidence showing the importance of the PGN pathway in early-life, and how its perturbation could contribute to atypical social development of offspring and altered expression of hypothalamic neuropeptides.

S4.3. Nutrients, microbiota and intestine, a triumvirate that controls cognitive brain functions

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The intestine, via the gut-brain axis, plays an essential role in regulating food intake and body weight. However, the implications of this nervous axis go beyond the regulation of energy balance, as signals originating from gut are also capable of modulating emotions and cognitive abilities. More specifically, numerous studies have highlighted the effects of nutrients and gut microbiota on cognitive performance.

We have already shown that these foods (notably fermentable fibers and proteins) are capable of regulating energy homeostasis via an intestine-brain signal. These macronutrients induce the activation of intestinal gluconeogenesis (IGN), which generates a "portal glucose" signal detected in the portal vein and relayed to the brain, in particular brain regions controlling energy homeostasis and stress-related behavior. In these situations, IGN has several beneficial anti-diabetic, anti-obesity and anti-anxiety effects. On the contrary, the specific deletion of IGN promotes the development of a pre-diabetic state and anxiety-like behaviors, associated with defects in hippocampal neurogenesis. As the hippocampus is a regulatory center of memory, we hypothesized that IGN might control cognitive functions using the same gut-brain circuit.

Therefore, to assess the effects of IGN on memory, we used mouse models with a genetic activation (I.G6pc^{overexp} mice) or deletion (I.G6pc$^{-/-}$ mice) of the intestinal glucose-6-phosphatase, the key enzyme of endogenous glucose production. We studied spatial memory in both mouse models, using the Morris water maze test. In this test, mice were placed in a circular swimming pool and had to learn the location of an escape platform (made invisible by immersion in white water). The platform was placed in one area, defined as a quarter of the pool. Mice relied on distal cues to navigate from start locations to locate the platform. After repeated trials, we measured the time to find the platform. One week later, the platform was removed and we measured the time spent by the mouse in the platform area, referred as memory trace. Hippocampal tissues were sampled to study molecular mechanisms.

Our results shows that the time to find the platform was lower in I.G6pc^{overexp} mice and increased in I.G6pc$^{-/-}$ mice, suggesting that the induction of IGN improves learning and the absence of IGN is sufficient to induce a learning deficit. Nevertheless, the memory trace was similar whatever the genotype.

In parallel, neurobiological studies were carried out to understand the molecular mechanisms involved. Consistent with improved learning abilities, increased expression of NeuN and NMDA-R in the hippocampus of I.G6pc^{overexp} mice suggests enhanced synaptic plasticity when IGN is induced.

Thus, as a relay for nutrients and microbiota, the gut is an important modulator of cognitive abilities. More specifically, one intestinal function, IGN, may be able to modulate the learning process. This should lead to a better understanding of the relationships between intestinal nutrient metabolism and cognitive processes, which are still poorly understood at the mechanistic level.

S4.4. Gut-derived metabolites as modulators of brain inflammation and behaviour

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Gut microbiota has been associated with mental health that can be influenced by several neurobiological processes including brain inflammation. Until now mechanisms remained poorly understood. In this regard, gut-derived metabolites (GDM) are interesting compounds that are produced by gut bacteria and, for some of them, can reach the brain and influence brain inflammation and behaviour. Thus, the aim of our work was to select GDM that are decreased in mental health disorders and to test whether a supplementation can have a protective effect in a mice model of depressive-like behaviour. We used chronic social defeat stress (CSDS) model that is known to induce behavioural disturbances and brain inflammation. Using, primary microglia culture we further dissected the anti-inflammatory activity of the selected GDM. We observed that a phenolic GDM is able to alleviate proinflammatory cytokines production under lipopolysaccharides stimulation in primary microglia culture. We also observed that oral supplementation with this GDM was able to rescue CSDS-induced weight gain as well as anxiety and depressive-like behaviour. Our results revealed that phenolic GDM can efficiently modulate microglia inflammatory activity *in vitro* and prevent chronic stress induced behavioural disturbances.

Communications Orales

OC.1. Microglial inflammasome is involved in the regulation of food intake

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Context. Overfeeding causes significant cellular stress in all organisms, linking nutrition and inflammation. Indeed, regular consumption of high-calorie high-fat foods triggers a systemic immunological response that alters pancreas, liver and adipose tissue functioning, and promotes metabolic disorders such as obesity. Recent studies have shown that this inflammatory response also occurs in the brain. Furthermore, the food-related brain inflammatory response can be detected very early, at the time scale of a meal. However, the physiological value of the postprandial inflammatory response (PIR) in the brain is totally unknown.

Aim. This study involves characterizing the PIR in the hypothalamus, a brain structure that coordinates physiological responses to maintain body energy homeostasis and that is highly sensitive to postprandial metabolic signals, in mice.

Results. Using immunostaining, 3D reconstruction and morphometric analysis, we showed that microglia, resident immune cells in the brain, can change their shape rapidly after a meal and this becomes highly significant when the meal is very rich in fat. This results in an increase in cell volume, length and number of branches. In addition, cell sorting of hypothalamic microglia reveals a specific increase in IL-1β mRNA expression in these cells. To better understand the role of reactive microglia in the PIR, we generated *Asc*^{MgKO} mutant mice in which the *Asc* gene, an essential component of the intracellular inflammasome, is specifically deleted in microglia. Interestingly, *Asc*^{MgKO} mice have no phenotype and eat normally on a standard diet, but they become hyperphagic on a high-fat diet. This suggests that the postprandial microglial inflammatory response that appears during overfeeding has a positive valence, stimulating satiety and maintaining energy balance. The aberrant feeding behavior found in *Asc*^{MgKO} mice is not present in female mice, indicating sexual dimorphism in this physiological response.

Conclusion. Thus, the PIR appears in the hypothalamus after a meal, and is exacerbated by a fatty meal. It is characterized by the reactivity and pro-inflammatory phenotype of microglia which is found in males only. The brain PIR is a plasticity-related mechanism that has an adaptive physiological value, limiting food intake under caloric pressure.

OC.2. Mini-puberty could participate to the modulation of reproductive lifespan in female mice

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In female mammals, reproductive function is limited in time, notably due to the exhaustion of the stock of follicles present in the ovary. Reproductive senescence, named 'menopause' in humans may also result from hypothalamic aging. This physiological process is characterized by the elevation of basal gonadotropin levels, the decrease in the quality and quantity of oocytes, and the occurrence of irregular sexual cycles. However, the mechanisms underlying reproductive senescence, and in particular the possible contribution of early developmental events in this process is not clear. We hypothesized that mini-puberty, corresponding to a transient activation of the hypothalamic-pituitary-ovary (HPO) axis, with high secretion of LH, FSH and estradiol (E2), could regulate reproductive lifespan through its early action on the ovary and/or on the hypothalamus. To this purpose, we used a mouse model injected with a GnRH receptor antagonist (GonadoSTOP mice) to suppress LH, FSH and E2 levels specifically during mini-puberty. In these mice, estrous cycles were irregular as revealed by increased duration of diestrus 2, but fertility (time to conception and % of pregnant mice) was comparable to that of control females at 3, 4 and 5 months. However, late mating studies at 11 months revealed that 47% of GonadoSTOP mice versus 18% of control mice were pregnant (n=16-17 mice/group; $P<0.01$). Our RT-qPCR analyses of markers reflecting ovarian activity (Cyp19a1, Inhba, Inhbb, Amh, Gdf9, Sirt1, Fshr, Lhcgr and Lgals3) at 4 and 11 months revealed that ovarian aging occurred similarly in both groups, suggesting that increased reproductive longevity in GonadoSTOP mice does not have an ovarian origin. Control mice displayed higher basal LH levels at 11 months than at 4 months, in line with the advent of reproductive senescence. In contrast, 11-month-old GonadoSTOP mice displayed similar LH levels to 4-month-old CTR mice ($P>0.05$), and lower LH levels than 11-month-old control mice (n=6-10 mice/group; $P=0.0074$). Taken together these data suggest that transient and sustained activation of the HPO axis at mini-puberty may regulate key aspects of reproductive function with long-term impact on fertility, possibly by acting on hypothalamic neuronal networks contributing to reproductive longevity.

OC.3. Moody-fructose: effect of fructose consumption on mood disorders

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Introduction: The increase in fructose consumption has been highlighted by the ANSES institute as a potential public health concern in view of a large number of studies demonstrating the detrimental effect of fructose overconsumption on metabolic disorders (ANSES Saisine n°2012-SA-0186). However, the effects of this sugar on brain functions and mental health has been neglected; despite studies suggesting the potential impact that fructose consumption has on the development of mood disorders (De Souza et al., 2021). A brain hallmark of mood disorders is neuroinflammation, a process controlled by microglia. This raises the possibility that fructose contributes to neuroinflammation and mood disorders by acting directly on the brain. In support of this hypothesis, 1/ the brain is fully equipped to absorb and metabolize fructose; 2/ the selective-fructose transporter GLUT5 is expressed at the blood brain barrier and thorough the brain; 3/ within brain cells, GLUT5 is almost exclusively expressed in microglia (>90%, Payne et al., 1997). Thus, the aim of this project is to study 1/ the effects of fructose-enriched consumption on emotional behaviors, and 2/ the direct effects of fructose on the activity of microglial *in vitro*.
Materials and methods: Three-month-old C57/BL6J mice were given access to water or fructose solution (25% w/v) *ad libitum* for 8 weeks before performing behavioral tests to study emotional and memory behaviors. RNA-sequencing analyses has been performed on several brain areas in to order to determine brain pathways impacted by fructose feeding. Microbiota composition has also been analyses by 16S RNA-seq to correlate brain dysfunction to microbiota composition. In addition, primary microglia cells from adult mice were cultured in DMEM F/12 medium for 5 days before being exposed to fructose (500µM) in presence (or not) of the inflammation stimulator LPS (Lipopolysaccharide, 100µg/ml). Markers of inflammation and microglia homeostasis were measured by RT-qPCR.
Results and statistical analyzes: Our data show that a fructose-enriched diet induces anxiodepressive-like behaviors in mice in the forced swimming, splash test, elevated plus maze and novelty-suppressed food tests (emotional z-score: controls: 0 ± 0.19; Fructose: 2.17 ± 0.42), as well as memory deficits in the object location memory and Morris water maze tests (memory z-score: controls: 0 ± 0.2; Fructose: 1.64 ± 0.24). RNA-seq analyses show that these alterations in brain functions are associated with changes in expression of genes involved in carbon (glycolysis, TCA cycle) or lipid metabolism (KEGG pathways). Brain dysfunctions are also associated with gut dysbiosis. *In vitro*, we observed that fructose decreases the expression of the microglia homeostatic markers p2ry12 gene (-52.5 ± 4.6%) and glut5 (-79 ± 6%). Moreover, we found that fructose alters the expression of pro-inflammatory cytokines cytokines il-1b, il-6 and tnf-a in response to LPS treatment for 3 or 6h (decreased expression at 3h and increased expression at 6h). Fructose-induced changes in homeostatic markers or cytokines expression are blunted in microglia cultured from glut5-deficient mice.
Conclusion: Collectively, these data show that fructose induces behavioral alterations in mice and disrupts microglial physiology and inflammatory response. *In vivo* experiments in microglia-Glut5-deficient mice are currently in progress to determine whether changes behaviors and brain genes expression are dependent on fructose-induced microglia dysfunctions rather than due to alterations in gut microbiota.

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OC.4. An olfactory bulb GnRH neuronal population translates social relevant odors into reproductive behavior in male mice

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Pheromones influence reproductive physiology and behaviors in various species, humans included. Indeed, pheromone exposure in rodents accelerates sexual maturation, promotes estrous synchronization in females and induces a fast increase of serum LH levels in both sexes. The role of gonadotropin-releasing hormone (GnRH) neurons as the final neural output controlling reproduction fitness and fertility is well established among the different vertebrates. GnRH neurons are also known to integrate sex-related olfactory information to coordinate and optimize reproduction through another network involving olfactory and limbic areas in the brain. In addition, an extra-hypothalamic GnRH neuronal population has been identified, in both rodents and humans, in areas dedicated to olfactory processing and more specifically in the olfactory bulbs. We named this population $GnRH^{OB}$ neurons. We hypothesized that this newly identified population of GnRH neurons may convey olfactory and/or pheromonal information to participate in the neuroendocrine responses controlling reproduction. Using viral cell tracing and whole-head tissue-clearing and 3D-imaging, we show that $GnRH^{OB}$ neurons extend long projections into the vomeronasal organ, the main olfactory epithelium and into the median eminence. We confirmed by single-cell RNA sequencing of the entire olfactory bulb population that $GnRH^{OB}$ neurons express olfactory and pheromone receptors suggesting a possible detection of olfactory cues by the $GnRH^{OB}$ neurons. We then investigated whether $GnRH^{OB}$ neurons can be activated by opposite-sex smell. Using of *in vivo* calcium imaging coupled with two-photon microscopy we confirmed that olfactory and pheromonal stimulation can activate the $GnRH^{OB}$ somata and their processes. To confirm that this activation participates to the neuroendocrine responses, we tested whether $GnRH^{OB}$ neurons promote LH release. Our results showed that the chemogenetic activation of $GnRH^{OB}$ neurons triggers LH release in male mice. Using a combination of virogenetic experiments and electrophysiological recordings on hypothalamic slices we demonstrate that $GnRH^{OB}$ neurons are also connected to the GnRH population located in the rostral preoptic area and that chemogenetic activation of $GnRH^{OB}$ neurons triggers an increase of $GnRH^{POA}$ firing activity. Finally, the function of $GnRH^{OB}$ neurons was investigate through multiple olfactory and social behavior tests using chemogenetic and neuronal ablation approaches. Remarkably, our data demonstrate that male preference for female odors is enhanced upon chemogenetic activation of $GnRH^{OB}$ neurons, impaired after genetic inhibition or ablation of these cells and relies on GnRH signaling in the posterodorsal medial amygdala. Taken together, our study highlights a novel role for olfactory GnRH neurons as a central regulatory hub linking pheromonal stimulation with reproductive functions.

OC.5. Unmasking the role of hypothalamic Tbx3 in the pathophysiology of maternally inherited diabetes

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Exposure to a high-fat diet during pregnancy is a predisposing factor for metabolic and reproductive disorders in the offspring [1, 2]. In response to maternal hypercaloric stress, altered neurogenesis and impaired neurite outgrowth are observed in hypothalamic pre-opiomelanocortin (POMC) expressing neurons during early development [3, 4], which may contribute to the aetiology of maternally inherited metabolic and reproductive disorders. However, the key molecular and cellular underpinnings of altered neuronal development in the offspring following maternal metabolic cues have yet to be fully elucidated. Our data suggest that the *T-box 3* gene (*Tbx3*), which encodes a transcription factor maintaining Pomc neuronal identity [5, 6], is a critical node linking maternal metabolic stress with altered reprogramming of reproductive and metabolic health in the offspring. Maternal exposure to a high-fat diet during pregnancy and lactation resulted in higher fat mass accumulations and more significant impairments in glucose homeostasis in transgenic offspring mice carrying a specific loss of *Tbx3* expression in POMC-expressing precursors (POMC-Tbx3-KO) compared to wild-type (WT) controls. In WT mice, maternal high-fat diet altered the expression of neuronal identity markers in POMC-derived neurons, as assessed by lineage tracing. However, maternally-induced changes in cell identity were lost in POMC-Tbx3 KO mice. POMC-expressing precursors can give rise to fertility-regulating neurons that express kisspeptin (Kiss1) [7]. Therefore, we also investigated the cell-specific role of Tbx3 in the Kiss1 population by exposing conditional knockout mice carrying a specific loss of *Tbx3* in Kiss1 neurons to the maternal high-fat diet paradigm. Our data suggests that Tbx3 in Kiss1 neurons is not required to mediate the effect of maternal high fat diet on the transmission of metabolic disease. However, Tbx3-mediated actions in Kiss1 neurons are necessary for the proper development and maturation of sex organs in response to maternal high-fat diet. Thus, Tbx3 could be a potential mediator in the transmission of metabolic and reproductive consequences in mice following impairments in maternal metabolic health. Alterations in Tbx3-mediated mechanisms of identity maintenance in Pomc precursors may explain how maternal metabolic disease influences the correct programming of brain circuits that integrate metabolic and reproductive needs throughout adult life.

