Dijon 2017
Société de Neuroendocrinologie

42ème colloque de la Société de Neuroendocrinologie
Dijon / France
18 / 21 Septembre 2017

Program

+ Informations

Renseignements: https://www.societe-neuroendocrinologie.fr/
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## Program at a glance

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<td><strong>18/09/17</strong></td>
<td>13:00 - 16:00</td>
<td>Registration</td>
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<td>13:00 - 17:00</td>
<td>SNE scientific board meeting</td>
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<td>18:00 - 19:00</td>
<td>Conférence Grand Public: <em>Quand notre intestin parle à notre cerveau. Quand notre cerveau lui répond...</em></td>
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<td></td>
<td>20:00 - 21:00</td>
<td>Welcome mixer</td>
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<td><strong>19/09/17</strong></td>
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<td>08:45 - 09:00</td>
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<td>SNE general assembly</td>
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<td>Closing session</td>
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<td>Lunchbox</td>
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**Categories:**
- Formalities
- Conferences
- Official ceremonies
- Lunchs and refreshments
- Social events
Location
Dijon, France

Congress venue
- Registration
- Conférence Grand public
- Congress
- Welcome mixer
- Gala dinner

> Centre des Sciences du Goût et de l’Alimentation
> Théâtre des Feuillants
> School of Medicine / Centre des Sciences du Goût et de l’Alimentation
> Dijon City Hall
> Cellier de Clairvaux

Public transportation
The T1 tram line can get you efficiently to the SNE meeting. Find out a tram every 5-7 min ! The congress is only 20 min away from the Dijon Train Station.

Train station > Congress / School of medicine........... 20 min
By Tram T1 Dir Quetigny from Dijon Gare to CHU-Hôpitaux

Train station > Conférence Grand public / Théâtre des feuillants..... 9 min
9 min walk from Dijon Gare

Conférence Grand public > Welcome mixer / Dijon City Hall.............. 10 min
10 min walk from Dijon Gare

Congress > Gala dinner / Cellier de Clairvaux.......... 15 min
By Tram T1 Dir Dijon Gare from CHU-Hôpitaux to Godrans (12 min)
Then 3 min walk from Godrans to Cellier de Clairvaux
Conférence Grand Public

_Lundi 18 septembre, 18h00, Théâtre des Feuillants, Dijon_

Quand notre intestin parle à notre cerveau,
Quand notre cerveau lui répond...

30 minutes de présentation suivie de 30 minutes d’échange avec l’auditoire.

Avec
Pr Pierre Déchelotte (CHU Rouen) - Intestin, Microbiote et Dépression.
Dr Gilles Mithieux (INSERM Lyon) - Intestin, Nutriments et Comportement Alimentaire.
Pr Claude Knauf (Université de Toulouse) - Intestin, Hormones et Maladies Métaboliques.

Accès depuis la gare de Dijon
Program

Monday, September 18

13:00 - 16:00  Registration

13:00 - 17:00  SNE scientific board meeting

18:00 - 19:00  Conférence Grand Public: Quand notre intestin parle à notre cerveau...
Chaired by Luc Pénicaud (Toulouse/Dijon, France), Alexandre Benani (Dijon, France)
- Pierre Déchelotte (Rouen, France)
- Claude Knauf, (Toulouse, France)
- Gilles Mithieux (Lyon, France)

