Journée Scientifique de la SNE 2020

Webinaire

18 décembre, 8H45-16H

Programme et résumés

Chers amis et collègues,

L'année 2020 a été particulièrement difficile pour tous tant sur le plan personnel que professionnel. De nombreux projets de recherche ont été impactés par la crise sanitaire qui nous touche depuis le printemps et le congrès annuel prévu à Bordeaux en association avec la BSN a dû être reporté en 2021. Malgré cela le Conseil Scientifique de la SNE a souhaité organiser une journée scientifique en « distanciel » pour mettre en avant les différents travaux poursuivis ou achevés au cours de cette année si particulière. Notre objectif était triple :

- donner l'opportunité à chacun de présenter les travaux de son équipe au cours de sessions plénières ou de sessions posters,

- récompenser les travaux de jeunes chercheurs en octroyant pour la première fois un prix de thèse à un étudiant ayant soutenu sa thèse au cours des 12 derniers mois et un prix jeune chercheur de la SNE à un chercheur junior ayant déjà réalisé un ou plusieurs postdocs,

- le dernier objectif était de pouvoir réaliser une Assemblée Générale annuelle selon les statuts de notre société savante.

Cette journée a été rendue possible grâce aux moyens techniques et à la disponibilité des agents de la DSI-INRAE. Nous tenons à les remercier et plus particulièrement messieurs Jacques Foury, Jean-Michel Hourdon et Eric Maldonado.

Nous espérons que cette journée vous satisfera et que vous excuserez tout éventuel bug technique. Nous comptons sur votre participation à l'Assemblée Générale de 15H à 16H qui est un des moments forts de la vie de la SNE. En vous donnant d'ores et déjà rendez-vous à Bordeaux du 22 au 24 septembre 2021.

Bonne journée à tous

Nicolas de Roux, Alexandre Benani, Xavier Fioramonti, Laurence Dufourny

Pour le Comité d'Organisation

8H45-9H : Introduction par N. de Roux- Modalités techniques

9H-11H : Session de communications orales « Métabolisme »- **modérateurs : X. Fioramonti et A. Benani**

-Julie Brossaud (INRAE UMR1286, Bordeaux) « Rôle de la 11 beta hydroxystéroïde deshydrogénase dans les effets centraux du diabète de type 1 chez l'enfant »
-Judith Estrada-Meza (INSERM U1213, Lyon) « La néoglucogenèse intestinale module le développement néonatal des circuits hypothalamiques régulant le métabolisme énergétique et permet le maintien de la composition corporelle à l'âge adulte »
-Stéphane Leon (INSERM U1215, Bordeaux) « In vivo lineage tracing reveals POMC neuronal heterogeneity in response to metabolic stress»
-Monica Imbernon (INSERM U1172, Lille) « Tanycytes Control the Hypothalamic Uptake and Metabolic Actions of Liraglutide»
-Mouna El Mehdi (INSERM U1239, Rouen) « Identification of a novel peptidergic system that relays insulin signaling in the brain to regulate glucose homeostasis: the 26RFa/GPR103 system»

-Manon Duquenne (INSERM U1172, Lille) « Implication of leptin receptors expressed in hypothalamic tanycytes in the central control of energy homeostasis»

11H-12H : Session prix – **modérateurs: N. de Roux et Y. Anouar**

-**Prix de thèse de la SNE 2020 : Nour Mimouni** (INSERM U1172, Lille) « *The Domino effect of PCOS:* Fetal exposure to Anti-Müllerian Hormone triggers a transgenerational epigenetic transmission of Polycystic Ovary Syndrome defects in adulthood»

-**Prix Jeune Chercheur SNE 2020 : Charlotte Vanacker** (Univ. Michigan, USA) « A role for preoptic area astrocytes in regulating gonadotropin-releasing hormone (GnRH) neuron activity and luteinizing hormone (LH) release»

12H-13H : Session posters : 4 « salles » de présentation en parallèle

12H-12H30 :

-**Yassine Bentefour** (GIGA Neurosciences, Liège) **«** Rôle du VMHvl dans le comportement sexuel chez la souris femelle »
-Ashley Castellanos-Jankiewicz (INSERM U1215, Bordeaux) « Hypothalamic bile acids signaling through the TGR5 receptor protects from obesity »

-Freddy Jeanneteau (IGF, Montpellier) «Correction of vasopressin deficit in the lateral septum ameliorates social deficits of mouse autism model»
-Suzanne Ducroq (IBPS, Paris) «Behavioral effects of chronic adult exposure to low doses of phthalates in male mice. »

12H30-13H :

-Hervé Tostivint (MNHN, Paris) «Conserved role of urotensin II-related peptide 2 (URP2) signaling to control spine straightness in bony vertebrates»
-Nolwenn Adam (IBPS, Paris) «Effects of adult phthalate exposure on female reproductive behaviors and neuroendocrine responses»
-Justine Vily-Petit (INSERM U1213, Lyon) « Les effets bénéfiques de la néoglucogenèse intestinale dépendent de l'activation d'un axe nerveux intestin-cerveau-tissus périphériques impliquant le neuromédiateur CGRP et le système nerveux sympathique »
-Sébastien Bouret (INSERM U1172, Lille) « Maternal Non-Nutritive Sweeteners Exposure Rewires Hypothalamic Circuits Causing Metabolic Defects»

13H-15H : Session de communications orales « Reproduction »- modératrices C. Cornil et M. Montero-Hadjadje

-Lina Riachy (INSERM U1239, Rouen) « Contribution of super-resolutive techniques to the understanding of molecular mechanisms governing neuroendocrine secretion »
-Lucas Court (GIGA Neurosciences, Liège) « Effect of chronic intracerebroventricular administration of an aromatase inhibitor on the expression of socio-sexual behaviors in male Japanese quail»
-Delnia Ahmadpour (INSERM U1130, Paris) « Adult exposure to low doses of di(2-ethylhexyl)phthalate alone or in an environmental phthalate mixture disrupts the blood brain barrier and its close environment in male mice»
-Thomas Bahougne (INCI, Strabourg) « Impact of Circadian Disruption on Female Mice Reproductive Function»
-Clémence Delcour (INSERM U1141, Paris) « Onze nouveaux variants rares de MKRN3 identifiés chez 58 patients avec puberté précoce centrale isolée, issus de 35 familles. »
-Lydie Naulé (Harvard Med. School, Boston, USA) « Elucidating the Role of MKRN3 in Puberty Initiation Using both *in vivo* and *in vitro* Approaches »