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OC.6. Consequences of brain aromatase knock-out on cell proliferation, differentiation and behavior in zebrafish

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Amongst its pleiotropic physiological effects, 17β-estradiol (E2) regulates neuroplasticity. The key enzyme involved in E2 synthesis from testosterone is aromatase. In the brain of teleost fish, aromatase B (AroB) is encoded by the *cyp19a1b* gene. Previous data from the laboratory have shown that, in zebrafish, *cyp19a1b* is strongly expressed in radial glial cells that actively divide to generate newborn cells, suggesting the central role of E2 in neuroplasticity.
The aim of this work was to investigate the impact of AroB deletion on neurogenesis and behavior using specific AroB-KO juvenile and adult zebrafish.
Swimming activity was investigated in 1 month juveniles and several behavioral traits were studied in 3 month adults. We do not observe a statistically significant difference between AroB-KO and wild-type juveniles fish. At the later stage, statistical analyses revealed that AroB-KO males were hypoactive compared to WT counterparts, and AroB-KO females showed a similar trend. In addition, AroB-KO males displayed decreased boldness compared to their WT counterparts, while females were not statistically impacted.
Proliferation and differentiation of dopaminergic and serotoninergic neurons were investigated in juveniles and adults by immunofluorescence and by RT-qPCR.
In juveniles, proliferation was quantified in three regions, the ventral pallium of the telencephalon (equivalent to amygdala), the medial pallium of the telencephalon (equivalent to hippocampus) and the diencephalic nucleus of posterior recess. A significant decrease in cell proliferation was described in the nucleus of posterior recess in AroB-KO animals compared to WT whereas no differences were observed in the ventral and medial pallium. A significant decrease of PCNA expression, a marker of cell proliferation, was also highlighted by qPCR in the whole brain of AroB-KO juveniles compared to WT animals. For the serotoninergic neurons, we observed a significative increase of the number of serotoninergic neurons in the paraventricular organs of AroB-KO animals compared to WT. The effect of *cyp19a1b* gene deletion on dopaminergic neurons is under analysis.
In adults, our results highlighted region-dependent effects of AroB deletion on cell proliferation in both male and female AroB-KO compared to WT. While an increase in the number of proliferating cells was observed in the olfactory bulbs of AroB-KO animals, the other regions of the telencephalon and diencephalon showed a statistically significant decrease. qPCR performed on adult brains showed a significant decrease in PCNA transcript expression in both AroB-KO adult males and females compared to wild-type. No change in the number of dopaminergic and serotoninergic neurons was observed in adult male and female olfactory bulbs, telencephalon and diencephalon.
Our results suggest that an alteration in local E2 production impacts brain proliferation and is associated with a modification of behavior.
A BRB-Seq study is currently undertaken to identify genes affected by AroB deletion in juveniles and adults. Quantification of monoamines and their metabolites by HPLC will also improve the knowledge about the link between AroB and behavior.
This work was supported by the French National Research Agency (FEATS project; ANR-19-CE34-0005-05) and a PhD fellowship.

OC.7. EphrinB3 in POMC neurons and control of energy and glucose homeostasis

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In the hypothalamus, proopiomelanocortin (POMC)-expressing neurons have been primarily described as controlling feeding behavior, but accumulating evidence also showed a crucial role of these neurons in the control of glucose homeostasis, notably hepatic glucose production and insulin secretion. For this, and in addition of being able to sense peripheral signals such as leptin, POMC neurons receive direct upstream excitatory (glutamate) and inhibitory (GABA) inputs coming from a plethora of brain areas. A recent study from our group specifically showed that manipulating during development, a well-known actor of glutamatergic synapse formation called EphrinB1, reduced the number of glutamatergic inputs into POMC neurons, impaired their excitability and insulin secretion in response to hyperglycemia. Interestingly, EphrinB1 and two other members of EphrinB family, EphrinB2 and EphrinB3, are still expressed in POMC neurons of adult mice. While we described a role of EphrinB1 in the development of excitatory inputs into POMC neurons, we still do not know to which extend these three proteins could participate to the control of energy and glucose homeostasis through synaptic plasticity of POMC neurons.

Here, we focused on EphrinB3, that showed the highest level of expression in POMC neurons of adult male mice compared to EphrinB1 and EphrinB2. To specifically study the role of EphrinB3 in the synaptic plasticity of POMC neurons and subsequently in the control of energy and glucose homeostasis, we used a viral approach to overexpress Efnb3 (gene encoding EphrinB3) in POMC neurons in male adult mice (Pomc-Efnb3-OE). First, we evaluated physiological parameters such as body weight, body composition, food intake, glycemia, and glucose tolerance, and measure the spontaneous postsynaptic excitatory currents (sEPSC) of POMC neurons in animals fed a regular chow diet. Despite an increased AMPA/NMDA-dependent sEPSC in POMC neurons, POMC-Efnb3-OE mice displayed a mild phenotype under chow diet, with a tendency of increased insulin in fed condition. Then, as exposure to high fat diet (HFD) is known to modulate synaptic plasticity of POMC neurons, these mice were exposed to HFD for several weeks, and similar parameters were assessed. Interestingly, in both chow and HFD conditions, Pomc-Efnb3-OE male mice showed altered AMPA-dependent sEPSC of POMC neurons compared to control mice. In addition, Pomc-Efnb3-OE male mice displayed greater body weight gain, associated with an increased fat mass compared to control mice. In order to identify the molecular mechanisms underlying such altered physiological outcomes, we performed a single cell RNA-sequencing experiment on sorted POMC neurons in both Pomc-Efnb3-OE and control mice.

This project aims to provide novel mechanisms underlying the control of energy and glucose homeostasis through POMC neurons activity.

OC.8. Ontogeny of the caudal neurosecretory system in zebrafish revealed by a transgenic (*uts2a: gfp)* line

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The caudal neurosecretory system (CNSS) is a neuroendocrine complex whose existence is restricted to fishes. In teleosts, the neuroendocrine neurons of the CNSS, called Dahlgren cells, are located in the terminal segments of the spinal cord, and project to a neurohemal organ, the urophysis, from which several neuropeptides, including urotensins, are released. Very little is currently known about the development of the CNSS. The aim of the present study was to study the ontogeny of this system by using the zebrafish (Danio rerio) as a model. For this purpose a Tg(*uts2a : gfp*) fluorescent reporter line was constructed, in which green fluorescent protein (GFP) expression is driven by the urotensin 2a (uts2a) gene promoter. As expected, the Tg(*uts2a : gfp*) line recapitulates faithfully the endogenous expression profile of the *uts2a* gene and hence allows us to visualize the Dahlgren cells. With this line, the first GFP^+ cells could be detected as early as the hatching period in the caudal spinal cord. A number of GFP+ cell processes appeared to project rostrally towards the brain, but most of them were targeted towards the urophysis anlage. The morphogenesis of the urophysis could be indirectly followed thanks to the accumulation of GFP^+ fibers within it. In order to visualize the neurovascular interface within the urophysis, a Tg(uts2a : RFP) line was crossed with a vascular endothelial reporter expressing GFP in kdr -positive cells. The study of this new line is currently under investigation. In conclusion, the present work provides the basis for further studies on the mechanisms of development of the CNSS in zebrafish.

Tostivint H, Girardot F, Parmentier C, Pézeron G. Biol Aujourdhui. 2022;216(3-4):89-103

Symposium Jeunes Chercheurs

Recherche translationnelle en Neuroendocrinologie

SJC.1. High Anti-Müllerian Hormone (AMH) at mini-puberty induces PCOS-like traits in the offspring

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Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder affecting up to 18% of women of reproductive age worldwide. The condition was originally classified as a reproductive disorder, being the most common cause of anovulatory infertility in women. Today it is well known that PCOS has other long-term health repercussions, including obesity, type-2 diabetes, and increased risk for cardiovascular disease (1). Despite the detrimental consequences on women's health, PCOS has no cure, thus the development of treatment options is an urgent need. It is well known that hyperandrogenism together with high circulating anti-Müllerian hormone (AMH) levels, two to three times higher in women with PCOS as compared to healthy women, are common features in PCOS women (2, 3). Altered intra-ovarian AMH signaling severely affects follicular growth, dominant follicle selection, and ovulation in PCOS. In addition, AMH levels are also higher during pregnancy in women with PCOS as compared to control women (4, 5), and exposure to high AMH levels in utero leads to PCOS-like traits in adult female mice (4, 6). Clinical studies have also shown that daughters and sons of women with PCOS display elevated AMH levels during mini-puberty (7). Based on these findings we wondered whether high AMH levels during mini-puberty might set the stage to develop PCOS in later life. To test this hypothesis, we exposed post-natal mice of both sexes to high AMH from p12 to p14, which corresponds to mini-puberty in mice. This treatment induces the appearance of reproductive and metabolic PCOS-like traits during adulthood. Overall, our study shows that exposure to aberrant AMH levels during mini-puberty is sufficient to induce long-lasting reproductive and metabolic defects in both sexes and highlight a new preclinical mouse model of PCOS that can be used in future mechanistic studies to dissect PCOS etiology.

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SJC.2. The role of dysregulated ghrelin/LEAP-2 balance in eating disorder: a translational study in anorexia nervosa

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Objectives. Anorexia nervosa (AN) is a multifactorial psychiatric disorder with social, psychological, genetic and environmental risk factors. Growing evidence suggest an uncovered role of metabolic factors such as appetite regulators in the physiopathology of AN, both as consequences of chronic under nutrition and as maintenance factors of the disorder [1]. The ghrelin system is a key regulator of appetite and food intake across species with elevated ghrelin levels during chronic undernutrition in rodents and patients suffering from acute AN. LEAP-2, a recently discovered ghrelin antagonist, appears to be up-regulated in obesity and opposes to the orexigenic drive of ghrelin [2,3]. The ghrelin/LEAP-2 ratio could be a novel interesting insight to reflect the regulation of appetite in AN. We provide the first translational study exploring the ghrelin/LEAP-2 regulation in long-term food restriction in both mice and in a longitudinal clinical cohort of patients suffering from AN.

Methods. Using a translational strategy, we compared the regulation of ghrelin/LEAP-2 ratio during food restriction and after refeeding 1/ in female mice exposed to a paradigm reproducing the environmental context of AN, combining physical activity with a running wheel and quantitative chronic food restriction during 14 days followed by 10 days of progressive refeeding (n=8/group); 2/ in an ongoing longitudinal study of patients with AN evaluated before and after 4 months of intensive refeeding during an inpatient rehabilitation program (n=23) as well as 6 months after hospital discharge. We measured longitudinal changes in concentrations of ghrelin and LEAP-2 in each individual at both time-points with ELISA immunoassays.

Results. 1/ Long-term food restriction in mice was associated with increased ghrelin levels ($p<0.001$), decreased LEAP-2 levels ($p=0.002$) and increased ghrelin/LEAP-2 ratio ($p<0.001$) compared to *ad libitum* fed controls. Refed mice had lower ghrelin levels ($p<0.001$) and higher LEAP-2 levels ($p=0.008$) compared to restricted state. The ghrelin/LEAP-2 ratio decreased with refeeding ($p<0.001$). 2/ Patients with AN displayed increased plasma levels of ghrelin on admission than after refeeding ($p<0.01$) but also higher LEAP-2 levels ($p=0.016$) with similar ghrelin/LEAP-2 ratio at both time-points. Longitudinal follow-up is ongoing to evaluate the clinical status of patients on a long-term scale (*i.e.* stable or unstable weight gain) with specific associated ghrelin/LEAP-2 ratio.

Conclusions. The metabolic regulation of appetite during food restriction favors an orexigenic drive of ghrelin as exhibited in food-restricted animals. This metabolic profile is reversed by refeeding. However, we provide clinical evidence that the ghrelin/LEAP-2 ratio is not regulated with nutritional status in AN, as it is in the case of a physiological adaptation to food restriction. This innovative transversal and translational paradigm enabled us to identify abnormal metabolic adaptation to nutritional status in patients with pathological eating. Proper adaptation of eating behavior to nutritional status is a key in the physiopathology of nutritional status and understanding the impact such dysregulation remains a critical challenge. Our future goal is to explore the link between metabolic alteration and clinical status in AN in order to identify biomarkers of remission useful in individualized care.

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SJC.3. Functional validation of oxytocin-like bacterial proteins produced by Lactobacillus species

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Oxytocin is a neuropeptide hormone with multiple physiological functions playing a key role in promoting social interactions. Deficient oxytocin signaling was associated with stress-related and autism spectrum disorders (ASD). Recently, gut microbiota was shown to be involved in the regulation of brain function and behavior but the molecular mechanisms remain largely unknown. Moreover, our recent studies showed that immunoglobulin G (IgG) plays a role in oxytocin carrier protein. In this study, we hypothesized that specific gut bacteria may produce bacterial proteins with molecular mimicry to oxytocin which can modulate oxytocin signaling via the production of oxytocin-reactive IgG and be relevant to ASD. Using a proteomic approach, we found that several Lactobacillus (L.) species display increased levels of oxytocin-like immunoreactivity as compared to E. coli. Then, using the mass-spectrometry, 3 potential oxytocin-like bacterial proteins have been identified in Lactobacillus salivarius. C57Bl6 mice have been immunized with the identified oxytocin-like recombinant Lactobacillus proteins resulting in increased plasma levels of oxytocin-reactive IgG. Moreover, increased oxytocin levels were found in the hypothalamus of immunized mice. Immunization of BTBR mice, an animal model of ASD, with the same bacterial proteins, resulted in an improvement of social behavior and a decrease of anxiety-like behavior and hyperactivity. The effect was sex-dependent, most of significant changes observed in males. We conclude that specific Lactobacillus strains may improve social behavior by synthesizing oxytocin-like antigenic proteins which stimulate the production of oxytocin-reactive IgG playing a role of oxytocin carrier proteins *i.e.* stabilizing and enhancing oxytocin signaling. These results open new possibilities for the ASD treatment.

SJC.4. Beneficial effects of chrononutrition on high fat diet-related memory impairments – a translational study

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The consumption of a diet rich in fat and sugar (HFHS) not only affects the metabolism and cardiovascular system but also the brain functions. The adolescence, is an age group specifically at risk of developing obesogenic-induced cognitive alterations given the brain maturation processes occurring over this age range. In rodent models, we demonstrated that the consumption of HFHS during adolescence affects hippocampal-related memory processes, whereas the same diet consumed at an adult age does not result in memory deficits. Fascinatedly, previous research has shown the beneficial effects of time-restricted feeding (TRF, food access during the active phase only) on both animals and humans' physiology and metabolism. Therefore, the present study aimed to test the effects of TRF on memory as well as food intake, hippocampal gene expression and dendritic spine dynamics in HFHS-fed mice during the peri-adolescence period. TRF prevented memory alterations in several hippocampal-related memory tasks in HFHS-fed mice. By using indirect calorimetry chambers, we found that HFHS-fed mice displayed a disrupted eating pattern characterised by an increased total calorie and food intake during the light/inactive phase, resulting into a phase-advance of food intake rhythm compared to normal chow-fed mice (NC). TRF on HFHS-fed mice restored this calorie/food intake phase shift, without reducing total food intake compared to *ad libitum* HFHS-fed mice. RNA sequencing analyses performed on hippocampal tissues revealed that around a thousand genes were found to be differentially regulated in *ad libitum* HFHS-fed mice during the consolidation of a memory task compared to NC-fed mice, with this differential gene expression being restored with TRF. Using *in vivo* multi-photon imaging within the hippocampus we found that the density of dendritic spines in pyramidal neurons was increased in *ad libitum* HFHS-fed mice and restored to NC levels by TRF in HFHS-fed mice. In order to fulfil the clinical purposes of this project, calorie intake and meal timing are currently being assessed in a cohort of adolescent patients with obesity over a 6-day digital food record, to assess the magnitude of food intake phase shift occurring among obese adolescents. Following this home-based assessment, TRF is being tested in the same cohort of patients undergoing 4 weeks of nutritional reeducation at a specialised clinic. The present translational study offers promising new insights on molecular, morphological, and behavioural changes induced by TRF in individuals with obesity.