20:00 - 21:00  Welcome mixer
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<td>09:00-10:00</td>
<td>Jean-Louis Nahon, Valbonne, France</td>
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<td>The melanin-concentrating hormone: tale of a gene that becomes a gene that becomes a gene...</td>
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<td>10:30-11:00</td>
<td>Vincent Laudet, Banyuls-sur-mer, France</td>
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<td>Evolution of thyroid hormone signalling</td>
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<td>11:00-11:30</td>
<td>Frédéric Flamant, Lyon, France</td>
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<td>Using mouse genetics to analyze the multiple functions of thyroid hormone nuclear receptors</td>
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<td>11:30-12:00</td>
<td>Barbara Demeneix, Paris, France</td>
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<td>Thyroid Hormone and neural stem cells</td>
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<td>12:00-12:30</td>
<td>Hugues Dardente, Tours, France</td>
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<td>Thyroid hormone and seasonal rhythmicity</td>
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<td>Chaired by Julie Bakker (Liège, Belgium), Matthieu Keller (Tours, France)</td>
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<tr>
<td>14:00 - 14:30</td>
<td>Ivan Rodriguez, Geneva, Switzerland Innate behaviors in mammals: from genes to circuits</td>
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<td>14:30 - 15:00</td>
<td>Thomas Hummel, Dresden, Germany Sex differences in olfactory responses in humans: fMRI studies.</td>
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<td>15:00 - 15:30</td>
<td>Matthieu Keller, Tours, France Male odors modulate the female gonadotrope axis in ungulates</td>
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<td>15:30 - 16:00</td>
<td>Anne Didier, Lyon, France Effects of aging on olfactory perception</td>
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<td>16:00 - 16:30</td>
<td>Coffee break</td>
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<td>16:30 - 19:00</td>
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<td>18:00 - 19:00</td>
<td>Social hour</td>
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### Symposium 3: Uncovering the roles of microRNAs in neuroendocrine regulations

Chaired by Joëlle Cohen-Tannoudji (Paris, France), Andrea Messina (Lausanne, Switzerland)

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<tr>
<th>Time</th>
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<th>Title</th>
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<tr>
<td>08:30 - 09:00</td>
<td>Yongsoo Park, Izmir, Turkey</td>
<td>MicroRNA exocytosis by vesicle fusion in neuroendocrine cells</td>
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<tr>
<td>09:00 - 09:30</td>
<td>Andrea Messina, Lausanne, Switzerland</td>
<td>A miRNA-embedded genetic network drives the rise in hypothalamic GnRH before puberty</td>
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<td>09:30 - 10:00</td>
<td>Bruno Quérat, Paris, France</td>
<td>Role of miRNAs in mediating hypothalamic GnRH action on pituitary gonadotrope hormones</td>
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<td>10:00 - 10:30</td>
<td>Mohammed Taouis, Orsay, France</td>
<td>Hypothalamic miRNA contribute to leptin-dependent central regulation of energy homeostasis</td>
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<td>10:30 - 11:00</td>
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<td>Coffee break</td>
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<td>11:00 - 13:00</td>
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<td>Short talks chosen from abstracts</td>
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Chaired by Alexandre Benani (Dijon, France), Paolo Giacobini (Lille, France)

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<tr>
<td>11:00 - 11:10</td>
<td>Stéphanie Goyon, Strasbourg, France</td>
<td>The unexpected level of plasticity of the oxytocinergic system</td>
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<tr>
<td>11:20 - 11:30</td>
<td>Angelopoulou Eleni, Strasbourg, France</td>
<td>Electrical Firing Characteristics of RFRP neurons in mice</td>
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</table>
Fernando Cázarez-Márquez, Strasbourg, France
Kisspeptin and RFP3 modulate lean and fat mass in the Phodopus Sungorus via two different hypothalamic neuronal pathways

Manon Duquenne, Lille, France
A role for tanycyte exocytosis in the central control of energy homeostasis?

Sarah Geller, Lausanne, Switzerland
Hypothalamic astrocytes and tanycytes are prominent producers of lactate compared to cortical astrocytes but seem to involve different precursors: A potential distinct role in the regulation of energy balance and reproductive function

Laëtitia Merle, Dijon, France
Impact of a high fat high sucrose maternal diet on the progeny’s olfactory system

Katharina Stobbe, Valbonne, France
The Role Of CCL5 Signaling In The Central Inflammation Associated With Obesity.