15H-16H : Assemblée Générale de la SNE

Session 1 : Métabolisme

Rôle de la 11 beta hydroxystéroïde deshydrogénase dans les effets centraux du diabète de type 1 chez l'enfant

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Avec l'apparition du diabète de type 1 (DT1) de plus en plus précoce chez les enfants, se pose le problème du retentissement du diabète sur le fonctionnement du système nerveux central de ces jeunes patients alors que le développement et la maturation de leur cerveau se poursuivent. Ainsi, le DT1 est associé à une diminution de leurs capacités de mémorisation, d'attention et d'apprentissage. Si l'équilibre glycémique peut être en lien direct avec la survenue de ces troubles centraux, l'exposition accrue aux glucocorticoïdes (GC) peut également être responsables d'atteintes hippocampiques pouvant les expliquer. Nos données récentes convergent vers l'hypothèse d'une suractivité de l'enzyme 11 beta hydroxystéroïde-deshydrogénase de type 1 (11HSD1), capable de réguler la biodisponibilité tissulaire des GC par conversion de la forme inactive en forme active (cortisol chez l'homme ou corticostérone chez les rongeurs). En effet, nous avons mis en évidence (i) chez des enfants DT1, une élévation de l'activité de la HSD1; (ii) chez le raton rendu diabétique insulinoprive, une suractivité de la HSD1 hippocampique, des déficits de mémoire dépendante de l'hippocampe et des anomalies de structure hippocampique et une diminution de la neurogenèse. Nous avons récemment montré que les atteintes centrales ne sont que partiellement prévenues lors du traitement par l'insuline, rappelant la situation clinique des enfants diabétiques. De plus, nous avons confirmé le rôle central de l'enzyme dans la survenue des troubles cognitifs et mnésiques du DT1 grâce à l'utilisation d'un inhibiteur spécifique de l'activité 11HSD1 permettant de prévenir ces déficits en l'absence de traitement par insuline. L'étude de souris knock-down pour le récepteur de l'insuline au niveau hippocampique montre que l'insuline ne module pas directement l'activité HSD1. Nous sommes donc en train d'explorer les rôles respectifs du glucose et de l'insuline dans la modulation de l'activité 11HSD1 grâce à un modèle ex-vivo de tranche hippocampique.

La néoglucogenèse intestinale module le développement néonatal des circuits hypothalamiques régulant le métabolisme énergétique et permet le maintien de la composition corporelle à l'âge adulte

Judith Estrada-Meza[1], Jasmine Videlo[1], Clara Bron[1], Adeline Duchampt[1], Cécile Saint-Béat[1], Marine Silva[1], Fabienne Rajas[1], Sébastien Bouret[2], Gilles Mithieux[1], Amandine Gautier-Stein[1],

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La néoglucogenèse intestinale (NGI) régule l'homéostasie énergétique notamment en modulant la signalisation hypothalamique de la leptine. Chez les nouveau-nés, la NGI est induite pendant la fenêtre temporelle de croissance axonale hypothalamique, qui dépend d'un pic de leptine néonatal. Nous

avons émis l'hypothèse que le pic néonatal de NGI régule également le développement des circuits hypothalamiques régulant le métabolisme énergétique.
Nous avons induit génétiquement la NGI néonatale en sur-exprimant la sous-unité catalytique de la glucose-6-phosphatase au jour postnatal 1 ($I_{overexp}G6pc1_{P1}$). Les circuits hypothalamiques ont été étudiés en mesurant la densité de fibres *Agouti-related protein* (AgRP) et *Pro-opiomelanocortin* (POMC) dans les noyaux hypothalamiques de souriceaux $I_{overexp}G6pc1_{P1}$. Le métabolisme a été étudié chez des souris adultes $I_{overexp}G6pc1_{P1}$ soumises à un régime riche en graisses et en sucrose (HF/HS) pendant 2 mois. Les mêmes expériences ont été menées chez des souris avec une induction de la NGI à P12 ($I_{overexp}G6pc1_{P12}$) pour déterminer si les effets étaient limités à la fenêtre néonatale.
Les souriceaux $I_{overexp}G6pc1_{P1}$ présentent une augmentation de la densité des fibres AgRP dans le noyau paraventriculaire antérieur (PVHant) par rapport aux témoins. Ceci est associé à une augmentation de 67% de la phosphorylation de STAT3 (induite par injection intra-péritonéale de leptine) dans l'hypothalamus des souriceaux $I_{overexp}G6pc1_{P1}$ par rapport aux témoins. De plus, les souris adultes $I_{overexp}G6pc1_{P1}$ présentent une diminution de 30% de leur masse grasse, une augmentation de 10% de leur masse maigre, et une meilleure signalisation hypothalamique de la leptine après 2 mois de régime HF/HS. L'induction de la NGI à P12 n'affecte pas ces paramètres.
La NGI exercerait donc un rôle neurotrophique sur les projections AgRP vers le PVHant, spécifiquement au début de la période néonatale. Ce rôle est associé à une meilleure sensibilité hypothalamique à la leptine et une protection de la composition corporelle de l'individu sous un régime HF/HS.