SJC.5. Exocytosis is regulated by a subset of miRNAs in pheochromocytoma, a neuroendocrine tumor

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Pheochromocytomas (PCC) are rare neuroendocrine tumors of the adrenal medulla gland that secrete high levels of catecholamines into the bloodstream, leading to clinical manifestations such as hypertension. Catecholamine hypersecretion is caused by both the tumor burden and dysfunction of exocytosis, the final step of the regulated secretory pathway (RSP)[1]. However, the etiology of the hypersecretoty activity of PCC is poorly understood. Several studies suggest that miRNAs, as post-transcriptional inhibitors, play a role in the control of the RSP. To investigate this possibility, we developed first a bioinformatics workflow based on interaction (STRING-DB), prediction (mirDIP, miRabel[2]) and co-expression (oncomiR) analysis software. Applied to miRNA (GSE29742) and gene (GSE19422) expression data in PCC, this workflow allowed us to identify an interaction subnetwork in which 34 miRNAs and 46 genes, involved in the exocytosis process, are deregulated in PCC compared to the normal adrenal medulla. In order to determine the effect of these miRNAs in exocytosis, we developed a secretion assay based on a luminescent reporter (*h*GH1-Nluc) specifically expressed in the dense-core secretory vesicles of the rat PCC cell line (PC12-2luc). Thus, under resting and stimulated (Ba^{2+}) conditions, we observed that 16 of the 34 transfected identified miRNAs inhibit exocytosis activity (-30% to -71%). A kinetic analysis with miR-34a-5p, one of the most connected miRNAs of the interaction subnetwork, shows that its overexpression induces a dose-dependent inhibition of the secretion of the luminescent reporter both in resting conditions (-67%) and upon stimulation of PC12-2Luc cells (-39%). This finding confirms the inhibitory effect of miR-34a-5p on exocytosis. Experimental validation of miRNA:mRNA interaction at the transcriptomic level (RT-qPCR) is in progress. Characterization of this interaction subnetwork involved in the control of the RSP will provide new insight into the deregulation of secretion mechanisms in neuroendocrine tumors such as PCC.

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SJC.6. Chronic shifts impact the female mice reproductive axis, fertility and offspring development

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In female mammals, the reproductive function displays cycles whose regularity, depending on a functional circadian system, is an important parameter for fertility. Indeed, the preovulatory luteinizing hormone (LH) surge occurs at the transition between the resting and active period, at the end of the follicular phase, to promote successful fertilization by coordinating ovulation with the activity onset, when sexual arousal is maximal. The timing of the LH surge depends on both the positive feedback from estrogen produced by mature ovarian follicles and a circadian signal generated by the master circadian clock located in the suprachiasmatic nuclei (SCN) in the hypothalamus [1]. The synchronization of the hormonal and circadian signals is essential because light-induced disruption of circadian rhythms can negatively impact female reproductive function in both animals and humans [2].

In this context, we have investigated the consequences that a chronic exposure to shifted light/dark cycles could have on the female mammal's gonadotropic axis. Adult female mice were either kept in regular light/dark cycles or exposed to a model of shift work conditions (10-hour light phase advance for four days followed by 10-hour light phase delay for three days) during four weeks. The activity of the SCN vasopressin-containing neurons, the daily activity of the kisspeptin neurons located in the rostral preoptic area of the third ventricle, and the daily LH secretion during proestrus were then compared between both groups of mice.

Exposure to chronic shifts reduced the number of vasopressin neurons known to transmit the daily information to the kisspeptin neurons, abolished activation of kisspeptin neurons typically observed at the resting/active period transition and reduced the amplitude and altered the timing of the preovulatory LH surge. Furthermore, when female mice exposed to chronic shift were mated with a male for five days, they showed a decrease in fertility, observed by a reduced gestation rate, without any effect on the number of pups per litter, the birth weight of the pups and the mortality rate at 5 days after the birth. In contrast, male mice born to dams exposed to chronic shifts displayed increased body mass during growth and female mice showed delayed puberty with a delayed vaginal opening.

Chronic exposure to shifted light/dark cycles impacts the hypothalamic network regulation of the preovulatory LH surge in female mice, leading to fertility troubles and impairing offspring development. In future experiments, we will investigate whether peripheral clocks within the hypothalamic-pituitary-ovarian axis are also altered by chronic shifts. Altogether, these experiments will provide a better understanding of the central and peripheral mechanisms by which circadian disruption alters the female mammal reproductive function, and may pave the way for potential therapeutic interventions to reduce some of the negative effects of shift work on women's fertility.

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Posters

Communication Flash

P1. Phenotypic characterization of a novel mouse model of Prader-Willi syndrome with genetic invalidation of both *Magel2* and *Necdin*

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Prader-Willi syndrome (PWS) is a multigenic disorder caused by the loss of seven contiguous paternally expressed genes located in the human 15q11-q13 region (chromosome 7 in mice). Mouse models with inactivation of all PWS genes display 100% lethality within the first postnatal week and have, therefore, not been very helpful in understanding the physiopathology of this syndrome. Mouse knockout (KO) models for each candidate gene were then generated. Among them, Necdin- and Magel2-KO mouse models display distinct phenotypes mimicking part of the PWS clinical features. However, none of these single KO mice recapitulate all the symptoms observed in PWS patients, which is expected since the PWS phenotype is likely the result of multiple genes that are lost and usually interact with each other at the cellular and molecular levels. Consistent with this idea, preliminary and published data suggest that there is functional interaction and potential redundancy between *Necdin* and *Magel2*. In the present study, we used a newly generated double KO (DKO) mouse model to explore the effect of a combined deletion of *Magel2* and *Necdin*. Male and female DKO mice display normal body weights throughout postnatal life. DKO mice exhibit a lower body length at weaning that catches up during adulthood. Male, but not female, DKO mice have a lower fat mass and higher lean mass only at three months of age. A delayed onset of puberty is also observed in both male and female DKO mice. We are currently examining the effect of the combined loss of *Magel2* and *Necdin* on various behavioral outcomes (e.g., feeding behavior, social behavior, and cognition). Together, these data indicate that mice with a genetic deletion of both Magel2 and Necdin display a phenotype that surprisingly resembles that of single KO mice and still does not recapitulate the obesity of patients with PWS.

P2. Kisspeptin analog C6 treatment improve reproduction parameters in mice with hyperprolactinemia

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In mammals, kisspeptin is a major positive regulator of the hypothalamichypopohysogondial (HPG) axis, offering an attractive strategy for controlling reproduction. The Molecular Reproductive Neuroendocrinology team designed a kisspeptin-10 (Kp-10) analog, called C6, which has pharmacological advantages over endogenous Kp-10. In this study, C6 was tested in an infertile hyperprolactinemic mouse model to assess its ability to restore HPG axis function and ovarian cyclicity. These results suggest a partial restoration of ovarian cyclicity, LH secretion and the expression level of genes involved in reproduction by treating hyperprolactinemic mice every other day. The administration of C6 could thus be an alternative to women with hyperprolactinemia resistant to dopaminergic treatment.

P3. Ccl5 chemokine, a key regulator of neuroinflammation and type 2 diabetes associated with nutritional obesity

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Background: Obesity is a major public health problem impacting nearly 13% of the world population. The main cause of this disease is a deregulation of food intake resulting from an excess of lipids contained in a hyperlipidic diet. This high-fat-induced obesity is now considered as a low-grade chronic inflammatory state that has inhibitory effects on insulin action. It has recently been shown that obesity is associated with central inflammation in the hypothalamus, an important region of the brain, with the secretion of inflammatory cytokines (IL-1β, IL-6, TNF-α) and chemokines (CCL5).

Objective: In this work, we evaluated the role of the chemokine CCL5 in the pathophysiology of obesity and/or T2DM.

Experimental strategy: Our study was carried out on male mice invalidated for the CCL5 gene ($CCL5^{-/-}$ mice) or its receptor CCR5 ($CCR5^{-/-}$) and wild-type (control) mice. Animals were fed a standard diet (SD; 5% fat) or a hyperlipid-hypercaloric diet (HFD, high-fat diet; 40% fat) for 30 weeks. Blood, adipose tissues, liver, brain samples were collected and physiological tests were performed to explore metabolic parameters, glucose homeostasis and peripheral/central inflammatory response.

Results: First, we confirmed the presence and cell origin of CCL5 and its receptor CCR5 in the hypothalamus by *in situ* hybridization in control mice. Then, we showed that metabolic parameters and glucose homeostasis seem to be less disturbed in the invalidated mice compared to the control mice under HFD. Finally, we demonstrated that the invalidated mice under HFD show a lower peripheral and central inflammation than control mice.

Conclusion: These findings demonstrate that the absence of CCL5 and its receptor CCR5 seems to protect against the development of obesity and type 2 diabetes.

P4. The effect of time-related feed distribution on dromedary behavior and physiology under natural LD cycle conditions

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In natural environments, several environmental cues are known to can affect circadian rhythms. The impact of food distribution time on animal welfare has not been well studied for large mammals such as the dromedary. The aim of this study was to investigate the influence of time switching of feed distribution on the dromedary's locomotor activity, wake/sleep cycle, and rumination under natural conditions of the light/dark cycle. The study was conducted in two 21-day phases, during which food was distributed at either 10:00 in the morning or 22:00 in the evening. Behavioral and polysomnographic methods were utilized to record sleep, while activity was recorded using a validated scoring method. Video analysis revealed that during the first phase, dromedaries showed a diurnal activity pattern with significantly more activity during the day (8.8 hours) than at night (1.5 hours). However, when camels were fed at 10 pm, the rhythmicity of locomotor activity was disrupted and the animals shifted from a diurnal to a twilight activity pattern, with almost similar activity rates during the day (5.1 hours) and night (4.1 hours). Results showed that during the first phase, rumination was rhythmic, with a peak during the night (more than 5 hours) and less during the day (0.8 hours). The rhythmicity of rumination persisted even when feed was distributed at 10 pm, with a peak during the night (5 hours) and a slight increase during the day (1.5 hours). In addition, the total duration of rumination (6.6 hours) for 24 hours remained constant when the two phases were compared. Polysomnographic results obtained during the last three days of each phase showed that sleep architecture differed between the two phases, with an increase in wakefulness duration (6 hours) during the night in phase II with feeding à 10 pm compared with that (4 hours) of the first phase I (feeding at 10 am). Additionally, a slight decrease in overnight rumination duration in phase II (5.3 hours) was observed compared to phase I (6.2 hours), while total rumination duration remained the same (7.3 hours). Regarding sleep, REM sleep duration did not change between the two phases (0.7 hours). However, the drowsiness duration increased during the day (from 0.3 to 0.5 hours) and decreased during the night (from 2 to 1 hour) when comparing phases I and II, respectively. Finally, NREM sleep duration decreased from 1.6 hours during Phase I to 1.4 hours during Phase II. Time of feeding affects the physiology and sleep of the camel, a diurnal desert species. Feeding should be managed during daytime to avoid any disturbance of circadian rhythms and sleep.

Keywords: Dromedary, circadian rhythms, feeding, locomotor activity, rumination, sleep, polysomnography.

Communication Flash

P5. Long-term functional consequences of perinatal exposure to perfluoroalkyl substances

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In the rodent subventricular zone (SVZ), neural stem cells (NSCs) reactivate during postnatal development to produce new neurons and oligodendrocytes throughout life. SVZ-NSCs generate neuroblasts that migrate towards the olfactory bulbs where they differentiate into mature interneurons, that participate to olfactory behaviors. Furthermore, few NSCs give rise to oligodendroglial precursor cells (OPCs) that migrate toward the surrounding white matter, notably the corpus callosum, where they differentiate into myelin-forming oligodendrocytes. We previously demonstrated that the SVZ, especially during early postnatal window, is a key brain region sensitive to developmental endocrine disruption that may induce long-term functional consequences in the adult.

PFAS are a group of synthetic and persistent chemicals found in our daily environment from the early development to aging. Perinatal exposure to these persistent organic pollutants is of particular concern because of their well-established risks on human health, notably by promoting infertility, metabolic disorders and cancerogenesis. Due to their hydrophobic features, our hypothesis is that PFAS may accumulate in lipid-rich structures such as myelin, thus impairing myelin function.

Our aim was to assess whether a perinatal exposure to perfluorooctane sulfonate (PFOS) and/or perfluorooctanoic acid (PFOA) could alter neurodevelopment and myelin formation. To this end, pregnant mice were exposed to either PFOS or PFOA (20mg/L) *via* drinking water during late gestation and lactation (from E15 to P21). Mass spectrometry analysis revealed that PFOS, and to a lesser extent PFOA, accumulated in the myelin sheath of P21 pups. Characterization of SVZ-derived neuro-gliogenesis showed that PFOS, but not PFOA, i) decreased cell proliferation, ii) promoted SVZ-oligodendrogenesis at the expense of neurogenesis and iii) blocked OPC differentiation and maturation in the corpus callosum. Moreover, we examined whether perinatal and transient exposure to PFAS could have long-term functional effects in adult mice. To this end, we demonstrated that olfactory memory is impaired in PFOS- and PFOA-exposed mice, suggesting that PFAS permanently affect the production of new olfactory interneurons. However, the deleterious effect of PFAS on olfactory memory was more pronounced in mice exposed to PFOS. Of note, motor coordination was not affected by either PFOS or PFOA. Further, we will examine the long-term effects of PFOS and PFOA perinatal exposure on neurogliogenesis and myelin integrity in adult mice using transmission electron microscopy. Altogether, our data demonstrate that perinatal exposure to PFOS, but not PFOA, disrupts neurodevelopment with irreversible functional consequences later in life, even in absence of continued exposure.

Communication Flash

P6. Metabolic and neuroinflammatory consequences of hypothyroidism in two mouse strains with different metabolic adaptability capacities, the C57BL/6J and the WSB/EIJ strains

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Thyroid hormones (TH), among their pleiotropic actions, play a central role in the regulation of metabolism and in cognitive functions. Indeed, hypothyroidism is associated with a decrease in energy expenditure and lipid metabolism, and an impairment of memory. Metabolic deregulations induced by a high fat diet (HFD) generate peripheral inflammation, which promotes the development of neuroinflammation in various brain regions. This inflammatory state can disrupt neuronal homeostasis, leading to the alteration of synaptic plasticity and to memory disorders. Furthermore, a link between hypothyroidism and the development of neuroinflammation has previously been shown, particularly in the hippocampus, a brain structure rich in TH receptors.

Our objective was to evaluate whether metabolic deregulations induced by hypothyroidism favor the development of neuroinflammation and thereby promote memory deficits. We compared the response to induced hypothyroidism in two mouse strains, the wild-derived WSB/EiJ mouse strain characterized by an obesity resistance due to its high metabolic flexibility phenotype and the C57BL/6J mice, prone to HFD-induced obesity. Adult mice were fed with a low-iodine diet supplemented with 6-n-propyl-2-thiouracyl (PTU) for 7 weeks to induce hypothyroidism.

Our results show that hypothyroidism, characterized by a decrease in serum T4 levels, led to metabolic deregulations, as an alteration of lipid metabolism in the liver of both strains. However, the decrease in hepatic lipid synthesis was compensated in WSB/EiJ mice by a mobilization of lipid reserves from white adipose tissue, but not in the C57BL/6J mice. No peripheral inflammatory response to hypothyroidism was observed in both strains. In the hippocampus of C57BL/6J mice treated with PTU, the decrease in intracellular T3 availability was accompanied by an activation of glial cells, a hallmark of neuroinflammation, associated with an impairment of spatial memory. In contrast, no inflammatory response was observed in the hippocampus of WSB/EiJ mice, which appeared to maintain their thyroid status by locally increasing T3 availability via compensatory mechanisms.

Our results shed the light on the fact that serum thyroid status does not always reflect central thyroid status. Moreover, they demonstrated that the described link between hypothyroidism and neuroinflammation does not seem to be the consequence of metabolic deregulations induced by hypothyroidism but rather of an unbalance in the central thyroid signaling. Thus, our results emphasize the importance of maintaining central thyroid homeostasis to protect against the development of neuroinflammation, and in extension, of neurodegenerative diseases, given that neuroinflammation favors cognitive and memory impairments.