Danaé Nuzzaci, Dijon, France
Changes in neuroglial interactions on POMC neurons at the meal scale

Ashley Castellanos Jankiewicz, Bordeaux, France
Role of the bile acids – TGR5 receptor system in the hypothalamic regulation of energy balance

Marie-Pierre-Moisan, Bordeaux, France
High glucocorticoid signaling as a marker of depression in obese patients

13:00 - 14:00 Lunch

14:00 - 15:00 SNE General Assembly
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<td><strong>Chaired by Valérie Simonneaux (Strasbourg, France), Marie-Pierre Moisan (Bordeaux, France)</strong></td>
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<td>15:00 - 15:20</td>
<td><strong>Konstantina Chachlaki, Lille, France</strong></td>
<td>Prix Servier</td>
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<td><strong>Ophélie Le Thuc, Munich, Germany</strong></td>
<td>Prix Servier</td>
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<td>15:40 - 16:00</td>
<td><strong>Marion Rincel, Bordeaux, France</strong></td>
<td>Prix Servier</td>
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<td>16:00 - 16:20</td>
<td><strong>Pauline Campos, Montpellier, France</strong></td>
<td>Prix SNE</td>
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<td>16:30 - 17:00</td>
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<td><strong>Young investigators’ Symposium : Innovative tools and models</strong></td>
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<td><strong>Chaired by Ophélie Le Thuc (Munich, Germany), Céline Cansell (Valbonne, France)</strong></td>
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<td>17:00 - 17:15</td>
<td><strong>Yael Grosjean, Dijon, France</strong></td>
<td>Impact of Heterodimeric Amino acid Transporters on metabolism : input of a genetic model.</td>
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<td>17:15 - 17:30</td>
<td><strong>Paolo Giacobini, Lille, France</strong></td>
<td>3D imaging of solvent-cleared brains: a new tool to study development and organization of the hypothalamic-pituitary-gonadal axis in different species</td>
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<td>17:30 - 17:45</td>
<td><strong>Brian Lam, Cambridge, United Kingdom</strong></td>
<td>Deciphering the heterogeneity of hypothalamic POMC expression neurons by single-cell RNA sequencing</td>
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<td>18:00 - 18:15</td>
<td><strong>Manon Torres, Marseille, France</strong></td>
<td>Le &quot;RNA pull-down&quot;, une nouvelle technologie pour identifier les ARN circadiens cibles du long ARN non codant Neat1</td>
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<td>18:15 - 18:30</td>
<td><strong>Oliver Brock, London, United Kingdom</strong></td>
<td>The glycoprotein-deleted rabies system : how to monosynaptically dissect a neuronal brain circuitry ?</td>
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20:00 - 23:00  Gala dinner

Gala dinner at Cellier de Clairvaux, Dijon
By tram T1 Di J Gare from CHU-Hôpitaux to Godrans (12 min)
Then 3 min walk from Godrans to Cellier de Clairvaux
Thursday, September 21

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<td>08:30 - 09:00</td>
<td>Luc Pénicaud, Toulouse/Dijon, France</td>
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<td>Brain glucose sensing: an epic tale of new mechanisms</td>
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<td>09:00 - 09:30</td>
<td>Bernard Thorens, Lausanne, Switzerland</td>
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<td>Brain glucosensing, glucose homeostasis and feeding behavior</td>
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<td>09:30 - 10:00</td>
<td>Sebastien G. Bouret, Los Angeles, USA</td>
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<td>Development of neuroendocrine systems controlling body weight and glucose homeostasis</td>
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<td>10:30 - 11:00</td>
<td>Jean-Denis Troadec, Marseille, France</td>
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<td>Glial endozepines reverse high fat diet-induced obesity by increasing hypothalamic sensitivity to peripheral leptin</td>
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<td>11:00 - 11:30</td>
<td>Daniela Cota, Bordeaux, France</td>
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<td>The role of the endocannabinoid system in the CNS regulation of energy balance</td>
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<td>11:30 - 12:00</td>
<td>Nelly Pitteloud, Lausanne, Switzerland</td>
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<td>FGF21: a potential link between reproduction and metabolism</td>
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**The melanin-concentrating hormone: tale of a gene that becomes a gene that becomes a gene...**

Jean-Louis Nahon
Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), UMR 7275 CNRS-UNS/UCA, Valbonne, France

Mammalian melanin-concentrating hormone (MCH) is a hypothalamic neuropeptide that displays multiple functions, mostly controlling feeding behavior and energy homeostasis and regulating sleep, the stress axis, emotion, and reward processes. In this Lecture I will address four questions related to: 1) what are the effects of MCH on cerebrospinal fluid circulation in the mammalian brain?; 2) how immune factors could regulate the expression of MCH?; 3) which receptors could mediate the action of MCH in “humanized” mice?; 4) what consequences of MCH gene duplication during primate evolution on brain functions?