In vivo lineage tracing reveals POMC neuronal heterogeneity in response to metabolic stress

Stéphane Léon, Vincent Simon, Samantha James, Nathalie Dupuy, Daniela Cota, Carmelo Quarta

INSERM U1215, Bordeaux

The brain is critically involved in the regulation of energy balance and glucose homeostasis. Depending on the levels of energy available in our body, the activity of a group of hypothalamic neurons expressing the neuropeptidergic marker proopiomelanocortin (POMC) changes and it plays a key role in maintaining energy balance. When POMC neuronal activity is altered, this can lead to impaired energy homeostasis and therefore to obesity. However, POMC neurons are highly diverse, and whether or not such heterogeneity is implicated in the development of diet-induced obesity (DIO) remains unknown. Here, we used a lineage-tracing approach in combination with immunofluorescence and fluorescent in situ hybridization to characterize the peptidergic heterogeneity of murine POMC neurons under basal conditions or during DIO. Using this strategy, we successfully 'traced' with a reporter protein POMC neurons in adult mice, thereby allowing to study these neuronal cells independently from the expression of their main marker POMC. Three subpopulations could be identified using this approach: neurons expressing high levels of POMC (POMC-high), neurons with low POMC expression (POMC-low), and neurons with no POMC expression (POMC-'ghost'). Notably, chronic exposure to a high-fat diet (HFD) led to alterations in the peptidergic machinery of the POMC-high subpopulation, but did not affect POMC-low or POMC-ghost neurons. Thus, our preliminary data suggest that DIO leads to selective functional alterations in specific POMC neuronal clusters. The approach proposed may be used in the future to study the relationship between POMC neurons heterogeneity and obesity pathogenesis.

Tanycytes Control the Hypothalamic Uptake and Metabolic Actions of Liraglutide

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Liraglutide, an anti-diabetic 59 and agonist of the glucagon-like peptide-1 (GLP1) receptor, has recently been approved for the treatment of obesity in individuals with or without type-2 diabetes. Despite its broader metabolic benefits, it is unclear whether liraglutide regulates insulin secretion through specific brain centers or directly via pancreatic beta cells. Here, we demonstrate that liraglutide enters the hypothalamus of mice through specialized ependymoglial cells called tanycytes, bypassing the blood-brain barrier (BBB). Blocking tanycytic transcytosis by cell-specific expression of botulinum neurotoxin impedes the liraglutide-induced activation of hypothalamic neurons and its metabolic effects, including oral glucose-stimulated insulin secretion, mimicking celiac vagotomy. Finally, unlike native GLP1, liraglutide fails to enhance glucose-stimulated
insulin-secretion from human islets. Collectively, these data suggest that tanycytes shuttle liraglutide into the brain, where it exerts its anti-obesity effects and induces insulin secretion in response to dietary glucose uptake.

Identification of a novel peptidergic system that relays insulin signaling in the brain to regulate glucose homeostasis: the 26RFa/GPR103 system

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The neuropeptide 26RFa and its receptor, GPR103, form a peptidergic system expressed by the hypothalamic nuclei involved in the control of energy balance. 26RFa stimulates food intake and exerts its orexigenic activity by modulating the NPY/POMC system of the hypothalamus. Our team has recently shown that the 26RFa/GPR103 system is also expressed at the periphery where it is involved in the regulation of glucose homeostasis, with 26RFa acting as an incretin. In addition, the orexigenic and incretin activities of 26RFa are strongly disrupted by a high-fat diet leading to obesity and diabetes. It is now well established that the cooperation between the hypothalamus and peripheral organs contributes to the regulation of carbohydrate homeostasis. All the observations made to date indicate that 26RFa could play a role linking the hypothalamic regulation of energy balance and glucose homeostasis, and could be an interesting therapeutic target to prevent/cure type 2 Diabetes.
In this context, the objective of my thesis was to understand the role of 26RFa in the central regulation of glucose homeostasis.

For this, we firstly characterized the impact of the absence of 26RFa on the regulation of glucose homeostasis in a recently generated mutant mouse model for 26RFa. Mice lacking 26RFa show an alteration in glucose tolerance associated with a decrease in insulin secretion and an increase in hepatic glucose production. In addition, these mice show histological alterations of the endocrine pancreas, resulting in an increase in the number and size of pancreatic islets and a decrease in the insulin content of β-pancreatic cells. These observations confirm the important role of 26RFa in the regulation of glucose homeostasis.
In a second step, we studied the involvement of the 26RFa/GPR103 neuropeptidergic system in the hypothalamic regulation of glucose homeostasis. Central administration of 26RFa (i.c.v.) improves glucose tolerance in a manner similar to peripheral administration (i.p.). This effect is associated with increased insulin secretion from the pancreas. In addition, insulin strongly stimulates the release of 26RFa from surviving hypothalamic explants and directly activates a subpopulation of 26RFa neurons expressing the insulin receptor. Finally, insulin looses its central anti-hyperglycemic effect when a 26RFa receptor antagonist, GPR103, is centrally administered, and in 26RFa mutant mice. All these data indicate that 26RFa is an actor in the central regulation of glucose homeostasis by relaying some central effects of insulin. In particular, we have shown that central 26RFa relays the effect of insulin on its own secretion by the pancreas but does not relay its effect on hepatic glucose production.
These data place the 26RFa/GPR103 neuropeptidergic system at the core of the regulation of glucose homeostasis, as a major player in the cooperation between the hypothalamus and the periphery.

Implication of leptin receptors expressed in hypothalamic tanycytes in the central control of energy homeostasis

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The control of energy balance that allows for the maintenance of body mass requires a continued dialogue between the periphery and the hypothalamus in the brain. The access of peripheral hormones to that structure is essential to the proper functioning of neural circuits that regulate energy balance. However, little is known about the transport mechanisms of circulating metabolic signals into the hypothalamus. The median eminence, a hypothalamic structure forming the floor of the 3rd ventricle, contains specialized ependymoglial cells called tanycytes. Tanycytes have been shown to shuttle metabolic signals such as leptin into the cerebrospinal fluid, via transcytosis. Identifying the molecular mechanisms involved in this transport is essential to our understanding of the phenomenon of central hormone resistance found in obese and type 2 diabetes patients. After an infusion of a recombinant fusion protein (TAT-Cre) into the 3rd ventricle of leptin receptor gene-floxed mouse model (LepR(loxP/loxP)), we investigated the role of the LepR in tanycytes on the central control of energy homeostasis in mice. Our results show that selectively impairing LepR expression in tanycytes increases body weight, adiposity, cholesterolemia, triglyceridemia and decreases noradrenaline serum concentration. It's associated with an increase of food intake, peripheral but not central leptin anorectic effect and glucose intolerance. Pancreas and adipose tissue activity of our model is also affected. Altogether, these data demonstrate for the first time the key role of tanycytes in the central

control of energy regulation *in-vivo*, the involvement of LepR expression in tanycytes for circulating leptin action in the metabolic brain.