P7. Impact of a moderate reduction in protein intake on eating behavior and neuronal activation in the dorsal vagal complex and ventral striatum in rats

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Context and objectives - The adjustment of dietary protein intake to requirements is ensured by a very fine brain control that is still poorly understood. It includes the detection of protein intake that relies on essential amino acids (EAA) detection in several part of the brain and integrates protein status and metabolism (Heeley and Blouet 2016) to modulate eating behaviour in consequence. In a recent study, we have shown that a moderate reduction in the protein content of the diet induces hyperphagia and increases the appetence for protein (Champeil-Potokar et al 2021). The hyperphagia reflects the body's need to obtain amino acids whatever the excess in caloric intake. There is no consensus on this so-called "protein leverage" effect, but it may constitute an increase in the risk of obesity when individuals reduce their protein intake by switching to low animal protein diets. It is therefore important to better understand the impact of a low protein diet on the activation of brain areas involved in satiety (dorsal vagal complex, DVC), and reward (Nucleus Accumbens (NAcc) and Olfactory Tubercle (OT)). The aim of the study was to analyse the neuronal activation (c-fos) induced by a low protein (LP) meal or a high protein (HP) meal in the nucleus of the tractus solitarii (NTS), the NAcc and OT, of rats chronically fed with a low protein diet (LP) or with a normal protein diet (NP).
Methods - Six week-old rats were fed a 6% protein (in percent of energy) diet (LP rats, n=16) or a 20% protein diet (NP rats, n=16) during 2 months. The composition of the 2 diets was similar for lipids (6% of energy as soy oil), fibers, vitamins and minerals; protein source was casein and its variation was compensated by maize starch. LP and NP diets were isocaloric. Rats growth and daily food consumption were followed, and their appetence for protein determined in dietary tests allowing self-selection between pellets containing 6% (LP), 20% (NP) or 55% (HP) protein. Two hours before sacrifice, and after 4h of fasting, rats were given a 5g meal of LP or HP pellets. At sacrifice, brains were fixed, frozen and sliced for immunohistochemistry of neuronal activation (c-fos) in reward areas (NAcc and OT) and in the brain stem DVC to evaluate the impact of the meal. The DVC was also immunolabeled for glia to evaluate microglial activation in the area postrema and the deployment of the astroglial barrier between the AP and NTS.
Results - Reduced protein intake in LP rats induced an increase in food consumption (+24%, $p<0.01$) leading to an increase in body weight (+5%, $p<0.01$) and visceral adiposity (+30%, $p<0.01$) compared to NP rats. Choice tests showed a strong ($p<0.001$) dietary and olfactory preference for high protein (HP) pellets over LP or NP pellets in LP rats as well as in half of the NP rats (NP2). The other half of NP rats (NP1) did not show a preference for HP pellets. Consumption of a meal of HP pellets increased c-fos neuronal activation in the ventromedial region of the NTS as compared to LP pellets whereas LP or HP meal induced similar c-fos activation in the NAcc of LP or NP rats. c-fos activation in the medial OT (encoding the positive valence of odors) tended to increase in LP rats in response to HP vs. LP meal (+38%, $p=0.10$), and in NP2 vs. NP1 rats (+44%, $p=0.10$ in response to LP or HP meal).
Conclusion - Our results show that meal-induced neural activation in the satiety and reward regions varies with meal protein content, animal protein status, and individual appetence for protein.

Champeil-Potokar G.; et al.; J Nutr. 2021 May, 151, 1311-1319 Heeley N.; Blouet C.; Front. Endocrinol. 2016, 7, 148(1-11)

P8. Annexin A1 as tanycyte signal for tanycyte/neuron communication

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Energy balance requires accurate crosstalk between the periphery and the central nervous system. In the hypothalamus, specialized ependymoglial cells called tanycytes line the walls and the floor of the third ventricle and extend their processes into the brain parenchyma. Thanks to their strategic location, tanycytes integrate peripheral signals about the metabolic state and modulate neuronal function accordingly. However, how tanycytes communicate with neurons remains largely unknown.

Our pilot experiment highlighted Annexin A1 (ANXA1) as a candidate tanycyte signaling molecule modulating hypothalamic neuronal activity and gene expression in response to energy balance. Here, we show that ANXA1 is expressed along the third ventricle in the mediobasal hypothalamus, mainly by dorsal tanycytes and typical ependymal cells. Interestingly, mice challenged with a fasting-refeeding paradigm display an increase in tanycytic ANXA1 expression during refeeding. In contrast, ANXA1 expression decreases in mice fed a high-fat high-sucrose diet for eight weeks. Our *in vitro* experiment also demonstrates that dexamethasone and glucose levels regulate ANXA1 expression and subcellular localization. Notably, ANXA1 colocalizes with the CD9 marker in the presence of glucose, suggesting an exosomal secretion pathway. Finally, intracerebroventricular injections of ANXA1 N-terminal active peptide induce cFos cellular activation in hypothalamic neurons and reduce food intake.

Our results suggest that tanycytic ANXA1 expression is regulated by the metabolic and/or inflammatory status and can induce neuronal activation in the hypothalamus. This study constitutes the first step in our effort to understand how tanycytes modulate neuronal function and energy balance.

Communication Flash

P9. The regulation of glucose and energy homeostasis by a hypothamalic subpopulation of neurons, expressing 26RFa and orexins

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26RFa (QRFP) is a biologically active neuropeptide known to promote feeding behaviour and to exert both a central and peripheral antihyperglycemic effect that is associated with an increase of insulin production by the pancreatic islets. Recently, we found that the hypothalamic 26RFa-expressing neurons are key relays of the central insulin action allowing the regulation of glucose homeostasis.

To go further, we investigated, in the present study, the effect of a chemogenetic activation of the 26RFa-expressing neurons in the Lateral Hypothalamic Area (LHA), in 26RFa deficient and 26RFa-expressing mice. Surprisingly, we found that the chemogenetic stimulation of the 26RFa neurons induces prohyperglycemic and anorexigenic effects, opposite to those of a central administration of 26RFa. The same effects were found in 26RFa deficient mice, suggesting therefore that another peptidergic system in these neurons is responsible for this reduction of glucose tolerance and food intake. Interestingly, double labelling RNAscope® experiment revealed that a subpopulation of 26RFa neurons in the LHA also express the orexins, another orexigenic neuropeptides. However, we recently found that a central injection of orexin induces an antihyperglycemic effect similar to that observed with 26RFa. Interestingly, the chemogenetic activation of 26RFa neurons also decreases the mRNA expression of both the 26RFa and orexin systems, thus explaining the suprisingly prohyperglycemic and anorexigenic effects, induced by the activation of this neuronal population.

To conclude, the present data promotes the first evidence that the neuronal networks regulating glucose and energy homeostasis involve the 26RFa/orexin-expressing neurons of the LHA. In addition, our data reveal that 26RFa and orexin systems are down-regulated when these LHA neurons are activated, inducing therefore a prohyperglycemic effect. In agreement with the observation that orexin neurons are sensitive to low blood glucose, a hyperglycemia could conversely inhibit this subpopulation of neurons, leading to the activation of 26RFa/orexins neuropeptidergic systems, ensuring therefore the maintenance of energy and glucose homeostasis.

El Mehdi M.; Takhlidjt S.; Devère M.; Arabo A; Le Solliec MA.; Maucotel M.; Bénani A.; Nedelec E.; Duparc C.; Lefranc B.; Leprince J.; Anouar Y.; Prévost G.; Chartrel N.; Picot M. Diabetologia 2022, 65(7):1198-1211

Communication Flash

P10. Intranasal effects of an analogue of the gliopeptide ODN on mice food-intake and hypothalamic POMC expression

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Compelling evidence shows that endozepines, including DBI and its processing fragment ODN, contribute to the regulation of energy balance by driving a critical relay in brain glucose sensing [1]. Produced by glial cells, ODN exerts a potent anorexigenic effect by targeting hypothalamic pathways via the activation of a still unknown G protein-coupled receptor (GPCR) [2]. Preliminary investigations revealed that acute intranasal (i.n.) administration of OP (2 µg), an ODN-derived analog, induces a significant reduction in food intake in fasted mice 30 minutes after their treatment. Co-instillation of OP with the ODN-GPCR antagonist, cyclo[DLeu^5]OP, nullified the OP-induced reduction in food intake.

Since i.n. administration of OP mimics the anorexigenic effect of an intracerebroventricular (i.c.v.) administration of ODN, we herein studied the distribution of c-Fos labeling in the hypothalamus of mice fed a standard diet. Animals instilled with OP (2 µg) displayed a strong increase in c-Fos labeling as early as 30 minutes after i.n. treatment in the paraventricular, arcuate and ventromedial nuclei that remained significant 60 minutes post-treatment. Similar c-Fos labeling was previously reported in the same hypothalamic areas after i.c.v. injection of OP/ODN. Moreover, we showed that i.n. administration of OP (20 µg) in fasted mice significantly enhances the expression of proopiomelanocortine, supporting that OP application led to action potentials discharge and activation of POMC neurons.

Finally, to determine whether the anorexigenic effect of OP is partially due to visceral discomfort associated to the i.n. route of administration, a conditioned taste aversion assay was performed in normally fed mice. No significant differences in saccharin (0.3%) consumption between OP-treated and vehicle-treated animal was observed, unlike mice that received an intraperitoneal injection of LiCl. These results indicate that the reduction in food intake observed in OP-instilled mice was not due to visceral malaise.

In summary, the present preliminary results show that OP i.n. instillated activates the same hypothalamic areas and the same molecular target as through i.c.v. injection and that its i.n. administration does not display any aversive component. Taken as a whole, these data strengthen the therapeutic potential of the ODN system to treat human eating disorders. Supported by Inserm (U1239) and the Région Normandie.

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P11. Sex-dependent hypothalamic response in a diet-induced obesity murine model

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The hypothalamic inflammation is a key pathophysiological mechanism that links chronic consumption of lipids to the development of obesity and associated metabolic complications (Lee *et al*., 2020). However, the sex-dependent effects remain poorly studied. Therefore, we aimed to evaluate the sex-dependent hypothalamic response to a high fat diet (HFD) in mice.

Male (M) and female (F) C57BL/6 mice received a standard diet (SD) or HFD (60% kcal from fat) for 14 weeks (W14, n=12/group). The hypothalamic mRNA expression of neuropeptides involved in the regulation of food intake (NPY, AgRP, POMC) and inflammatory markers (IL-1β, TNFa, IL-6, iNOS, FIZZ-1, CD11b, IBA-1, P2RY12) was measured by RT-qPCR. In addition, immunostaining directed against IBA-1 and GFAP were performed in the arcuate nucleus. Results were compared with *t*-test or Mann-Whitney tests.

Both M-HFD and F-HFD mice exhibited a decrease in the mRNA levels encoding NPY and AgRP ($p<0.05$ and $p<0.01$, respectively) whereas the mRNA level encoding POMC was only increased in F-HFD mice ($p<0.05$). The genic expression of CD11b was increased in F-HFD mice ($p<0.05$) whereas the iNOS expression was down-regulated in M-HFD mice ($p<0.01$). The ratio IBA-1/P2RY12 was increased in M-HFD mice ($p<0.05$) while it remained unaffected in F-HFD mice. Finally, preliminary results of immunostaining suggest a reduction of IBA1+ cell number in F-HFD mice ($p<0.01$).

Our data reveal a sex-dependent hypothalamic response to the high fat diet. The underlying mechanisms and consequences need to be further studied.

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Lee, C.H., Suk, K., Yu, R., Kim, M.-S. Cellular Contributors to Hypothalamic Inflammation in Obesity. Mol Cells 2020, 43, 431–437

P12. Molecular target characterization of intranasally bioactive anorexigenic neuropeptide ODN analogue

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Obesity is an epidemic that threatens global well-being as it is so common among the world's population that it has now overtaken undernutrition and infectious diseases as the most significant contributor of ill health. It is eminent that we find therapeutics and alternatives to tackle this world health crisis. In this context, our team in Rouen found octadecaneuropeptide (ODN) and its analogue (OP) have been able to reduce the food intake and increase weight loss in animal models. This anorexigenic effect of ODN/OP is mediated through activation of a still unidentified G Protein Coupled Receptor (GPCR) present at least in glial cells. It is to be noted that, thus far, only leptin sensitive neuronal populations (AC and NTS regions of brain regions) have been known to orchestrate energy homeostasis and weight loss. Therefore, this unexplored glial mechanism of action of ODN/OP through an unidentified GPCR has high therapeutic values. Not to mention, more than three fourth of currently marketed drugs are targeted through GPCRs. Thus, the major focus of this study is to identify and to functionally characterize the receptor in an *in vitro* model. The gliopeptide ODN is an endozepine (EZ) and are ligands for the benzodiazepine (BZ) binding sites. It is known for its micromolar negative allosteric modulation for $GABA_A$ R to induce its well documented anxiogenic effect. However, released by astrocytes, ODN stimulates astrocytes activity at nanomolar concentrations to induce intracellular calcium release and this autocrine effect is attenuated in the presence of pertussis toxin, a known inhibitor of G proteins. Our team have therefore developed an antagonist for this ODN-GPCR, $cyclo_{(1-8)}[DLeu^5]OP$. Using this ODN-GPCR antagonist, we demonstrated that the anorexigenic effect of ODN or OP is mediated by this ODN-GPCR. However, we have recently demonstrated that OP is ineffective in food intake and weight loss in the presence of leptin antagonist or in leptin deficient ob/ob mice indicating a strong co-correlation of leptin mediated pathways in this ODN-GPCR induced anorexigenic effect. To reiterate, this unexplored mode of action of ODN/OP via ODN-GPCR may pave paths to alternative therapeutics for obesity and energy homeostasis related disorders. Briefly, we aim to identify the molecular target (ODN GPCR) of ODN/OP in the mouse brain. With prior experience in identifying a GPCR for an ant venom peptide using a GPCR library; we performed a GPCR screening for ODN using TANGO-GPCR library. In addition, we have also utilized inter-disciplinary based approaches using chemistry and molecular biology for the pursuit of identifying the GPCR, in which, a tailor-made tag (Cy3) borne by ODN/OP (probe) was covalently transferred to the ODN-GPCR in cultured astrocytes for its subsequent identification. We first synthesised the probe and functionally tested it against rat astrocytes by proliferation assay, ELISA assay (MCP-1) and BCL-2 expression by western blot analysis. We have also tested the effect of the probe in its well-known neuroprotective effect of ODN in 6-OHDA induced toxicity of rat astrocytes by qRT-PCR of Caspase-3. Next, we have used Cy3 antibodies to detect the transfer of Cy3 from the probe to the ODN-GPCR using fluorescent microscopy and western blot. In summary, our preliminary data indicate that we are one-step closer to identifying the ODN-GPCR or one of its partners.

P13. The unexpected molecular mechanism of action of Chromogranin A in neuroendocrine secretion

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In neuroendocrine cells, neurohormone secretion is supported by secretory granule exocytosis upon cell stimulation. Chromogranins are a family of regulatory glycoproteins at the crossroads between many steps of neurohormone secretion, such as neuropeptide aggregation and sorting but also secretory granule biogenesis occurring at the level of the Golgi. We recently demonstrated that, among the members of this family, chromogranin A (CgA) interacts with a Golgi membrane phospholipid called phosphatidic acid (PA), through a PA-binding domain (PABD), thereby facilitating membrane remodeling and deformation at the origin of secretory granule formation (Carmon *et al*., 2020). A recent study demonstrating the role of CgA in the fusion pore expansion, which controls the last step of secretory granule exocytosis and then neurohormone release (Abbineni *et al*., 2019), our current research aimed to investigate whether PA could be the membrane molecular partner of CgA in the regulation of neurohormone secretion. Using confocal microscopy and transmission electron microscopy to analyze plasma membrane sheets, we observed the colocalization of CgA with membrane proteins of secretory granules, dopamine b-hydroxylase and vesicle-associated membrane protein 2, at exocytotic sites, indicating that CgA interacts with secretory granule membrane until exocytosis. To analyze the ability of CgA to interact with PA during exocytosis, we used total internal reflection fluorescence microscopy, which allows the tracking of plasma membrane dynamic events, and we observed COS7 cells overexpressing fluorescent either wild type CgA (WTCgA) and PABD-deleted CgA (CgAΔPABD). Interestingly, PABD deletion resulted in a significant reduction of the duration of CgA release coupled to an increase of the number of exocytosis events, suggesting that CgA controls fusion pore opening through its interaction with PA. Furthermore, we analyzed neurosecretion of chromaffin cells using carbon fiber amperometry to finely measure catecholamine release after CgA-GFP and CgAΔPABD-GFP overexpression. In a preliminary study, we observed that WTCgA, but not CgAΔPABD, overexpression led to a higher catecholamine charge of secretory granules and to an increased time to reach maximal catecholamine release, suggesting that CgA improves neurosecretion through its interaction with PA. The current use of newly synthesized fluorescent PA could help to detect and analyze CgA/PA interaction and its role in neurosecretion dynamics in living neuroendocrine cells. Moreover, CgA being expressed in hypothalamic cell lines and in some murine hypothalamic neurons located in areas controlling glucidic homeostasis, we plan to study the impact of targeted CgA gene invalidation on neurohormone secretion, and its potential impact on mouse metabolic phenotype.