A key feature at the basis of these four “surprising” stories with no evident link, but MCH itself, is a misunderstood concept that drives most of the scientific discoveries: serendipity!

First, the MCH functions were attributed to neuronal circuits expressing MCHR1, the single MCH receptor in rodent. However, MCH fibers were also found localized close to the ependymal cells lining the brain ventricles. Developing new techniques to measure and analyze the ependymal cilia beat frequency (CBF) in acute mouse brain slice preparations, we showed that the CBF is modulated by MCH (application, hypothalamic stimulation or activation/inhibition of MCH-expressing neurons using in vitro optogenetics). Simultaneously, the volume of both the lateral and third ventricles is increased in MCHR1-KO mice compared to their wild-type littermates. However, our results demonstrated a dynamic mode of MCH's effects on the spontaneous CBF of ependymal cells. This novel mechanism of action of a neuropeptide could contribute to maintain cerebro-spinal fluid homeostasis and allows long-term regulation of neuroendocrine functions.

Second, the chemokines and their cognate receptors are expressed in several neuronal populations, including MCH neurons, suggesting potential roles in the regulation of feeding behavior. A study demonstrated that a chemokine named CCL2 triggers neuroinflammation with down-regulation and weight loss that is reversed by CCR2 antagonists. However, MCH neurons and receptors are absent from perfused hypothalamic explants. These effects are reversed by the CCR2 antagonist and in CCR2-deficient mice. We conclude that CCL2 loss-regulation in the hypothalamus could regulate LPS-induced weight loss and loss of appetite. Based on these and other studies chemokines are emerging potential therapeutic targets for treating dysregulation of the energy balance.

Third, two G protein-coupled MCH receptors are described in fish and a single one in mammals. In rodents, there is a single MCH receptor. However, we could not anticipate which MCH receptors and corresponding neuronal pathways could be the over-expressed under conditions of broad MCH antagonism in human models. Therefore, we generated a mouse model mimicking the human MCH receptor network and allowing for isoform isoresponse. As targeted knock-in (KI) approach in mice, complete phenotype characterization is currently carried out and should provide important clues about the roles associated with the MCHR2 signaling in a “humanized” mouse model.

Finally, two genes, named PMCHL1 and PMCHL2, have been submitted to strong regulatory constraints during Hominoid evolution. Strikingly, most of the PMCHL1 gene transcripts correspond to long non-coding RNAs (lncRNAs). Furthermore, in situ hybridization experiments demonstrated co-localization of MCH mRNA and PMCHL1exons1-2 IncRNA in the cortex of macaque, adjacent to neurons expressing MCHR2. This suggests a functional relationship between the expression of a “primate-specific” IncRNA and its gene template in order to provide a new ligand source for MCHR2.
The onset of puberty and the regulation of fertility in mammals are governed by a complex neural network, primarily in the hypothalamus, that converges onto the gonadotropin-releasing hormone (GnRH)-producing neurons, the master regulators of gonadotropin secretion and postnatal gonadal growth and function. For the GnRH neurons to exert their pivotal role in the establishment of a fertile phenotype, their activity and neurosecretory capacity needs to be precisely regulated by upstream pathways. As early as in the 1990's nitric oxide (NO) was presented as a key molecule in the preovulatory GnRH/LH surge and results from several recent studies have suggested the interaction of neuronal nitric oxide synthase (nNOS or NO) and GnRH neurons. Even though NO has now been long recognized as a key player in the central hormonal regulation of ovulation during adulthood, no one had considered the possibility that it could be an early master regulator of GnRH neurons before puberty, hence participating in the definition of the neuroendocrine axis. Challenging this idea we identified for the first time a series of mutations on the Nos1 human gene in patients with Constitutive hypogonadotropic hypogonadism (CHH), as well as in probands related to Constitutional delay of growth and puberty (CDGP). This exciting finding not only highlights a key role of nNOS in the establishment of a fertile phenotype but importantly, paves the way for a better understanding of conditions of human idiopathic infertility. This aim was supported by evidence supporting a novel role of hypothalamic NO signaling in the regulation of the GnRH neuronal population during embryonic development. NO is required for the proper migration of GnRH cells. Embryonically, during the perinatal period, it controls GnRH neuronal activation of the GnRH neurons as well as the neuronal firing activity, coordinating the events leading to the sexual maturation and the establishment of a fertile phenotype. We also hope that our results will expand our understanding of how these cellular mechanisms are regulated in the embryonic and postnatal development, and will possibly provide opportunities for therapeutic strategies against debilitating conditions.