Prix de thèse de la SNE 2020 :
Nour El Houda Mimouni

The Domino effect of PCOS: Fetal exposure to Anti-Müllerian Hormone triggers a transgenerational epigenetic transmission of Polycystic Ovary Syndrome defects in adulthood.

Nour El Houda Mimouni [1], Isabel Paiva [2], Anne-Laure Barbotin [1], Fatima Ezzahra Timzoura [1], Damien Plassard [3], Stephanie Le Gras [3], Gaetan Ternier [1], Pascal Pigny [4], Sophie Catteau-Jonard [1,5], Virginie Simon [1,5], Vincent Prevot [1], Anne-Laurence Boutillier [2] and Paolo Giacobini [1, 6]

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Polycystic ovary syndrome (PCOS) is the most common endocrine and metabolic disorder affecting women in reproductive age. Women with PCOS exhibit 2-3x higher levels of circulating Anti-Müllerian Hormone (AMH) as compared to healthy women and it is unclear if the elevation of AMH is a bystander effect or is driving the condition. Moreover, PCOS has a strong heritable component, however the etiopathology of the disease and the mechanisms underlying its transmission remain to be elucidated. Therefore, it is crucial to unravel contributions of intrauterine environmental factors that might induce epigenetic changes leading to increased susceptibility to PCOS later in life. Here, we measured AMH levels in a cohort of pregnant women with PCOS and control women revealing that AMH is significantly more elevated in the former group versus the latter. We then treated pregnant mice with AMH to model our clinical findings and investigated the neuroendocrine phenotype of their female progeny across multiple generations.

Prenatal AMH-treated (PAMH) female offspring recapitulated the major PCOS cardinal neuroendocrine reproductive features, namely hyperandrogenism, elevation in LH pulse frequency and oligo-anovulation, and a persistent rise in the GnRH neuronal firing activity in adulthood. This new preclinical PCOS model showed that fetal exposure to excess AMH drives a transgenerational transmission of reproductive and metabolic PCOS alterations across multiple generations via altered landscapes of DNA methylation.

Collectively, our results challenge the concept of PCOS originating in utero and appear to consolidate the role of AMH as a trigger of the pathogenesis. This work further points to PAMH mouse model as an excellent preclinical tool to investigate both neuroendocrine disturbances of PCOS and how developmental programming effects are transmitted, while offering a therapeutic avenue for the treatment of the disease.

Prix Jeune Chercheur SNE 2020:
Charlotte Vanacker

A role for preoptic area astrocytes in regulating gonadotropin-releasing hormone (GnRH) neuron activity and luteinizing hormone (LH) release

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GnRH neurons regulate fertility via episodic GnRH release, which triggers LH release. GnRH neurons are surrounded by astrocytes, which can detect local neurotransmitter release and modulate neurotransmission. Prostaglandin E2 (PGE2), which is primarily of astrocyte origin within the hypothalamus, increases GnRH neuron firing and LH levels in rodents. We hypothesized that the stimulation of astrocyte signaling near GnRH cell bodies alters LH release and GnRH neuron activity. To test this, we injected AAVs bearing DREADDs (designer receptor exclusively activated by designer drugs) and mCherry driven by the glial fibrillary acidic protein (GFAP) promoter, expressed primarily in astrocytes, bilaterally in the medial preoptic area of GnRH-GFP male mice. 91% of infected cells expressed the astrocyte marker S100β while only 7% expressed the neuronal marker NeuN (none were GnRH neurons). The DREADD utilized activates Gq signaling in response to clozapine-N-oxide (CNO). Intraperitoneal injection of CNO (0.3mg/kg) induced a marked increase in LH in Dq-mCherry infected mice (n=10, baseline, 0.7±0.08 ng/mL; post-CNO 3.3±0.4 ng/mL) whereas no change was detected in mice with control virus expressing only mCherry. Extracellular recordings were used to monitor firing activity of GFP-identified GnRH neurons in brain slices from these mice. CNO increased firing rate by ≥50% in 8 of 9 (89%) GnRH neurons from Dq-mCherry infected mice (baseline 0.12±0.05 Hz, CNO 1.01±0.29 Hz, n=9, $p<0.0001$) compared to controls (baseline 0.20±0.09 Hz, CNO 0.19±0.08 Hz, n=12). GnRH neurons located outside the hit did not respond to CNO (baseline 0.38±0.22 Hz, CNO 0.23±0.12 Hz), suggesting this effect may not propagate. Preliminary data suggests CNO induced firing in 5 of 10 (50%) cells when pretreated with PGE2 receptor antagonists (antag 0.17±0.05 Hz, antag+CNO 0.41±0.21 Hz, n=10). These data provide evidence that activating Gq signaling in astrocytes in the region containing GnRH cell bodies increases GnRH neuron firing and LH release.