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P14. Transgenerational alterations of energy balance caused by a mixture of endocrine disrupting chemicals in rats

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The prevalence of obesity has been rising worldwide for several decades. Obesity is associated with multiple risk factors, such as a lack of physical exercises, unbalanced diet, but also genetic or environmental factors such as developmental exposure to endocrine disrupting chemicals (EDC). Our recent data have indicated that transgenerational exposure to a mixture of EDC disrupted the hypothalamic control of puberty and reproduction in F3 female rats. The aim of the current study is to characterize the effects of a transgenerational exposure to such mixture of EDC on energy balance in male rats. Wistar dams were orally exposed to a mixture of 13 anti-androgenic or estrogenic EDC at environmentally relevant doses 2 weeks before mating, during gestation and lactation. Third-generation males (F3) were then exposed to a high-fat diet (HFD, 45% fat) between 3 and 6 months of age (n=14 for standard diet controls (CNN) and EDC (ENN), n=13 for HFD controls (CHFD) and EDC (EHFD)).
F3 males ancestrally exposed to EDC showed a significantly higher body weight than the control group at 3 months of age, before exposure and throughout the HFD treatment period. This increased weight gain (Mean body weight ± SD: CNN: 570.2 ± 24.1 g; CHFD: 593.5 ± 29.3 g; ENN: 628.5 ± 38.1 g; EHFD: 630.5 ± 48.1 g) was associated with a significant increase in food intake (CNN: 34.7 ± 0.8 g; CHFD: 21.9 ± 1.1 g; ENN: 36.7 ± 3.02 g; EHFD: 25.8 ± 4.4 g). Consistently, the increase in the ratio of gonadic white adipose tissue weight over body weight (WATg) (Mean WATg weight ± SD: CNN: 0.011 ± 0.001 g; CHFD: 0.013 ± 0.002 g; ENN: 0.013 ± 0.003 g; EHFD: 0.014 ± 0.002 g) and average adipocyte size (Mean adipocyte size ± SD: CNN: 3051 ± 369 µm²; CHFD: 4020 ± 627 µm²; ENN: 4507 ± 341 µm²; EHFD: 4304 ± 1127 µm²) was affected by EDC and HFD exposure. F3 males ancestrally exposed to EDC and exposed to HFD showed a significant decrease in testicular weight (Mean testicular weight/ body weight ± SD: CNN: 0.0033 ± 0.0004 g; CHFD: 0.0033 ± 0.001 g; ENN: 0.0027 ± 0.0005 g; EHFD: 0.0028 ± 0.0003 g) and FSH plasma levels (Mean FSH levels ± SD: CNN: 3.42 ± 0.35 ng/ml; CHFD: 3.73 ± 0.97 ng/ml; ENN: 3.21 ± 0.54 ng/ml; EHFD: 3.10 ± 0.28 ng/ml) although testosterone, LH levels and sperm count were not affected.

In conclusion, transgenerational exposure to a mixture of EDC worsened the obese phenotype of F3 male rats induced by HFD. We hypothesize that such effects could be explained by a disruption of hypothalamic circuits controlling food intake.

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P15. Impact of nighttime light pollution on female metabolism in a diurnal animal model

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Circadian rhythms are cyclic changes close to 24 hours, which allow the adaptation of the biological functions of an individual to the predictable variations of its environment. Metabolic functions are closely related to these rhythms through reciprocal interactions (1). Humans are increasingly exposed to night light pollution, in particular workers with atypical schedules. Experimental and epidemiological studies indicate that shift work has a negative impact on general health (2) and notably it increases the risk of metabolic disorders (3). However, during shift work, it is still difficult to separate the direct impact of light at night (LAN) from the impact of desynchronization of the circadian system. Also, some studies show gender differences in relation to light pollution (4).

The objective of this project is to evaluate the metabolic impact of exposure to LAN or chronic shift work in female diurnal rodents, *Arvicanthis ansorgei*, whose daily temporal organization is close to that of women.

A cohort of female *Arvicanthis* (n=24) was tested for glucose tolerance and monitored in metabolic cages (food intake, locomotor activity) during the baseline condition (standard cycle of 12 h light and 12 h dark (LD)), the first week of exposure, and after one month of exposure to LAN (n=8), chronic jetlag (n=8), or the control condition (standard LD, n=8). The weekly jetlag protocol corresponds to 10-h phase delayed LD for 4 days then 10-h advanced LD for 3 days (corresponding to standard LD) and the weekly LAN protocol to 4 days with 6 h of light added during the middle of the night followed by 3 days in standard LD.

During the first experimental week (acute effect), exposure to LAN or jetlag protocol led to differences in eating behavior between the different protocol days. Indeed, in jetlag, the number of daytime meals and the percentage of daytime meals decreased throughout the week even when returning to standard LD. In the LAN protocol, the number of night meals and the percentage of night meals increased progressively during the days with light during the night and then decreased when back to standard LD. At the end of the first week, daytime activity was reduced in jetlag group compared to the control group.

After one month of exposure (chronic effect), analyses were performed on the last day of the week protocol when all animals were exposed to standard LD (during at least 3 days). Individuals exposed to chronic jetlag displayed decreased glucose tolerance, increased fasting blood glucose and increased nighttime food intake, compared to the control group but also to their own baseline condition. In contrast, individuals exposed to LAN did not show detectable effects on these parameters. These results suggest that chronic jetlag exposure has greater deleterious effects on glucose metabolism and eating behavior than chronic LAN after one month.

Further research will focus on the neuroendocrine mechanisms underlying the metabolic and circadian perturbations at the central and peripheral levels, and on the impact of chronic LAN and jetlag on female fertility.

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P16. Early exposure of zebrafish (Danio rerio) embryos to the endocrine disrupters clotrimazole and ethinylestradiol: long-term toxicological consequences on neuroplasticity and behavior

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Estrogen receptors are widely expressed in the brain of many species and experimental results highlighted the effects of estradiol (E2) on neuronal plasticity and behavior. The brain is therefore a prime target for endocrine disruptors (EDs) interacting with estrogen signaling. In our study, we focused on 2 EDs that are widely used in the pharmaceutical industry, accumulate in aquatic environments at concentrations up to 1ng/L, and contaminate living organisms: i) Ethinylestradiol (EE2), a potent estrogen receptor agonist, often found as the main bioactive molecule in contraceptive pills and ii) Clotrimazole (CLO), a broad-spectrum antifungal agent used in the topical treatment of dermatological and gynaecological infections and known to inhibit key enzymes of steroidogenesis, including aromatase that converts testosterone into E2. While acute exposure to these 2 chemicals during development in zebrafish is known to affect several neurobiological parameters, including the estrogen-dependent brain aromatase expression and activity, the long-term effects have not been investigated. Our objectives were therefore to better understand the long-term impact of early exposure to EE2 and CLO on zebrafish brain, focusing on the proliferation and differentiation of dopaminergic neurons and behavior.
Embryos were exposed to 4 ng/L of EE2, 250 µg/L of CLO, the vehicle 0.01% DMSO as solvent control, or water (negative control), from 2 to 120 hours post-fertilization. Fishes were then maintained under standard housing conditions until they reached adulthood. Spatial memory, associative learning capacity by classical conditioning, exploratory abilities, anxiety, sociability and aggressiveness were sequentially investigated in 3 months adults before euthanasia and head sampling. Initial analyses performed on individual behavioral variables showed trends towards increased exploration in EE2 and CLO-exposed male fish. We then subjected the entire dataset, including 22 behavioral variables, to a supervised classification model (random forest) which led to the selection of 10 variables that contributed most to differentiating the treatments while reducing redundancy. A Principal Component Analysis showed that the treatments separated well along axis 1 (EE2 and CLO treatments vs. solvent and negative controls), explained by 4 variables related to the level of swimming activity and exploratory abilities. A Kruskal-Wallis test highlighted a significant increase in these traits in males exposed to EE2 but not in females. Following behavioral analysis, immunofluorescence was performed on olfactory bulbs, telencephalon, preoptic area, and hypothalamus sections to determine the potential effects of EE2 and CLO on proliferative activity (mouse monoclonal antibody directed against human proliferating cell nuclear antigen-PCNA), and on dopaminergic neurons (rabbit polyclonal antibody directed against tyrosine hydroxylase-TH). We observed a significant increase in cell proliferation in CLO and EE2-treated fish compared to solvent controls in the dorsal telencephalon, in both males and females. Besides, the average number of TH+ cells in the telencephalon was significantly lower in CLO-treated fishes compared to solvent controls and no significant effect of EE2 was observed. Altogether, we show long-term behavioral alterations upon early exposure to EE2 and CLO which may be explained by regional effects on neurogenesis and dopaminergic system. This work highlights the need to further document their effects on cellular and molecular mechanisms that orchestrate behavior.

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P17. Role of the hepatic oestrogen receptor alpha and the hepatokine LECT-2 in the liver-brain axis and the hypothalamus regulation

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The energy homeostasis requires communication between the hypothalamus and peripheral organs. The latter send messages about their metabolic status to the hypothalamic arcuate nucleus, which integrates these signals. In this context, this access of signals to the arcuate nucleus is a key step in the regulation of the energy balance. The modulation is done in part via the fenestrated vessels of the Median eminence - arcuate nucleus interface (ME-ARH) whose permeability is regulated after a 24-hour fast and according to the animal's oestrous cycle, linking oestrogens, energy metabolism regulation (1). A player in this permeability could be the liver, with its expression of the alpha receptor (ERα) which regulates the metabolism as well as the secretion of hepatokines. We showed that the deletion of that receptor in female mice is enough to blunt the nutritional-dependant permability of the ME-ARH. From these results, we conclude that hepatic ERα is involved in energy metabolism regulated by the liver-brain axis and that among the different molecules secreted by the liver under the influence of oestrogens, there is a factor allowing the modulation of the permeability of ME-ARH. We hypothesis that the factor is the hepatokine LECT-2, known with its receptor TIE1 to regulate the fenestration of the liver's sinusoidal endothelial cells, more specifically they are able to increase the capillarisation (meaning less fenestrations) (2). The fenestrations in the liver and in the ME-ARH being similar, we aim here to elucidate if LECT-2-Tie1 could explain the phenomenon observed in our female KO mice.

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P18. The combined effect of perinatal TBBPA exposure and adulthood Western diet on metabolic control and central inflammation

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The growing obesity rate is not only the result of sedentary lifestyle and Western high fat high sucrose (HFHS) diet, but environmental exposure to endocrine disrupting chemicals (EDCs) may also contribute to this global phenomenon. Some EDCs called obesogens target adipose tissue and its function as well as central pathways regulating food intake and energy expenditure (1). Moreover, since obesity contributes to central inflammation, which can lead to neurodegeneration (2), we studied the combined effect of perinatal exposure to an EDC and adult life HFHS diet on metabolic health and the inflammatory status of the brain. Organisms are especially vulnerable to EDCs during the perinatal period when hormonal signaling orchestrates the setup of endocrine axes (3). We studied the widely used brominated flame-retardant tetrabromobisphenol A (TBBPA), which perturbs thyroid hormone signaling (central player in metabolic control) and acts as an obesogen, promoting adiposity, as shown in *in vitro* experiments and in zebrafish larvae (4,5).

Pregnant dams received 10 mg/kg/d TBBPA or vehicle for 4 weeks (last week of gestation through end of lactation). The progeny followed a HFHS diet from 2 to 6 months of age. We had four study groups of C57BL/6J male mice: vehicle+control diet, vehicle+HFHS diet, TBBPA+control diet and TBBPA+HFHS diet. The perinatal TBBPA exposure did not affect the circulating thyroxin levels at adulthood, but did increase the weight gain with both control and HFHS diets. The perinatal TBBPA exposure perturbed also other metabolic markers such as the circulating insulin and highdensity lipoprotein (HDL) levels. To study the consequences of the TBBPA exposure and HFHS diet at the central control level, we studied the inflammatory status of the brain (hypothalamus and hippocampus) and the total transcriptome with spatial resolution using the Visium 10x Genomics technology in the hypothalamus. We focused on two hypothalamic nuclei deeply involved in metabolic regulations, the arcuate nucleus and the paraventricular nucleus (controlling thyroid axis).

The spatial transcriptomics results allow us to determine the molecular pathways specifically affected by the different treatments in each hypothalamic nucleus involved in the central control of metabolism. In parallel, we were able to measure the combined effect of a perinatal exposure to an EDC and adulthood HFHS diet on the inflammatory status of the brain, especially in the hippocampus, giving clues to a major contributor to increasing incidence of neurodegenerative diseases. This will unravel the mechanisms by which the perinatal TBBPA exposure coupled to HFHS diet interferes with the setpoint adjustment of the thyroid axis or other hormonal pathways, and therefore alter the adult's ability to cope with metabolic challenges.

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P19. Transgenerational effects of endocrine disruptors combined to an i*n utero* exposure to high fat diet

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Endocrine disrupting chemicals (EDCs) are ubiquitous environmental pollutants that can alter puberty, fertility or energy balance. Our laboratory recently documented the effects of transgenerational exposure to EDCs on the hypothalamic control of puberty and reproduction[1]. Given the increase in prevalence of obesity worldwide and the impact of gestational high fat diet on metabolic risk in the descendance[2,3] , our current project aims at characterizing the effect of transgenerational exposure to a mixture of EDCs combined with gestational high fat diet (HFD). F0 dams were orally exposed to a mixture of 13 EDCs at environmentally relevant doses or to oil (controls), 2 weeks before mating, during gestation and lactation. F2 dams were than exposed to HFD (45% fat) or a normal diet during gestation and the first week of lactation. We exposed 7 F2 dams in each groups. Data were analyzed by two-way ANOVA.

Gestational exposure to HFD was associated with a significant lower weight in F3 pups between PND7 and PND40 ($p < 0,0001$). This phenotype was not significantly worsened by ancestral exposure to EDCs. In addition, we observed an increased neonatal mortality rate in rats exposed to HFD compared to normal diet ($p < 0,05$).

F3 females exposed to HFD *in utero* showed a significantly greater anogenital distance at P10 ($F=47,83$; $p < 0,0001$) and a shorter time between vaginal opening and first estrus ($F=5,501$; $p=0,02$) compared to controls. Gestational exposure to HFD also altered the estrous cyclicity in adult F3 (n=7/groups). Females cycled less regularly when exposed to HFD *in utero* with a significant decrease in time spent in proestrus ($F=9,516$; $p=0,0036$). This phenotype was not worsened by ancestral exposure to EDCs.

F3 males gestationally exposed to HFD showed significant pubertal delay, characterized by the age of balanopreputial separation ($F=43,60$; $p < 0,0001$). The same group presented a significant decrease in testicular weight at P25 ($F=13,69$; $p = 0,0012$) and at 7 months (n= 7/groups, $F=33,99$; $p < 0,0001$), but those effects were not worsened by ancestral exposure to EDC. The combination of transgenerational EDC exposure and in utero HFD induced a testicular descent et earlier age descent (n=7/group). No difference in gonadotropin or testosterone levels or sperm count were measured between the control, HFD or EDC groups at 7 months (n=7/groups).

In conclusion, we have shown that gestational exposure to HFD affected postnatal growth as well as pubertal development in male and female rats.

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P20. Anatomical distribution of the chemotrophic factors slits in the developing and adult hypothalamus

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Metabolic diseases such as obesity and type II diabetes are increasing at alarming rates. Experimental evidence indicate that obesity could result from defects in the development and function of neuroendocrine systems controlling energy metabolism, particularly the hypothalamus. The hypothalamus architecture relies on a series of molecular events defining functional circuits. In particular, growing axons must decide which direction to follow to innervate a particular region. It is known that the speed and direction of axonal growth are defined by diffusible axonal guidance signals acting remotely chemoattractant or chemorepulsive. Among these factors, Slits have been implicated in axon repulsion through their binding to their receptors Robo. There are three homologs of Slit in mammals: *Slit1*, *Slit2*, and *Slit3*. However, the role of chemotrophic factors, such as *Slits*, in the development and plasticity of the hypothalamus and the anatomical distribution of their receptor *Robo1-4* has not been investigated. This project aims to characterize the anatomical distribution of Slits in the mouse hypothalamus throughout postnatal life using the RNAscope technique. Data show that *Slit1* mRNA is expressed ubiquitously in hypothalamic nuclei (e. g., the arcuate, ventromedial and dorsomedial nuclei). The expression of *Slit3* mRNA is more restricted and only found in the ventromedial nucleus and endothelial cells of the median eminence. *Slit2* mRNA is also highly expressed in the ventromedial nucleus, but also in tanycytes, which are ependymoglial cells known to play an important barrier role. The expression pattern of Slit1, 2 and 3 was relatively comparable between pups at postnatal day 5 and 10 and adult animals. The Slit receptors *Robo1* and *Robo2* are expressed ubiquitously throughout the hypothalamus, including in tanycytes, but *Robo1* is more expressed in tanycytes compared to *Robo2*. In contrast, *Robo3* is not detected in the hypothalamus and *Robo4* expression is restricted to endothelial cells in the median eminence and hypothalamic parenchyma. Based on these observations, we are currently examining the functional impact of tanycytic *Slit2* expression on metabolic regulations and their barrier properties. This neuroanatomical work provides new insights into the potential role of Slits in hypothalamic development and plasticity.