#2. Role of the hypothalamic inflammation in energy balance disruptions

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The hypothalamus is a key brain area for the regulation of energy homeostasis as it controls, among others, food intake and energy expenditure. Hypothalamic neurons and glial cells act together to regulate, both in time and space, the metabolic functions of the hypothalamus. Thus, a causal link between hypothalamic inflammation and feeding behavior deregulations, such as anorexia and obesity, could exist.

We aimed to identify the molecular mediators linking central or systemic inflammation and the hypothalamic neuropeptidergic systems involved in the regulation of energy homeostasis, with a particular focus on chemokines.

On one hand, we identified the chemokine CCL2 (CC-motif chemokine ligand 2) and its receptor CCR2 (CC-motif receptor 2) as essential signaling elements in the weight loss associated to high-intensity inflammation induced by the central injection of bacterial lipopolysaccharide, via inhibition of Melanin-Concentrating Hormone neurons.

On the other hand, we took interest in the inflammatory response in the hypothalamus following consumption of high-fat diets, leading ultimately to weight gain or even obesity. We found, in mouse models of nutritional obesity, that (1) the chemokine CCL5 (CC-motif chemokine ligand 5) would promote weight gain, possibly by increasing the activity of hypothalamic MCH neurons; (2) the nature of the fats which enter the composition of a high-fat diet has an impact on the developmental kinetic and magnitude of the inflammatory profile, and (3) excessive lipid consumption can induce very early astrogliosis and microgliosis in the medio-basal hypothalamus.

Taken together, our results, which identify chemokines as potential therapeutic targets in the treatment of deregulation of feeding behavior, highlight the need of targeting hypothalamic inflammation in these pathologies.
Psychiatric disorders, such as anxiety and depression, are highly comorbid with gastrointestinal disorders, such as the irritable bowel syndrome. In addition, a history of stressful experiences in childhood is a major risk factor for the development of both psychiatric and gastrointestinal disorders, suggesting that they share common pathophysiological bases. The intestinal barrier plays a key role in the maintenance and its maturation, as for the brain, mainly takes place during the early postnatal period. Maternal separation in rodents is a well-characterized model of early-life stress associated with cognitive impairment, increased anxiety, and hyperresponsiveness of the hypothalamic-pituitary-adrenal (HPA) axis in the adult, but also long-lasting gastrointestinal dysfunctions. Recent studies indicate that maternal separation produces an increase of intestinal permeability in pups, a process mainly regulated by epithelial tight junction opening, under the control of the myosin light chain kinase (MLCK), and allows the passage of luminal elements, including bacteria, from the intestinal epithelium. Recent hypotheses suggest that gut bacteria influence brain function and behavior and therefore contribute to the development of psychiatric disorders. However, despite the increased gut permeability reported in several psychiatric disorders, the role of intestinal epithelial barrier dysfunction remains unexplored. Using pharmacological and transgenic strategies, we examined the impact of gut permeability changes on brain and behavior. First, we report that chronic administration of a MLCK inhibitor (ML-7, 5 mg/kg; i.p.) during development prevents some detrimental effects of maternal separation reported at adulthood, such as the impaired spatial memory, anhedonia, and altered negative feedback of the HPA axis to stress. Second, transgenic mice expressing a constitutively active form of the MLCK specifically in the gut (CA-MLCK mice), leading to chronic gut leakiness, exhibit spatial memory impairment in males and exacerbate anxiety-like behavior in females. In addition, both sexes display anhedonia and altered responsiveness of the HPA axis to stress compared with wild-type controls. Altogether, our results demonstrate 1) that early restoration of gut barrier function attenuates some of the long-term alterations associated with maternal separation in the gut and improves resilience to stress, and 2) that chronic gut leakiness in CA-MLCK mice is sufficient to affect behavior and neuroendocrine response to stress. These findings suggest that gut barrier dysfunction plays a critical role in the regulation of brain function, especially in a context of stress. Further investigation will shed new light on the molecular mechanisms involved in the brain-gut alterations reported here.
In vivo study of the hypophysiotropic TRH neuron: Well-known yet mysterious regulator of thyroid function