Session Posters

12H-12H30 : 4 posters en parallèle

Rôle du VMHvl dans le comportement sexuel chez la souris femelle

Yassine Bentefour 1, Julie Bakker 1

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La kisspeptine, une protéine synthétisée et sécrétée par deux populations neuronales différentes dans l'hypothalamus, régule la maturation sexuelle et l'ovulation. Des nouvelles preuves indiquent qu'elle joue également un rôle dans le comportement sexuel. Récemment,
il a été démontré chez la souris femelle que l'injection périphérique de la kisspeptine augmente l'expression du comportement de lordose et la préférence sexuelle vers le sexe opposé. Bien que les voies neuronales de la kisspeptine dans le contrôle du comportement sexuel doivent encore être élucidées, une cible potentielle de la kisspeptine pourrait être les neurones exprimant la forme neuronale du NOS (nNOS) au niveau de la partie ventrolatérale de l'hypothalamus ventromédial (VMHvl). En effet, il a été démontré que l'injection périphérique de la kisspeptine chez des souris nNOS-KO n'avait aucun effet sur la lordose, alors que l'injection du SNAP (donneur de NO) chez des souris Kiss-KO induisait une lordose à un niveau similaire à celle exprimée par des souris WT, indiquant que le nNOS est une cible en aval de la kisspeptine. Également, des études de traçage neuronal ont montré que les neurones nNOS du VMHvl reçoivent des fibres de kisspeptine, indiquant son potentiel implication dans le contrôle du comportement sexuel comme un noyau relais. Pour vérifier cette hypothèse, des souris femelles C57bl/6 étaient ovariectomisées et implantées avec un implant contenant de l'estradiol. Après une période de convalescence, les animaux ont subi une chirurgie stéréotaxique pour implanter des guides canules ciblant le VMHvl. Chez différents groupes d'animaux, la lordose et la préférence sexuelle ont été évaluées après administration des substances suivantes : Kp-10, L-NAME (un inhibiteur de nNOS), SNAP/BAY (un donneur de NO), et GnRH. Les résultats ont montré que la Kp-10 et le SNAP/BAY augmentent significativement la lordose, alors que la L-NAME engendre l'effet opposé. Cependant, les trois substances n'avaient aucun effet sur la préférence sexuelle. Plus intéressant encore, l'administration de la GnRH au niveau du VMHvl n'avait aucun effet sur la lordose ni la préférence sexuelle. Ces résultats montrent que le VMHvl est spécifiquement impliqué dans la lordose, et que l'activité de cette zone est sous le contrôle de la kisspeptine et le nNOS, et donc l'existence de deux circuits neuronaux sous-tendant la lordose vis-à-vis la préférence sexuelle.

Hypothalamic bile acids signaling through the TGR5 receptor protects from obesity

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Bile acids (BA) are cholesterol-derived molecules known for their lipid emulsifying properties during digestion. However, they also exert metabolic benefits through the activation of the G protein BA receptor TGR5 in peripheral tissues, which promotes thermogenesis in the brown adipose tissue, lipolysis in the white adipose tissue and improves glycaemia through increased glucagon like peptide 1 secretion, all of which are relevant for treating obesity. BA can cross the blood-brain barrier, and TGR5 is expressed the brain, specifically within the hypothalamus, whose neural pathways critically control energy metabolism. Whether BA have an anti-obesity action by targeting TGR5 in this brain region remains unknown. To address this question, we made use of pharmacological and genetic strategies coupled to the metabolic phenotyping of our murine models. Here we show that central administration of BA or of a specific TGR5 agonist in diet-induced obese mice decreases body weight and fat mass. These effects are possible through mechanisms involving reduced food intake and increased energy expenditure via activation of the sympathetic nervous system. Conversely, the downregulation of neuronal TGR5 expression in the mediobasal hypothalamus of adult mice favors the development of obesity and worsens established obesity by stimulating food intake and blunting sympathetic activity. Lastly, dietary BA supplementation, known to promote body weight loss through stimulating thermogenesis, hinders weight loss in the absence of neuronal TGR5 within the mediobasal hypothalamus. These findings identify hypothalamic neuronal TGR5 as a key mediator of a top-down neural mechanism offsetting obesity. With this evidence, we propose a shift in the current view of BA metabolic actions, which should now include a central perspective.

Correction of vasopressin deficit in the lateral septum ameliorates social deficits of mouse autism model

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Intellectual and social disabilities are common comorbidities in adolescents and adults with *MAGEL2* gene deficiency characterizing the Prader-Willi and Schaaf-Yang neurodevelopmental syndromes. The cellular and molecular mechanisms underlying the risk for autism in these syndromes are not understood. We ask whether vasopressin functions are altered by *MAGEL2* deficiency and whether a treatment with vasopressin can alleviate the disabilities of social behavior. We used *Magel2* knockout mice (adult males) combined with optogenetic or pharmacological tools to characterize disease modifications in the vasopressinergic brain system and monitor its impact on neurophysiological and behavioral functions. We find that the activation of vasopressin neurons and its projections in the lateral septum are inappropriate to perform a social habituation/discrimination task. Mechanistically, the lack of vasopressin impedes the deactivation of somatostatin neurons in the lateral septum, which predicts social discrimination deficits. Correction of vasopressin septal content by administration or optogenetic stimulation of projecting axons suppressed

the activity of somatostatin neurons and ameliorated social behavior. This preclinical study identifies vasopressin in the lateral septum as a key factor in the pathophysiology.

Behavioral effects of chronic adult exposure to low doses of phthalates in male mice.

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Phthalates are frequently detected pollutants in the environment due to their extensive use in the manufacture and processing of several products such as PVC plastic. This results in a large contamination of adult individuals in industrialized countries as well as wildlife. Our team has recently shown that adult exposure to di-2-ethylexyl phthalate (DEHP) at the tolerable daily intake dose (50 µg/kg/day) or a lower dose close to the environmental human exposure (5 µg/kg/day), alters the emission of ultrasonic vocalizations, lowers male attractiveness and delays the initiation of mating in male mice (Dombret et al. 2017). This vulnerability is not due to changes in circulating levels of testosterone or the integrity of the gonadotropic axis, but rather to a selective downregulation of the androgen receptor (AR) in the neural circuitry involved in the expression of sexual behavior. This receptor plays an important and complementary role with the estrogen alpha receptor in testosterone-induced activation of this behavior. Interestingly, a down-regulation of the AR was also observed in the hippocampus, an androgen-sensitive structure involved in cognitive behaviors. In this context, this study was designed to assess the neural effects of chronic adult exposure to DEHP alone at 5 or 50 µg/kg/day, or in an environmental phthalate mixture on sexual and cognitive behaviors in male mice. The obtained results confirm our previous observations that oral exposure to DEHP alters both the emission of ultrasonic vocalizations and copulatory behavior, with a diminished number of mounts, intromissions and thrusts, without affecting olfactory preference. They also show for the first time that similar effects are obtained for the environmental phthalate mixture, suggesting that DEHP drives the effects of this mixture. Cognitive behaviors were also altered by DEHP alone or in the phthalate mixture as evidenced by the impaired spatial memory, temporal order memory and novel object recognition. Experiments are in progress in order to determine the molecular targets of these alterations and measure the effects on functional and structural plasticity in the hypothalamus and hippocampus.