P21. Senescence of POMC neurons induced by SELENOT deprivation enhances neurosecretory activity

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Selenot is an ER-resident protein involved in the development of the central nervous system and the regulation of energy metabolism. Its expression in the brain is stable even in the presence of selenium deficiency, indicating that it probably plays an important role. We show in mouse hypothalamus by RNAscope that SELENOT is present in POMC neurons These neurons are involved in the regulation of food intake under the influence of satiety factors such as leptin. In a cell model of murine POMC neurons, we find that SELENOT expression is induced by leptin and oxidative stress. Removal of SELENOT from these cells depletes intracellular calcium stores. The absence of SELENOT also causes the cells to enlarge, adopting a flattened shape devoid of neuritic extension. Nuclei are larger in proportion to cell size, sometimes duplicated, and micronuclei are occasionally observed. Increased expression of p16 and p21 indicates arrest at the G1/S checkpoint. p16 is a hallmark of senescence, which is further confirmed by β-galactosidase positivity and induction of the pro-inflammatory cytokine IL-6. Counter-intuitively, intracellular levels of POMC and α-MSH are higher in cells lacking SELENOT than in control cells. On the other hand, the absence of SELENOT does not impair leptin-induced α-MSH secretion. Taken together, these data suggest that a defect in calcium homeostasis following SELENOT deprivation would activate senescence and increase neurosecretory activity.

P22. Aggrecan controls the spatial distribution of blood borne molecules in the arcuate nucleus

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A constant communication between the periphery and the brain is essential to maintain energy homeostasis. This communication is ensured by circulating hormones which reach the brain to inform it on the metabolic state of the individual. The transport of circulating hormones into the brain is facilitated by the fenestrated vasculature of the median eminence (ME), a circumventricular organ located close to the arcuate nucleus (ARH), a key center of metabolic regulations. However, once in the ME parenchyma, blood-borne molecules cannot freely diffuse into the ARH due to some unknown reasons. We suspect that a specialized extracellular matrix (PNN, perineuronal net), detected at the interface between the ME and the ARH, could account for the restricted molecule diffusion in this brain region. Our goals were to evaluate the role played by the PNN and its components in the restricted diffusion of blood-borne molecules between the ME and the ARH and to understand the physiological relevance of this restriction in the arcuate sensing of circulating metabolic hormones.

By staining albumin, an endogenous plasmatic protein, to track blood-borne molecule distribution between the ME and the ARH, we have highlighted that PNN deposition perfectly correlates with the sudden decrease of albumin diffusion at the ME/ARH border. We found that aggrecan (ACAN) is the most abundant PNN component detected at the ME/ARH border and ACAN deposit also correlates with the reduction of albumin diffusion. To evaluate the role of aggrecan in this specific diffusion of blood-borne molecules between the ME and the ARH, we studied the spatial distribution of albumin in mouse models of ACAN alteration either through genetic mutations (hypomorphic and knock-out) or through enzymatic digestion (stereotaxic injection of the aggrecanase ADAMTS-5). We showed that a disruption of ACAN deposit in this brain region induces a massive diffusion of albumin in the ARH, destabilizing its peculiar spatial distribution between the ME and the ARH. We found that ACAN mRNA are mainly expressed by NPY neurons located in the ventral part of the ARH and their down-regulation using a viral shRNA approach did not disturb ACAN proteins which have been already extracellularly deposited before infection, suggesting a low turnover for ACAN. The disruption of ACAN deposit by stereotaxic injection of aggrecanase (ADAMTS-5) led to an increase in food intake by mice after an overnight fasting period, suggesting that the abnormal spatial distribution of blood-borne molecules by ACAN digestion alters the arcuate sensing of these molecules. When mice are intraperitoneally injected with ghrelin, an orexigenic circulating hormones, after ACAN disruption, neuronal activation is improved in the ARH, notably in the dorsal area of this hypothalamic nucleus leading to an increased food-intake.

Altogether, our results bring to light the importance of the peculiar spatial distribution of blood-borne molecules in the ME/ARH region of the brain and reveal the role of aggrecan in the establishment of this specific distribution. Aggrecan is therefore a new actor in the regulation of the arcuate sensing of circulating metabolic hormones.

Communication Flash

P23. Characterisation of metabolic profiles of knock-out MCH-receptor1 mice and humanized MCH-receptor2 knock-in mice

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Melanin-concentrating hormone (MCH) is a cyclic neuropeptide involved in a number of neuronal functions with major roles in the regulation of food intake and energy expenditure as well as stress control. MCH gene overexpression leads to obesity and insulin resistance whereas MCH gene null mice are hypophagic and lean and are resistant to ageing-associated increases in body weight and insulin resistance.

Two MCH receptors, namely MCHR1 and MCHR2, have been found in human while only MCHR1 has been identified in rat and mouse. It can be predicted that a strategy aiming at blocking MCH action on MCH receptors could be a successful therapeutic approach in the treatment of obesity and/or mood disorders. However, it cannot be anticipated which MCH receptors and corresponding neuronal pathways would be the target of MCH antagonists in humans. Indeed, because of the lack of suitable animal models the biological functions of MCHR2 remained unknown up to date.

To resolve the issue, a transgenic mouse has been generated carrying the human MCHR2 (hMCHR2) gene by a targeted knock-in approach, and designed thereafter hMCHR2$^{Hprt/Hprt}$ mouse. The overall strategy consists in introducing the hMCHR2 gene (with regulatory flanking regions and a reporter cassette) in the HPRT gene, a unique docking site with permissive chromatin environment that supports promoter dependent transgene expression. Neuronal expression of the transgene in hMCHR2$^{Hprt/Hprt}$ mice has been demonstrated by monitoring of the spatial and temporal expression of the transgene and direct *in situ* hybridization/RNAscope. In agreement with MCHR2 mRNA distribution in Primates, we found strong expression of the transgene in defined brain areas of the adult hMCHR2$^{Hprt/Hprt}$ mice. Since this experimental mouse model mimics MCHR2 expression in primate species, functional characterization of the hMCHR2 transgene was thereafter attempted.

We compared four mice models with distinct genotype: (1) WT mice which express MCHR1 but not MCHR2, (2) hMCHR2$^{Hprt/Hprt}$ mice which express both MCHR1 and MCHR2, (3) MCHR1-/- that do not express either receptor and hMCHR2$^{Hprt/Hprt}$./MCHR1-/- mice which express only MCHR2. We submit these models to a control (CHOW) or a high fat diet (DIO) while following bodyweight and food intake. In a second time, mice were placed in calorimetric cages to evaluate deeply the role of each receptor in energy expenditure under CHOW or DIO condition.

First, our results show that both MCHR1$^{-/-}$ and hMCHR2$^{Hprt/Hprt}$ mice present an increase of food intake associated with a resistance to diet-induced obesity but also a slight glucose resistance. Second, preliminary results using calorimetric cages reveals that MCHR1-/- mice are hyperactive and present a higher basal energy expenditure than WT mice, but further experiment are needed to compare this phenotype within hMCHR2$^{Hprt/Hprt}$ mice.

Surprisingly, those results reveal that MCHR1-/- and hMCHR2$^{Hprt/Hprt}$ mice present very similar metabolic profiles. Both mice are hyperphagic and resistant to diet-induced obesity suggesting a potential inhibition of MCHR1 signaling by MCHR2 activation in hMCHR2$^{Hprt/Hprt}$ mice.

This work established that expression of both MCH receptors in mice may regulate feeding under regular or high fat diet, a situation that could be related to the interaction found between the *MCHR1* or *MCHR2* locus and Body Mass Index in general or depressive human populations. These consistent observations between species are remarkable and validate the phenotyping of a human-specific gene in a mouse model.

P24. The role of the suprachiasmatic nucleus in the transport of CSF-borne Tau by tanycytes into the circulation: putative implications for Alzheimer's disease pathology

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On one hand, Alzheimer's Disease (AD) is an age-related disease with an increase incidence on night workers, and similar to night workers AD patients present circadian rhythms disturbances observed in their sleep-wake cycle and hormonal rhythm. This has long been associated in AD with the loss of neurons of the suprachiasmatic nucleus (SCN), the mammalian central clock. On the other hand, one of the hallmarks of AD is the accumulation and aggregation of Tau protein in the brain, which triggers neuronal dysfunction and cell death. Although intracellular, Tau can be found in the brain parenchyma and in the cerebrospinal fluid (CSF), which is thought to be an intermediate destination in Tau clearance. One emerging hotspot for Tau CSF to blood exchanges has been the Median eminence (ME), a circumventricular organ (CVO) characterized by the presence of a highly permeable vasculature and adjacent to the third ventricle where ependymoglial cells called tanycytes are able to shuttle Tau protein from the CSF to the bloodstream. Intriguingly tanycytes from post-mortem AD patients showed morphological disturbances that could suggest an impaired tanycytic Tau transport.

Surprisingly tanycytes are also able to respond to circadian cues coming from the SCN. The rhythmic release of SCN-derived vasopressin (AVP) into tanycytes regulates the influx of circulating metabolic signals into the hypothalamus. In this work we aimed to better understand the communication between the SCN and tanycytes in the context of Tau protein clearance and AD.

So far, we have evaluated the tanycytic Tau transport using primary culture of tanycytes stimulated with or without AVP, the main neuropeptide in SCN-tanycyte communication; and we have partially assessed the circadian CSF clearance of Tau in freely moving animals.

P25. Role of CB1 receptors in POMC neurons in gating energy needs with competing behaviors

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Survival in natural habitats forces animals to constantly adapt their behavior according to their intrinsic needs and environmental conditions. For instance, a threatening situation will decrease the motivation to eat. However, an imbalance between competing behaviors can lead to maladaptive responses and consequent diseases, as is the case in humans where higher rates of metabolic and eating disorders are found in subjects with mental health issues. Therefore, there is a need to understand the molecular mechanisms regulating these competing behaviors. Within the brain, pro-opiomelanocortin (POMC) expressing neurons classically promote satiety during energy surfeit and have a role in the physiological adaptations that occur during stressful and fearful events. Cannabinoid type 1 (CB1) receptors are key physiological determinants of synaptic and behavioral functions. Recent findings from our lab have demonstrated that POMC neurons activity is regulated by CB1 receptors in relation to the body's energy state. Here, we hypothesized that CB1 receptors-dependent signaling in POMC neurons is at the intersection of fear and feeding responses. We have generated a mouse line that lacks CB1 only in POMC-expressing cells (POMC-CB1-KO). As compared to their control littermates, male and female POMC-CB1-KO mice did not show any relevant change in food intake in basal, unstressed conditions. However, when POMC-CB1-KO mice were in a fearful situation, they displayed blunted fear-induced suppression of feeding. Interestingly, this phenotype seems sexually dimorphic. While further studies are currently ongoing to decipher the potential underlying circuits involved in these responses, these results suggest that CB1 receptors in POMC neurons might play a key role in the balance between fear and feeding responses, two motivational states essential for survival.

Communication Flash

P26. Perturbation of maternal gut microbiota in mice during a critical perinatal window influences early neurobehavioral outcomes in offspring

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The gut microbiota is increasingly recognized as a key environmental factor that shapes host development and physiology, including neural circuits formation and function. Concurrently, there has been growing concern that early-life antibiotic exposure may alter brain developmental trajectories, increasing the risk for neurodevelopmental disorders such as autism spectrum disorder (ASD). Here, we assessed whether perturbation of the maternal gut microbiota in mice during a narrow critical perinatal window (last week of pregnancy and first three postnatal days), induced by exposure to a commonly used broad-spectrum oral antibiotic (ampicillin), influences offspring neurobehavioral outcomes relevant to ASD. Our results demonstrate that neonatal offspring from antibiotic-treated dams display an altered pattern of ultrasonic communication, which was more pronounced in males. Moreover, juvenile male, but not female, offspring from antibiotic-treated dams showed reduced social motivation and social interaction, as well as context-dependent anxiety-like behavior. However, no changes were observed in locomotor or exploratory activity. This behavioral phenotype of exposed juvenile males was associated with reduced gene expression of the oxytocin receptor (OXTR) and several tight-junction proteins in the prefrontal cortex, a key region involved in the regulation of social and emotional behavior, as well as a mild inflammatory response in the colon. Further, juvenile offspring from exposed dams also showed distinct alterations in several gut bacterial species, including *Akkermansia muciniphila, Lactobacillus murinus,* and *Parabacteroides goldsteinii.* Overall, this study highlights the importance of the maternal microbiome in early-life, and how its perturbation by a widely used antibiotic could contribute to atypical social and emotional development of offspring in a sex-dependent manner.

P27. Perinatal exposure to artificial sweeteners and microbiota-host derived metabolite

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Against the problematic of overconsumption of added sugars and resulting obesity and diabetes, low-calorie sweeteners have been the go-to substitute, for the sweet taste they provide without the calories. Nonetheless, little is known regarding the consequences of exposure to LCS during the perinatal period on the development of the offspring. Thus, we used a mouse model of perinatal exposure to aspartame, to investigate the consequences on offspring neurodevelopment of the hypothalamic melanocortin system, implicated in energy homeostasis. Interestingly, results indicate that male offspring showed increased fat mass and displayed glucose intolerance. Perinatal aspartame exposure also induced altered melanocortin system development and disrupted parasympathetic innervation of the pancreas. However, maternal aspartame intake does not affect directly the offspring's development for the following reasons: 1) Aspartame is rapidly metabolized after ingestion, 2) It is not detected in maternal breastmilk nor in the offspring serum, 3) Incubation of hypothalamic neurons *in vitro* with aspartame does not alter axonal growth. Metabolomic data from the lab showed that phenylacetylglycine (PAG) is the only metabolite commonly found in breastmilk of mice consuming aspartame as well as in their pups' plasma. We have found that exposing dams to this metabolite recapitulates metabolic and neurodevelopmental alterations associated with maternal aspartame consumption. Altogether, these data show that maternal aspartame consumption has long term effects on offspring metabolism and hypothalamic development, and that the plasmatic increase in gut microbial co-metabolite PAG may be the mechanism through which aspartame has deleterious perinatal effects

Park S, Belfoul AM, Rastelli M, Jang A, Monnoye M, Bae H, Kamitakahara A, Giavalisco P, Sun S, Barelle PY, Plows J, Jang C, Fodor A, Goran MI, Bouret SG. Maternal low-calorie sweetener consumption rewires hypothalamic melanocortin circuits via a gut microbial co-metabolite pathway. JCI Insight. 2023 May 22;8(10):e156397

P28. Neonatal transfer of maternal microbiota has a lasting effect on the feeding behavior of the offspring through the homeostatic and reward system

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The microbiome is known to impact nearly every aspect of host physiology in health and disease, as it has a substantial effect on metabolic function. Vertical transmission from mother to child can affect the physiology from one generation to the next when changes in the composition of the microbiota have occurred due to maternal diet or obesity.

Our objective was to study whether obesity or thinness during gestation and lactation would impact different maternal microbiota and whether the transfer of microbiota to the newborn would modify feeding behavior, independently of the metabolic alterations of the mother.

By using transplantation of vaginal, fecal, and milk-derived microbiota from OP and OR dams, which differed in taxonomic composition, into pups born to conventional Fischer F344 dams from birth to day 15 of life we demonstrated the programming of offspring feeding behaviour. Homeostatic and non-homeostatic regulation of food intake were investigated on young and mature animals.

Through metagenomic, metabolomic and transcriptomic approaches we search to identify markers or microbiota signatures associated to eating behaviour characteristics.

Early transfer of maternal microbiota was associated with specific feeding behavior traits that predisposed F-OP rats to a higher risk of overconsumption in later periods of life. The metagenomic analysis allowed us to identify a few species and the corresponding metagenomic functions were positively or negatively associated with the alteration of food intake parameters and cerebral functional pathways.

These results support the idea that neonatal transfer of gut microbiota can program feeding behavior, probably by acting early in life in shaping brain structures.

P29. Molecular mechanism underlying CaMK1D-dependent function in AgRP neurons

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Our laboratory recently discovered that calcium/calmodulin-dependent protein kinase 1D (CaMK1D), a genetic hot spot in type 2 diabetes, promotes ghrelin-mediated food intake in mice. Mechanistically, we found that CaMK1D acts in AgRP neurons to increase phosphorylation of CREB and expression of the orexigenic neuropeptides NPY/AgRP in response to ghrelin. To find other potential mechanisms downstream of CaMK1D signaling, we decided to address more globally CaMK1D-dependent transcriptional changes using RNA sequencing. To this end, we used an immortalized mature mouse hypothalamic GnRH neuronal cell line, the GT1-7 cell line. We generated an inducible expression system in GT1-7 cells allowing for doxycycline-induced re-expression of constitutive active CaMK1D in a CaMK1D knockout background. Comparing doxycycline- with control-treated cells, we observed that 25 genes were significantly regulated in a CaMK1D-dependent manner.