12H30-13H: 4 posters en parallèle

Conserved role of urotensin II-related peptide 2 (URP2) signaling to control spine straightness in bony vertebrates

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URP1 and URP2 are two neuropeptides of the urotensin II family identified in teleosts fish. In zebrafish, *urp1* and *urp2* are primarily expressed in the spinal cord and the hindbrain. In the spinal cord, their transcripts mainly co-localize in a small population of sensory neurons called cerebrospinal fluid-contacting neurons (CSF-cNs). It has been recently proposed that URP1 and URP2 are required for correct axis formation and maintenance. Their action is thought to be mediated by their receptor Uts2r3 specifically expressed in dorsal somites. In support of this view, it has been shown that *uts2r3* invalidation results in severe scoliosis in adult zebrafish, starting with a downward curvature. Interestingly, we observed that *[urp1;urp2]* double mutants are also scoliotic.
Whether the mechanisms underlying scoliosis development in zebrafish can be transposed to idiopathic scoliosis occurring in humans is an important concern. The fact that *urp1* and *urp2* are not present in mammals could appear as an important limitation of their role outside the world of fish.
In the present study, we report for the first time the occurrence of *urp2* (but not *urp1*) in a tetrapod species, the xenopus X. laevis. We show both in tadpole and adult frog that *urp2* mRNA-containing cells occur in close contact with the ventral side of the central canal along the whole spinal cord, whereas in the brain, they are located below the fourth ventricle, strongly suggesting that they correspond to CSF-cNs. We also identified the xenopus counterpart of *uts2r3* and we show that like in zebrafish it is expressed in the dorsal somitic musculature. Finally, we reveal that the gene knockout of xenopus *uts2r3* results in a severe spine curvature of the tadpoles.
Taken together, our results demonstrate that the URP2 signaling pathway first reported in zebrafish is an ancestral feature of bony vertebrates.

Effects of adult phthalate exposure on female reproductive behaviors and neuroendocrine responses

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Over the last decades, a massive increase of environmental contamination with endocrine disrupters has been recorded. Among these molecules, phthalates are of great concern since they are widely encountered in the environment, due to their use in plastic production. Among phthalates, di-2-éthylexyl phthalate (DEHP) is the most detected in the environment, but other phthalates are also present. These molecules have been extensively linked to reproductive dysfunctions, but little is known about their effects on neural systems that regulate reproductive behaviors. Our team demonstrated that adult exposure to low doses of DEHP disrupts male sexual behavior (Dombret et al., 2017). In the present study, our aim is to investigate the effects of phtalates on female reproductive behaviors. Adult female mice ingested food containing a vehicle, DEHP at 5 µg/kg/day (close to the environmental exposure) or 50 µg/kg/day (tolerable daily intake dose), or a phthalates mixture relevant to the environmental exposure. Results show that phtalate exposure lengthens the estral cycle, with shorter proestrus stage and longer estrus and diestrus I stages, suggesting a hormonal perturbation.
Behavioral tests were performed on ovariectomized mice, which were primed with estrogen and progesterone to induce their receptivity. Phtalate exposure altered the normal preference of female towards males. Moreover, it disrupted lordosis behavior, a position adopted by females during copulation. This was associated with a lower number of neurons expressing progesterone receptor in the key regions for olfactory signal integration and sexual behavior. Finally, we demonstrated that male mice were less attracted by females exposed to phthalates or by their urine. Male mice also emitted less courtship vocalizations when in the presence of a phtalate-exposed female. This suggests a perturbation in pheromonal emission.
These data indicate that adult exposure to low doses of DEHP alone or in an environmental mixture alters the neural structures involved in female reproductive behaviors.

Les effets bénéfiques de la néoglucogenèse intestinale dépendent de l'activation d'un axe nerveux intestin-cerveau-tissus périphériques impliquant le neuromédiateur CGRP et le système nerveux sympathique

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** INSERM1213 « Nutrition, Diabète et Cerveau », Lyon.*

La néoglucogenèse intestinale (NGI) est une fonction qui exerce des effets métaboliques bénéfiques en générant un signal glucose portal à destination de l'hypothalamus. Les régimes riches en fibres ou en protéines améliorent le métabolisme en induisant la NGI. Les objectifs de mes travaux étaient 1/ d'évaluer les effets protecteurs de la NGI per se, indépendamment d'une induction par les nutriments, sur le développement de l'obésité et ses complications, et 2/ comprendre les mécanismes nerveux centraux et périphériques impliqués. L'étude comparée de modèles murins d'invalidation et d'induction génétique de la NGI a permis de montrer que l'induction de la NGI per se protège de l'obésité, du diabète, de la stéatose hépatique, et des altérations du métabolisme du tissu adipeux induit par un régime hypercalorique. A l'inverse l'absence de NGI, même en régime standard, est suffisante pour induire ces troubles métaboliques. Les métabolismes du foie et du tissu adipeux sont tous deux en partie au moins régulés par des afférences sympathiques. De fait, la NGI augmente l'expression de la tyrosine hydroxylase dans le foie et le tissu adipeux brun, suggérant que la NGI pourrait exercer ses effets via un axe nerveux intestin-cerveau-tissus périphériques impliquant le système nerveux sympathique (SNS). Enfin, une perfusion portale de glucose mimant l'activation de la NGI a permis d'identifier le mécanisme par lequel le glucose portal active son signal dans l'hypothalamus. Ces effets requièrent: 1/ le neuropeptide CGRP (Calcitonin Gene Related-Peptide); et 2/ la phosphorylation de STAT3 (Signal transducer and activator of transcription 3) dans l'hypothalamus, notamment dans les neurones exprimant la proopiomélanocortine, ceci étant indépendant de la présence de leptine. Ces travaux suggèrent fortement que la NGI exerce ses bénéfices métaboliques en activant un circuit nerveux intestin-cerveau-tissus périphérique, impliquant le neuropeptide CGRP, la phosphorylation de STAT3 dans l'hypothalamus, et l'activation du SNS.

Maternal Non-Nutritive Sweeteners Exposure Rewires Hypothalamic Circuits Causing Metabolic Defects

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The prevalence of obesity and diabetes has reached an alarming rate, including among pregnant women. Non-nutritive sweeteners (NNS) have increasingly been used as an alternative to sugar in the attempts to deliver a sweet taste without the excessive caloric or sugar load. However, there is little evidence regarding their biological effects, particularly during critical periods of growth and development. In this study, we aimed to investigate the impact of maternal NNS consumption on neurodevelopmental and metabolic outcomes in the offspring. Review of the literature revealed that there was a positive association between maternal NNS intake and offspring BMI z-score, fat mass, and/or overweight and obesity. We then used a mouse model to further understand the biological mechanisms mediating the effects of maternal NNS exposure on the offspring's metabolism. Adult female wild-type mice were exposed to either water (control), aspartame (0.03%), or rebaudioside A (0.02%) throughout pregnancy and lactation. Adult male, but not female, offspring from both aspartame- and rebaudioside A-exposed dams displayed increased adiposity and developed glucose intolerance. Moreover, maternal NNS consumption reorganized melanocortin circuits and disrupts parasympathetic innervation of pancreatic islets. In addition, gut microbiota signature was altered in

the offspring of NNS-fed dams. Taken together, our data indicate that maternal NNS consumption permanently remodels critical components of the brain, gut, and pancreas axes that may contribute to lifelong metabolic dysregulations.

Session 2 : Reproduction

Contribution of super-resolutive techniques to the understanding of molecular mechanisms governing neuroendocrine secretion

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In neuroendocrine cells, chromogranin A (CgA) is a key actor for the biogenesis of secretory granules. These vesicular organelles ensure the secretion of neurohormones/neuropeptides and neurotransmitters upon cell stimulation. Recently, we demonstrated that CgA acts synergistically with phosphatidic acid (PA) at the Golgi membrane to trigger secretory granule formation (1). PA is a pleiotropic lipid recognized as a key mediator of secretory granule exocytosis and membrane fusion events in neurons and neuroendocrine cells. Using mass spectrometry, we identified several common mono- and poly-unsaturated PA species in Golgi and secretory granule membranes. Using a liposome flotation assay, we demonstrated that CgA predominantly interacts with PA (36:1) and PA (40:6) species. To further analyze this interaction in a membrane configuration, we first generated supported bilayer membranes from PA-enriched liposomes and we analyzed the impact of CgA on membrane topology by atomic force microscopy (AFM). Using this nanoscopic technique, which provides a three-dimensional surface profile and monitors molecular interactions, we demonstrated that CgA interacts with PA (36:1), and induces membrane deformation and remodeling in a concentration- and time-dependent manner. Recent studies revealing that CgA and PA play an important role in controlling fusion pore expansion during exocytosis, we decided to investigate if their interaction could regulate this process. Preliminary experiments using membrane sheets coupled to electron tomography revealed that CgA is detected at exocytotic sites of the plasma membrane after neuroendocrine cell stimulation. Current experiments are in progress to analyze the implication of CgA/PA interaction in the size and the kinetics of fusion pores in single cell membranes using AFM, STED nanoscopy and total internal reflection fluorescence microscopy (TIRFM). This presentation will demonstrate the considerable contribution of innovative super-resolutive techniques to the understanding of fine molecular aspects of neuroendocrine secretion.

(1) Carmon, Laguerre, Riachy et al. Chromogranin A preferential interaction with Golgi phosphatidic acid induces membrane deformation and contributes to secretory granule biogenesis. *FASEB J* (2020) 34, 6769–6790.

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Effect of chronic intracerebroventricular administration of an aromatase inhibitor on the expression of socio-sexual behaviors in male Japanese quail

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Aromatase converts androgens into estrogens in the brain of vertebrates including humans. This enzyme is also expressed in other tissues where its action may result in negative effects on human health (e.g., promotion of tumor growth). To prevent these effects, aromatase inhibitors were developed and are currently used to block human estrogen-dependent tumors. In vertebrates including quail, aromatase is expressed in a highly conserved set of interconnected brain nuclei known as the social behavior network. This network is directly implicated in the expression of a large range of social behaviors. Given the potential implication of brain aromatase in a variety of behavioral processes, the primary goal of this study was to characterize in Japanese quail the potential impact of brain aromatase on sexual behavior, aggressiveness and social motivation (i.e., tendency to approach and stay close to conspecifics). A secondary goal was to test the feasibility of long-term delivery of an aromatase inhibitor directly into the third ventricle via Alzet™ osmotic minipumps. We demonstrate that this approach results in the strongest inhibition of both copulatory behavior and sexual motivation ever observed in this species, while other social behaviors were variably affected. Sexual motivation and the tendency to approach a group of conspecifics including females clearly seem to depend on brain aromatase, but the effects of central estrogen production on aggressive behavior and on the motivation to approach males remain less clear.

Adult exposure to low doses of di(2-ethylhexyl)phthalate alone or in an environmental phthalate mixture disrupts the blood brain barrier and its close environment in male mice

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Di(2-ethylhexyl)phthalate (DEHP) is the most common endocrine disruptor displaying an anti-androgenic activity. In male, hypothalamus and hippocampus are androgen-sensitive areas controlling sexual behavior and some cognitive functions respectively. Androgens have been also shown to modulate the cerebrovascular function. We examined the effects in adult male mice of a subchronic oral exposure to low doses of DEHP alone or in a relevant mixture of phthalates, on the blood-brain barrier (BBB) and its environment, focusing on the mPOA and hippocampus.

BBB leakage in the mPOA and hippocampal CA1 and CA3 subregions was observed following exposure to low doses of DEHP alone or in the mixture of phthalates, underlined by an alteration of endothelial tight junction organization and modification in caveolae-mediated transendothelial transport. In the hippocampal DG, none of these modifications were observed. In the mPOA, BBB disruption was accompanied by a glial activation associated with an increase of iNOS labeling colocalized with GFAP in capillaries astrocyte end-feet. Activated microglia exhibited a higher immunoreactivity of the inflammatory molecule COX-2. In the hippocampus, alteration of the BBB observed in the CA1 and CA3 regions was only associated with microglial activation without COX-2 expression, none activated astrocytes were detected.