Among these genes, Calcium homeostasis modulator family member 6 (Calhm6) stood out as a potential target of CaMK1D signaling. Indeed, the levels of Calhm6 mRNA were significantly upregulated (8-fold) in absence of CaMK1D in GT1-7 cells, while levels were decreased when CaMK1D was re-expressed. Increased Calhm6 mRNA has also been confirmed *in vivo* in the hypothalamus of $CaMK1D^{-/-}$ mice in comparison to $CaMK1D^{+/+}$ mice. The physiologic relevance of this target was confirmed as GT1-7 cells stimulated with ghrelin readily attenuated Calhm6 mRNA levels in CaMK1D-dependent manner. Taken together, these findings provide new insights into CaMK1D signaling. Decreased Calhm6 expression in AgRP neurons may represent a novel mechanism promoting obesity and T2D, which we will need to confirm in the near future.

Communication Flash

P30. The Gut microbiota influences blood-CSF barrier structure and function in the hypothalamus

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A complex neuronal network in the hypothalamus regulates appetite and energy expenditure. To maintain energy homeostasis, hypothalamic neurons must rapidly sense and integrate a variety of peripheral signals (*e.g.*, hormones and nutrients). This is possible because of the close vicinity of hypothalamic neurons with the median eminence (ME), a circumventricular organ containing a specialized interface called the blood-cerebrospinal fluid (CSF) barrier. The ME blood-CSF barrier is characterized by fenestrated vessels, which are highly permeable to blood-borne molecules. It is also composed of tanycytes, which are specialized hypothalamic glial cells that line the floor of the 3rd ventricle and are joint together by tight-junction complexes to create a physical barrier with a typical honeycomb structure that prevents the diffusion of molecules from the ME parenchyma to the rest of the brain via the CSF. The gut microbiota is a complex and dynamic community of bacteria living within the gastrointestinal tract of the host. It is now well established that the gut microbiota plays an essential role in maintaining host energy homeostasis. Increasing amount of evidence also suggests that the gut microbiota may affect brain function and development, as well as blood brain barrier integrity. However, whether the gut microbiota influences the structure and function of the hypothalamic blood-CSF barrier is still unknown. To address this question, we examined the structure and function of the hypothalamic blood-CSF barrier using two complementary animal models with altered gut microbiota. First, we used germ-free (GF) mice, which are completely devoided of microbes throughout life. Second, we generated mice in which the gut microbiota composition was altered exclusively during adulthood using oral administration of a cocktail of antibiotics (ABX) with large spectrum of action and low bioavailability. Using immunohistochemical labeling and confocal microscopy, we visualized tight-junction protein zonula occludens-1 (ZO-1) as well as the fenestrated capillaries (MECA-32) in relation to the blood-CSF barrier of the ME. Additionally, we assessed the diffusion function of the ME blood-CSF barrier by assessing the penetration of Evans Blue dye in the hypothalamic parenchyma. The results indicate that the organization tanycytic honeycomb structures was disrupted in GF and adult ABX mice. However, alterations of the gut microbiota did not impair the density of fenestrated capillaries at the levels of the ME. The structural reorganization of the ME barrier was associated with the altered diffusion of the circulating Evans Blue dye into the parenchyma of the hypothalamus. Together, our results suggest that gut microbiota plays an essential role in maintaining the structure and diffusion function of blood-CSF in adult mouse hypothalamus.

P31. Hyperactivation of YAP in tanycytes stimulates cell neogenesis in the mediobasal hypothalamus

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The hypothalamus has recently been identified as a neural stem cell (NSC) niche in the adult mammalian brain in which newborn neurons have been shown to integrate hypothalamic circuits controlling energy homeostasis such as the circuit of the arcuate nucleus. The putative NSCs of this niche are tanycytes. Tanycytes are specialized radial ependymoglial cells lining the walls and floor of the third ventricle and are a key component for the regulation of reproduction and energy balance by modulating the neuropeptide secretion of hypothalamic neurons into the pituitary portal vasculature but also by regulating the exchanges between the blood, the brain and the cerebrospinal fluid thanks to their barrier and sensor properties. Nevertheless, the molecular mechanisms controlling their NSC properties remain unknown. One possible candidate for the regulation of these properties is the Hippo pathway, a major signaling pathway involved in organ growth and stem cell control both during development and adulthood. This pathway has recently been shown to control the proliferative capacity of adult retinal Müller glia, which share similar developmental origin, morphology and molecular profile with tanycytes. We therefore thought to explore whether the Hippo pathway is expressed in adult tanycytes and controls their NSC properties.
A RT-qPCR analysis on Fluorescence Activated Cell Sorting (FACS)-sorted tanycytes revealed that all core components of the Hippo pathway are expressed in adult male mouse tanycytes. A neuroanatomical study combining *in situ* hybridization and immunofluorescent stainings showed that tanycytes highly express Yes-associated protein (YAP), one of the main downstream mediators of the Hippo pathway. In order to evaluate the consequences of *in vivo* YAP hyperactivation in tanycytes, we used a viral approach based on the intracerebroventricular injection of an adeno-associated virus (AAV)1/2 coding for a constitutively active mutant form of YAP (YAP^{CA}). Mice were subsequently injected with the thymidine analog bromodeoxyuridine (BrdU) and sacrificed at a short (1 week) or long time point (2 months) in order to assess cell proliferation and differentiation, respectively. Immunodetection of BrdU showed that expression of YAP^{CA} in tanycytes markedly stimulated cell proliferation in the mediobasal hypothalamus. Co-immunodetection of BrdU and different lineage markers (Sox2, HuC/D, S100β, Olig2) has been performed and is currently being analyzed in order to determine the fate of newborn cells. Altogether, our results show that YAP regulates the proliferation of adult tanycytes and their ability to give birth to new parenchymal cells.

Communication Flash

P32. Dietary fatty acid composition impacts obesity development, neuroinflammation and associated glial reactivity

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Obesity is a serious public health problem. It is associated with "low-grade" systemic inflammation and many studies are devoted to understand the mechanisms causing obesity. Some of them showed that nutrient composition in obesogenic diets may influence the severity of disorders associated with obesity such as insulin-resistance and chronic inflammation. Others, showed that deregulation directly in the brain could be responsible for this obesity syndrome as a local hypothalamic inflammation was found in obese animals fed a high-fat diet (HFD) leading to eating disorders. Indeed, certain lipids are responsible for this inflammation in a dependent manner of the quality of their fatty acids. Polyunsaturated fatty acid (PUFA) can be Omega 6 (ω6) or Omega 3 (ω3) and the balance between them is really essential for the organism functioning. At the cellular level, an excess of nutrients leads to activation of astrocytes and microglia which have a preventive role at first but can become harmful in the long term. Here we hypothesized that obesogenic diets rich in fat and varying in fatty acid composition, particularly in ω6/ω3 ratio, have various effects on energy metabolism and neuroinflammation associated with glial reactivity. Mice were fed either a control diet containing or a HFD containing either low (LO), medium (ME) or high (HI) ω6/ω3 ratio. Mice from the HFD-LO group consumed less calories and exhibited less body weight gain compared to other HFD groups. Both HFD-ME and HFD-HI impaired glucose metabolism while HFD-LO partly prevented insulin intolerance and exhibited normal leptin levels despite higher subcutaneous and perigonadal adiposity. Only HFD-HI showed increased microglial and astrocytes reactivity and markers of inflammation in the hypothalamus. Our results show that impaired glucose metabolism and neuroinflammation are uncoupled, and support that diets with high ω6/ω3 ratio are responsible for neuroinflammation and glial cells reactivity associated with the consumption of diets rich in fat.

1. Nuzzaci D, Cansell C, Liénard F, et al. Cell Rep. 2020;30(9):3067-3078.e5. doi:10.1016/j.celrep.2020.02.029 2. Cansell C, Stobbe K, Sanchez C, et al. Glia. 2021;69(1):42-60. doi:10.1002/glia.23882 3. Thaler JP, Yi CX, Schur EA, et al. J Clin Invest. 2012;122(1):153-162. doi:10.1172/JCI59660

Communication Flash

P33. GnRH, a fertile new pathway for the regulation of food intake

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Food intake and reproductive function are essential for the survival of organisms and are interconnected. They are regulated within the same brain structure: the hypothalamus. In the hypothalamus, the reproductive function is orchestrated by GnRH neurons. The way in which metabolic state can modulate the function of GnRH neurons is well documented. Indeed, it is known that in the presence of a disturbed energy balance due to both an excess and a lack of energy reserves, reproduction and hence GnRH neuronal activity is shut down as is the case in obesity or anorexia nervosa. In contrast, the existence of an inverse link is much less clear. In a previous paper we demonstrated that an increase in the number of GnRH neurons in the hypothalamus leads to an increase in GnRH secretion and that mice with this alteration are larger and fatter than littermate controls (1). For the first time, this paper suggested that GnRH neurons might be involved in the regulation of energy metabolism. To investigate deeper this hypothesis we generated mice in which activity-dependent exocytosis, including GnRH secretion, is blocked by the Cre recombinase-dependent expression of the Clostridium botulinum neurotoxin serotype B light chain (*Gnrh1*::*cre*; iBot). *Gnrh1*::*cre*; iBot mice show a drastic decrease in food intake as well as deregulation of body weight and other metabolic parameters. A 15-day treatment restoring the physiological rhythm of GnRH secretion in these mice rescues these alterations. Beyond state-of-the-art approaches, such as ultrahigh field 17.2T magnetic resonance imaging, as well as more classical behavioral and physiological approaches are being used to untangle the role of GnRH in the regulation of food intake. Overall, our results show an involvement of the reproductive hormone GnRH in the regulation of eating behavior and raise the intriguing possibility that pulsatile GnRH therapy holds potential for the management of eating disorders.

Vanacker C et al. Neuropilin-1 expression in GnRH neurons regulates prepubertal weight gain and sexual attraction. EMBO J. 2020 Oct 1;39(19):e104633. doi: 10.15252/embj.2020104633

P34. Seasonality of neurogliogenesis in the mammalian nervous system

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The survival of a species depends on the ability of individuals to adapt to environmental constraints. It is now well established that seasonal variations in day length (photoperiod) result in changes in nocturnal melatonin production which, via a pathway involving thyroid stimulating hormone (TSH) from the pars tuberalis, regulates triiodothyronine (T3) production by the hypothalamic tanycytes [1]. Thus, hypothalamic T3 exhibits annual rhythms with higher value in long photoperiod (summer) than in short photoperiod (winter). This melatonin driven rhythm in TSH/T3 is essential for the seasonal synchronization of biological functions such as reproduction, but the mechanisms by which T3 acts on the hypothalamic circuits controlling the reproductive axis remain unknown. It has been shown in the ewe, a short-day breeder, that the large nocturnal peak of melatonin in short photoperiod is associated with an increased neurogliogenesis in two neurogenic niches of the central nervous system (sub-granular zone and hypothalamus) [2], and that the inhibition of this cell proliferation alters the timing of seasonal reproduction in the ewe [3].

The Syrian hamster is another seasonal species, which in contrast to the sheep, breeds in long photoperiod, thus under a short nocturnal melatonin peak signal. Therefore, in this study we wanted to assess whether a seasonal neurogliogenesis also occurs in the Syrian hamster, and if so to determine the seasonal signal(s) involved.

For this purpose, I investigated proliferative activity in the brain of adult male Syrian hamsters transferred from short to long photoperiod, and vice-versa. Animals were sacrificed at different time points following the photoperiodic transfer, 24 hours after the injection of a DNA intercalator, 5-bromo-2'-deoxyuridine (BrdU). I found that cell proliferation increased in the hypothalamus and in the sub-ventricular and sub-granular areas with a maximum one week following the transfer from long to short photoperiod, but not during the opposite short to long photoperiod transfer.

In order to investigate whether the increased proliferation in short photoperiod is due to the lengthening of the nocturnal peak of melatonin, I performed daily injections of melatonin at late evening in hamsters kept in long photoperiod (mimicking a short photoperiod-like peak of melatonin). I found that melatonin injection significantly increases cell proliferation in the hypothalamus and the sub-granular zone already after one week of treatment. Next, to determine whether the proliferative effect of melatonin depends on its inhibition of TSH production by the pars tuberalis, I performed a chronic icv infusion of TSH in hamsters transferred from long to short photoperiod (mimicking a long photoperiod TSH signal; [4]). I found that TSH treatment did not prevent the short photoperiod induced proliferative activity in the three neurogenic niches.

In conclusion, my study shows that cell proliferation in the hypothalamus increases following a transfer from long to short photoperiod in the hamster, similarly to the sheep, suggesting that it is independent of the seasonal reproductive physiology. I also found that the short photoperiod-induced cell proliferation is due to a lengthening of the nocturnal melatonin peak, independently of the central TSH signal. In future experiments, I will phenotype the neoformed cells and investigate the biological role of seasonal neurogliogenesis on the Syrian hamster's reproductive function.

1-Dardente H.; Simonneaux V. J Neuroendocrinol 2022:e13124. 2-Migaud M.; et al. J Biol Rhythms 2011;26(6):486–496. 3-Batailler M.; et al. Sci Rep 2018;8(1):6188. 4-Klosen P.; et al. FASEB J 2013;27(7):2677–2686

P35. Diet and estrus cycle influence Glis3 expression in tanycytes

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The mediobasal hypothalamus (MBH) is one of the key centers of communication between the brain and the periphery. The median eminence (ME) at the base of the third ventricle of the hypothalamus is one of the circumventricular organs (CVOs) characterized by fenestrated capillaries that allow the passage of the molecules between the blood and the central nervous system (CNS). Tanycytes, specialized ependymoglial cells lining the third ventricle of the hypothalamus, act as sensors and mediators of these peripheral homeostatic signals, adapting their structural organization and function in response to systemic cues. The morphology of tanycytes close to the pituitary-portal system make them ideal candidates to study the disrupted brain-periphery communication in a context of metabolic disorders, including obesity and type 2 diabetes. Despite potential sex-specific variations in the access to circulating factors in the brain, most studies have predominantly focused on male rodents, leaving the mechanisms driving sex-dependent changes in these cells largely unexplored.

Using single-cell RNA sequencing of the median-eminence and the periventricular region, we identified several transcription factors and genes with differential activity in tanycytes in both male and female mice based on diet (chow or high-fat diet for 10 weeks) as well as estrus cycle phase of the female mice. One of the noteworthy targets was Gli-similar (Glis) 3, a member of a subfamily of Krüppel-like zinc finger transcription factors. Current reports suggest that Glis3 is critical for pancreatic β cell development and insulin gene transcription as well as essential for thyroid hormone biosynthesis. Loss-of-function mutations in Glis3 cause neonatal diabetes, hypothyroidism and other congenital dysfunctions. In addition, genome-wide association studies have identified Glis3 as the susceptibility gene for both type 1 and type 2 diabetes, making it one of the ideal candidates to study its function in tanycytes.

Our single cell RNA-seq analysis revealed that Glis3 is enriched in tanycytes with differential expression in male mice depending on diet while in female mice based on the estrus cycle phase. To further investigate the functional role of Glis3 in tanycytes, we deleted Glis3 from tanycytes of male and female mice and subsequently assessed the metabolic consequences of tanycytic Glis3 inhibition, including changes in body weight, body composition, glycemia, and insulin resistance in both sexes. This study has allowed us to gain primary insights into the potential role of tanycytic Glis3 in sex-specific metabolic regulation and glucose homeostasis.

P36. A deep dive in the embryonic and adult GnRH system in mice

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INTRODUCTION/AIM

In mammals, the central control of reproduction relies on a scattered population of Gonadotropin-Releasing Hormone (GnRH) neurons. These neurons originate in the nasal region and migrate towards the brain during the embryonic development, to then settle in the hypothalamus and establish interactions with several cell populations. Defects in these processes can lead to reproductive disorders, sometimes associated with absent puberty and/or infertility. While the migration and interactions of GnRH neurons have been extensively studied in the last decades, it was often through conventional sectioning methods not well suited to the visualization of neuronal networks: new approaches are thus needed to better understand the processes at play in the physio-pathological development of the GnRH network.

METHOD/RESULTS

To overcome the limitations of classical histological approaches, we combined whole-mount immunostaining with solvent-based tissue clearing (iDISCO+) and light-sheet imaging on mice samples from several prenatal and postnatal stages. We visualize the ontogeny and migration of the GnRH neurons in mice from the 11th embryonic day to birth, and reveal the whole-brain distribution of these neurons and their interactors at early postnatal stages and in adult brains. By imaging decalcified and cleared heads of young and adult mice, we also highlight an intriguing extracerebral population of GnRH neurons in the nasal region.

CONCLUSIONS

The 3-dimensional approach used in this work proves to be a powerful tool to study the scarce and scattered population of GnRH neurons. Understanding the migration, distribution and connections of the GnRH system will provide new insights on the development of the central control of reproduction, and greatly help to investigate new roles for GnRH and its interactors in mammals.