Exposure to low doses of DEHP alone or in phthalate mixture disrupts the BBB leading to a dysfunctionality of this selective interface ensuring a tight regulation between the circulatory system, the immune system and the brain parenchyma. The differences observed in one hand between the mPOA and the hippocampus, and in the other hand within the same brain area, could be underlined at least in part by different expression patterns of AR but also of estrogen receptors (ERs). Brain

capillaries and their close environment are highly sensitive to phthalates and should be also considered as a relevant endpoint in risk assessment for these molecules.

Impact of Circadian Disruption on Female Mice Reproductive Function

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In female mammals, cycles in reproductive function depend both on the biological clock synchronized to the light/dark cycle and on a balance between the negative and positive feedbacks of estradiol, whose concentration varies during oocyte maturation. In women, studies report that chronodisruptive environments such as shiftwork may impair fertility and gestational success. The objective of this study was to explore the effects of shifted light/dark cycles on both the robustness of the estrous cycles and the timing of the preovulatory luteinizing hormone (LH) surge in female mice. When mice were exposed to a single 10-hour phase advance or 10-hour phase delay, the occurrence and timing of the LH surge and estrous cyclicity were recovered at the third estrous cycle. By contrast, when mice were exposed to chronic shifts (successive rotations of 10-hoursour phase advances for 3 days followed by 10-hour phase delays for 4 days), they exhibited a severely impaired reproductive activity. Most mice had no preovulatory LH surge at the beginning of the chronic shifts. Furthermore, the gestational success of mice exposed to chronic shifts was reduced, because the number of pups was 2 times lower in shifted than in control mice. In conclusion, this study reports that exposure of female mice to a single phase shift has minor reproductive effects, whereas exposure to chronically disrupted light/dark cycles markedly impairs the occurrence of the preovulatory LH surge, leading to reduced fertility.

Onze nouveaux variants rares de MKRN3 identifiés chez 58 patients avec puberté précoce centrale isolée, issus de 35 familles.

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Introduction : La puberté précoce centrale idiopathique (iPPC) est définie par l'activation prématurée de l'axe hypothalamo-hypohyso-gonadique en l'absence de lésions cérébrales. Une perte de fonction de la protéine makorin RING finger 3 (*MKRN3*) est la cause la plus fréquente de iPPC.
Objectif : Décrire les différents variants rares du gène *MKRN3* identifiées dans une cohorte de patients avec iPPC, en estimer la fréquence et identifier de nouveaux variants non décrits.
Patients et méthodes : Sélection des patients chez qui des variants du gène *MKRN3* ont été identifiées lors d'une analyse génétique pour PPC (cas index ou enquête familiale). Regroupement des patients par famille. Analyse in silico et confrontation avec les variants décrits dans la littérature.
Résultats : 20 variants rares de *MKRN3* ont été identifiés chez 76 personnes issus de 35 familles (58 patients avec iPPC et 18 de leur apparentés non atteints) : 8 insertions/délétions avec décalage du cadre de lecture, 12 variants faux-sens. Cinq variants étaient partagés par plusieurs familles et le variant le plus fréquent, p.Ala162Glyfs*15, a été identifié dans 9 des 35 familles (26%). Onze nouveaux variants ont été identifiés: 4 variants faux-sens et 9 Insertions/délétions. Lorsque cela a été possible, la transmission paternelle du variant rare a été confirmée. Près de la moitié des familles (47%) présentaient une insertion/délétion localisées dans une région de 10 pb.
Discussion : Nous avons identifié 11 nouveaux variants de *MKRN3* associés à la iPPC. Ce travail confirme le rôle inhibiteur de MKRN3 sur la commande hypothalamo-hypophysaire de l'axe gonadotrope durant l'enfance.

Elucidating the Role of MKRN3 in Puberty Initiation Using both *in vivo* and *in vitro* Approaches

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Makorin ring finger protein 3 (MKRN3) was identified as a regulator of puberty initiation with the report of loss-of-function mutations in association with central precocious puberty. In juvenile mice, *Mkrn3* is highly expressed in the hypothalamus, with a decrease in expression before puberty initiation. This suggests an inhibitory role of MKRN3 during the prepubertal period, but the mechanisms of action remain unclear. In this study, we used *in vivo* and *in vitro* approaches to identify hypothalamic targets of MKRN3. Using a *Mkrn3* deficient (*Mkrn3m+/p-)* mouse model, we showed an accelerated puberty onset in female mice and a trend towards early puberty onset in male, as shown by an advanced day of female first estrus and a trend towards earlier age of male preputial separation, in *Mkrn3m+/p-* compared to *Mkrn3+/+* animals. There was no difference in *Gnrh1* and *Kiss1* expression in the preoptic area, nor in *Kiss1*, *Tac3* and *Tac1* expression in the arcuate nucleus, between *Mkrn3+/+* and *Mkrn3m+/p-* mice during postnatal development. In parallel, we generated hypothalamic neurons from wild-type and CRISPR/Cas9-generated *MKRN3*-deficient isogenic human induced pluripotent stem cells (hiPSCs). *OCT4*-expressing hiPSCs differentiated with high efficiency into *NKX2.1*-expressing hypothalamic progenitors, then into *POMC-*, *KISS1-* and *TAC3*-expressing hypothalamic neurons. To further investigate possible targets of MKRN3 action, we used both *in vivo* and *in vitro* approaches and compared the proteome of *Mkrn3*-deficient and WT mice and the transcriptome of hypothalamic neurons. The results reveal differences in gene and protein expression in factors involved in extracellular matrix organization, cell adhesion and axon guidance pathways, which together control neural development and synaptic plasticity. These findings suggest that Mkrn3 may be important as a postnatal/prepubertal regulator of hypothalamic plasticity. Understanding the involvement of MKRN3 in these processes will provide new insights into pubertal disorders and may lead to the development of new treatment strategies.