P37. Effects of enterobacterial ClpB protein immunization on feeding and locomotor behavior and plasma levels of α-MSH and ClpB in mice with activity-based anorexia

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Caseinolytic protease B (ClpB) is a protein produced by the Enterobacteriaceae family of gut bacteria which was previously identified as an antigen mimetic of α-melanocyte-stimulating hormone (α-MSH), an anorexigenic neuropeptide [1]. Autoimmune reaction to ClpB resulting in production of α-MSH cross reactive antibodies was proposed as a key element in a pathophysiological model of anorexia nervosa (AN) [2]. To further validate this hypothesis, in the present study, we determined if ClpB immunization may influence feeding and locomotor behavior in mice exposed to activity-based anorexia (ABA), an animal model of AN. For this purpose, C57Bl6 male mice have been immunized with *E. coli* recombinant ClpB protein 3 weeks before their exposure to the ABA protocol consisting of 3h/day access to food but unrestricted access to drinking water and a running wheel. Ad libitum-fed ClpB-immunized and control mice have also been studied. Efficiency of ClpB immunization was confirmed by high levels of plasma anti-ClpB IgG. No significant effect of immunization on body weight was observed in both ad libitum and ABA mice. However, lower food intake during the 1st hour during food access was observed in ClpB-immunized ABA mice, which was due to the reduction of meal size. Remarkably, ClpB-immunized ABA mice displayed consistently increased food-anticipatory physical activity which was higher then in ABA controls. While plasma ClpB levels were not different among the groups before immunization, an increase of ClpB in ClpB-immunized vs. the control mice was observed in the last day of experiment in both ad libitum-fed and ABA mice. Plasma levels of α-MSH and α-MSH-reactive IgG were also increased in ClpB-immunized ABA mice. In conclusion, ClpB immunization aggravated the ABA phenomenon in mice by increasing physical activity but decreasing food intake. The underlying mechanism may involve increased production of anti- ClpB α-MSH cross-reactive antibodies which may serve as a carrier protein for α-MSH enhancing its anorexigenic and physical activity promoting effects. Taken together, the present results further support a role of autoimmunity against bacterial ClpB in the pathophysiology of AN.

1.Tennoune N et al., Bacterial ClpB heat-shock protein, an antigen-mimetic of the anorexigenic peptide α-MSH, at the origin of eating disorders. Transl Psych, 2014.4:p.e458
2.Fetissov S.O. and T. Hökfelt, On the origin of eating disorders: altered signaling between gut microbiota, adaptive immunity and the brain melanocortin system regulating feeding behavior. Curr Opin Pharmacol, 2019.48:p.82-91

P38. Identification of sensitive postnatal and pubertal windows related to the organization and plasticity of hypothalamic structures involved in reproductive behavior in mice

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Puberty, triggered by reactivation of the hypothalamic-pituitary-gonadal axis, is a critical developmental period leading to sexual maturity. Activation of GnRH secretion stimulates secretion of pituitary gonadotropins, which are necessary for gonadal functions and secretion of sex steroid hormones. Puberty is a time of intense brain plasticity associated with significant hormonal, neural, and behavioral changes. Sexual differentiation of the brain is known to be controlled by the sex steroids testosterone and estradiol. The pubertal increase in sex steroid secretion and the synaptic location of estradiol receptors suggest a key role for estradiol in shaping the neural networks essential for sexual maturation. The energy demand associated with this plasticity and the localization of estradiol receptors ERα and ERβ in mitochondria suggest a link between estradiol, neuroplasticity and bioenergetic regulation.

The aim of this study is to determine the sensitive windows of neuroplasticity and cellular metabolism of the hypothalamus during postnatal and pubertal development and identify sex differences. To this end, we focused on two hypothalamic structures involved in reproductive behavior. The ventromedial nucleus of the hypothalamus (VMH) is involved in the activation of lordosis behavior in females and in the regulation of aggressive behavior in males. The medial preoptic area (mPOA) is a key region for stimulating male sexual behavior, both appetitive and consummatory phases, and is involved in the inhibitory circuitry of lordosis behavior in females. Using C57Bl6/J male and female mice, we examined in these regions the developmental changes in neuronal morphology by Golgi labeling. The number and morphology of dendritic spines were examined every 10 days between postnatal day 10 (PND10) and PND60. We also examined the changes in gene expression of steroid hormone receptors, genes involved in neuroplasticity, as well as genes underlying mitochondrial activity and dynamics in the mPOA and mediobasal hypothalamus (MBH) every 5 days between PND10 and PND60 by RTqPCR.

The analysis that are underway will allow us to identify 1) the postnatal and pubertal developmental periods of strong hypothalamic changes in neuroplasticity and bioenergetic processes ; 2) the most sensitive hypothalamic regions to these changes and 3) potential sexual differences. These findings will provide a better understanding of the role of steroid hormones in the morphological and cellular changes that occur during pubertal development.

Communication Flash

P39. Gut-brain axis: neuronal characterization of portal glucose sensing

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Portal vein is rightly located to carry out nutrient sensing in blood circulating from the digestive tract to the liver. This anatomical position makes it a suited place to sense and relay nutritional information from the intestine to the brain by neural afferents, including glucose from intestinal gluconeogenesis (IGN), several hours after a protein or fibre-enriched meal.

The detection of glucose produced by IGN does not rely on the transporter GLUT2 but potentially involves the glucose sensor SGLT3 (sodium/glucose co-transporter 3). Portal glucose detection initiates a nervous signal targeting the brain through a pathway dependent on the neurotransmitter CGRP (calcitonin gene-related peptide) and independent on the ventral vagus nerve. Induction of IGN by nutritional means protects against obesity and type 2 diabetes, (by increasing satiety, insulin sensitivity and energy expenditure) and their deleterious effects on emotional behaviour.

However, the mechanisms linking detection, transmission and integration of the portal glucose signal remain to be characterized. Here, we decipher anatomical and functional features of the portal vein that may grant it its major role in glucose sensing.

Portal glucose detection was studied by immunostaining targeting SGLT3 in clarified male mouse tissues. Portal glucose targets were studied by C-FOS staining of the parabrachial nucleus (PBN) and solitary nucleus (NTS) on brains of control and CGRP-/- male mice, after portal glucose infusion mimicking IGN. The role of the spinal branch was studied by measuring the decrease in food intake induced by portal glucose infusion mimicking IGN in male mice after denervation of the coeliac ganglions.

In portal vein samples, immunostaining shows that SGLT3 colocalizes with the neuronal marker PGP9.5 (protein gene product 9.5) on nerve fibres contacting portal vein's lumen.

Portal glucose infusion mimicking IGN increases C-FOS staining in the pons of control mice (PBN: saline 59 ± 10 vs glucose 88 ± 7 $p<0.01$ and NTS: saline 42 ± 3 vs glucose 82 ± 12 $p<0.01$) but not in CGRP-/- mice (PBN: saline 60 ± 9 vs glucose 53 ± 7). The decrease in food intake induced by portal glucose infusion is lost after denervation of the spinal branch (Sham: 4.25 ± 0.5 vs 1.25 ± 0.3 g/24h $p<0.01$; Denervated: 4.1 ± 0.8 vs 3.4 ± 0.6 g/24h). These results should provide a comprehensive mechanism for glucose sensing, a key component of blood glucose regulation.

Soty et al., Cell Metabolism, 2017 Soty et al., Neuroendocrinology, 2020 Delaere et al., Molecular Metabolism, 2012 Stein, Mithieux, Nature Reviews Gastroenterology & Hepatology, 2023

P40. The phenol sulfotransferase 1A1 (ST1A1) is expressed by reactive astrocytes and exhibits unexpected accommodation towards large estrogenic compounds

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Sulfotransferases (SULTs) are phase II drug-metabolizing enzymes catalyzing sulfoconjugation with the co-factor 3'-phosphoadenosine 5'-phosphosulfate (PAPS). Steroid hormones and phenolic drugs can be substrates or inhibitors of SULTs that metabolize endogenous neuromediators or steroid hormones into inactive forms for excretion. We previously showed that astrocytes expressed SULT1A1 with increased expression in a murine model of white matter neuroinflammation (1). We here further investigated the expression of SULT1A1 isoenzyme (ST1A1) in the human brain in multiple sclerosis, an autoimmune neuroinflammatory condition of the CNS. Moreover, we developed improved *in silico* structure-based approaches to better refine the binding energy of estrogenic compounds to hSULT1A1(2). To delineate SULT1A1 expression in human brain, we performed immunofluorescence on fresh-frozen sections in the normal appearing white matter and inflamed brain areas from multiple sclerosis patients. As previously reported in mouse, SULT1A1 is weakly expressed in astrocytes of the normal appearing white matter whereas increased immunoreactivity is observed in reactive astrocytes in the diseased human brain. It was not found to be expressed in other neural types. We also explored the ligand binding and flexibility of SULT1A1 from available crystal structures by molecular dynamics (MD) simulations and structure-based docking-scoring approaches. MD simulations with excited normal modes (MDeNM) were applied to better understand the recognition of diverse estrogen-related compounds by SULT1A1. MDeNM allowed exploring an extended conformational space of PAPS-bound SULT1A1, and analyses on binding of the substrates estradiol and fulvestrant demonstrated that conformational changes of the PAPS-bound SULT1A1 could acccount for the new accommodation of SULT1A1 towards large substrates such as fulvestrant. Taken together, these data suggest that SULT1A1 particularly expressed in reactive astrocytes during autoimmune neuroinflammation may be indeed a local target for estrogen and analogs, in addition to estrogen sulfotransferase SULT1E1 expressed in peripheral organs. This work was supported by ANR ToxMe (MAM/ABN).

(1) Guillot F., Garcia A., Salou M., Brouard S., Laplaud D.A., Nicot A.B. J Neuroinflammation 2015, 12:130. (2) Dudas B., Toth D., Perahia D., Nicot A.B., Balog E., Miteva M.A. Sci Rep 2021, 11:13129

P41. Study of maternal mediated mechanisms in the epigenetic programming induced by maternal stress: transgenerational transmission and oxytocin

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During perinatal life, adverse environments like stress impact brain development. This process, called programming, is the consequence of complex interactions between genes and the environment. The perinatal stress (PRS) rat model is characterized by enhanced HPA axis activity and reduced oxytocinergic tone. The enhancement of maternal behavior using carbetocin was able to reverse the PRS phenotype (Gatta et al., 2018).
Here we investigated in the PRS rat model, the intergenerational and transgenerational transmission of maternal stress, and explored if transmission of maternal stress and PRS phenotype to the next generations could be reversed by enhancing maternal behavior through oxytocinergic activation with carbetocin (CBT; Oxytocin agonist). For that, we used the maternal line and postpartum treatment in the F0 mothers to examine the effects of maternal stress and reversal by CBT on F1 generation up to the F3 in both mothers and offspring. The results we obtained, indicate the persistency of reduced maternal care in stressed dams in F0 and F1 generations, and the correction by CBT of maternal behavior, as well as the persistency of changes in genes and behavior in descendants of F1, F2 and F3 generations and correction by CBT.
Indeed, stress in F0 dams reduced maternal behavior, increased the latency of first contact after 15 min of separation from pups and reduced hippocampal gene expression of GRM7 and BDNF in F0 and F1 PRS dams. In the offspring, stress-induced high corticosterone levels and lower risk-taking behavior in F1, F2 and F3 PRS males. qPCR analysis in the hippocampus of F1 males showed a major down-regulation in stress/anti-stress genes in PRS adult males, and this down-regulation was also intergenerationally transmitted to the F2 PRS males. Postpartum CBT treatment corrected the reduction of maternal care, as well as the reduced licking latency in F0, F1 and F2 mothers. In the F1, F2 and F3 male offspring, CBT increased risk-taking behavior in the elevated-plus maze. These findings show intergenerational transmission of PRS deficits at the behavioral and molecular levels.
This study contributes to investigating the behavioral epigenetic mechanisms at the core of the transgenerational transmission of PRS and understanding the role of oxytocinergic activation in protecting the offspring *via* the mothers.

P42. Developmental exposure to environmental plasticizers alters sexual behavior in both male and female mice

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Phthalates are among the most detected organic pollutant in the environment due to their massive use in plastic production. Di-2-ethylexyl phthalate (DEHP), the most abundant phthalate, and other substances from the same family have been extensively studied for their reproductive effects. However, whether they affect the neural regulation of reproduction still needs to be investigated, in particular at environmentally relevant doses of these substances. In previous studies, the team showed that adult exposure of mice to phthalates severely disrupted male and female sexual behavior, associated with a neural downregulation of the androgen or the progesterone receptors, respectively [1, 2].

In the present study, we investigated the effects of developmental exposure to phthalates on reproductive behavior. For this purpose, dams ingested food contaminated with low doses of DEHP alone, or in an environmental phthalate mixture. The treatment was maintained during the gestational and lactational periods and ended at weaning. Analyses of male and female F1 offspring showed reduced anogenital distance and delayed pubertal initiation (age at preputial separation in males; age at vaginal opening and first estrus in females). Analyses of adult F1 mice showed that several components of sexual behavior were impaired. Both sexes displayed a lower ability to attract a sexual partner, although males' ultrasonic vocalizations were unchanged. Males also showed a disturbed mounting behavior, with a higher mating duration and fewer males reaching ejaculation. Females showed a disrupted olfactory preference, and their lordosis quotient was greatly reduced. The neural mechanisms underlying these behavioral alterations are currently under investigation, and preliminary data will be presented.

This data shows that reproductive behaviors are highly vulnerable to environmental doses of phthalates. Sex differences will be discussed, as well as a comparison with adult exposure. The importance of such data in the field of risk assessment for human health will also be presented.

[1] Dombret, C. et al., Environ. Health Perspect. 125, 097001 (2017) [2] Adam, N., Brusamonti, L. & Mhaouty-Kodja, S. Environ. Health Perspect. 129, 017008 (2021)

Index des auteurs

~ D ~

~ E ~

~ F ~

~ G ~

~ H ~

~ J ~

~ K ~

~ L ~

Programme simplifié

Mardi 26 septembre 2023		
13:00-18:00	Inscription	*Hall, Bât. CURIB*
14:00-17:00	Conseil scientifique SNE	*Salle 066, Bât. CURIB*
18:30	Cérémonie d'ouverture	*Amphithéâtre Franklin, Bât BLONDEL*
19:00	Conférence grand public	*Amphithéâtre Franklin, Bât. BLONDEL*
20:00-21:00	Cocktail de bienvenue	*Hall, Bât. CURIB*

Mercredi 27 septembre 2023		
08:30-10:30	**Symposium 1**	*Amphithéâtre, Bât. CURIB*
10:30-11:00	Pause-café	*Hall, CURIB*
11:00-12:00	**Présentations « flash »**	*Amphithéâtre, Bât. CURIB*
12:00-13:30	Déjeuner + Posters	*Hall, CURIB*
13:30-15:30	**Symposium 2**	*Amphithéâtre, Bât. CURIB*
15:30-16:00	Pause-café	*Hall, CURIB*
16:00-17:00	Assemblée Générale SNE	*Amphithéâtre, Bât. CURIB*
17:00-18:00	**Table ronde « Sciences et Société »**	*Amphithéâtre, Bât. CURIB*

Jeudi 28 septembre 2023		
08:30-10:30	**Symposium 3**	*Amphithéâtre, Bât. CURIB*
10:30-11:00	Pause-café	*Hall, CURIB*
11:00-12:00	**Communications orales**	*Amphithéâtre, Bât. CURIB*
12:00-13:30	Déjeuner + Posters	*Hall, CURIB*
13:30-14:30	**Lecture Jacques Benoit soutenue par Karger**	*Amphithéâtre, Bât. CURIB*
14:30-15:30	**Communications orales**	*Amphithéâtre, Bât. CURIB*
15:30-16:00	Pause-café	*Hall, CURIB*
16:00-17:00	**Symposium Jeunes Chercheurs**	*Amphithéâtre, Bât. CURIB*
17:00-18:00	**Session Posters**	*Hall, CURIB*
18:30-19:00	Concert Carillons	*Cathédrale de Rouen*
20:30	Dîner de gala	*Restaurant « Au Bureau »*

Vendredi 29 septembre 2023		
08:30-10:30	**Symposium 4**	*Amphithéâtre, Bât. CURIB*
10:30-11:00	Pause-café	*Hall, CURIB*
11:00-12:00	**Prix SNE et Prix de Thèse**	*Amphithéâtre, Bât. CURIB*
12:00-12:30	Cérémonie de clôture	*Amphithéâtre, Bât. CURIB*