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The homeostasis of the hypothalamus-pituitary-thyroid axis is indispensable for normal growth, metabolism, reproduction and intelligence. Hypophysiotropic TRH neurons of the paraventricular nucleus (PVN) play a primary role in the regulation of thyroid hormone (TH) production through release of TRH in the median eminence (ME) to stimulate pituitary TSH release. Despite the fact that the involvement of TRH neurons in the control of THs levels is undoubtable, their native behavior leading to TH homeostasis remains largely unknown. Here, we study the critical role of the pattern of activity of the hypophysiotropic TRH neurons in regulating the secretion of TSH in behaving animals. Using viral and optogenetic technologies, we first generated a mouse model in which the TRH neurons could be activated in conscious animals using blue light delivered to the hypothalamus. Transgenic TRH-Cre mice were injected in the ME with a Cre-dependent adeno-associated virus (AAV) to target Channelrhodopsins (ChR2) in the hypophysiotropic TRH neurons and a fiber optic was implanted in the PVN. With this model, we determined the patterns of activation of TRH neurons necessary to elicit TSH secretion. We then aimed to record the native electrical activity of TRH neurons and developed a state-of-the-art system allowing us to monitor the activity of genetically targeted TRH neurons, using calcium imaging as a proxy for spike dynamics. We injected an AAV in transgenic TRH-Cre mice to target the genetically encoded calcium indicator, GCamp6m, to the hypophysiotropic TRH neurons. Using gradient-index (GRIN) lenses and a 2g head-mounted microscope, we were able to perform deep brain imaging of multiple TRH neurons at the single cell-resolution level in freely moving mice. Results showed that hypophysiotropic TRH neurons are interacting with each other within local networks and modulate their activities throughout the day. We are currently dissecting the role of these networks using ex vivo slice preparations. Importantly, using tail blood sampling and a recent in house TSH ultra-sensitive ELISA, we are able to correlate the TRH neuron/terminal population activity with the level of TSH secreted by pituitary thyrotrphs. Finally, with the unique advantage of each mouse being its own control, we aim at comparing the TRH neuron activities coupled with TSH outputs in euthyroidic versus hypothyroidic mice. To summarize, we have developed new tools that are used to link, for the first time, the activity of endocrine TRH neurons and the resulting pituitary TSH secretion in health and disease. This work is supported by grants from FRM DEQ2015033732 and INSERM-Commissariat Montpellier.
#1. The unexpected level of plasticity of the oxytocinergic system

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Oxytocin is a neuropeptide found in many species. Oxytocin is mainly released by the paraventricular nucleus of the hypothalamus (PVN) and was first described in the control of parturition and lactation. Currently, this neuropeptide is well-known to impact a wide range of complex, various emotional behaviors, among which positively valenced emotions, such as social recognition and maternal care, and negatively valenced emotions, such as pain [1] and anxiety or fear [2]. In this context, it has been shown that oxytocin can activate a subpopulation of GABAergic interneurons in the lateral part of the central amygdala (CeL) that projects to its medial part (CeM) [3]. Their excitation leads to the inhibition of a subset of neurons which project to the brainstem [4] [5]. Here, we hypothesized that the efficiency of amygdala microcircuit modulation by oxytocin may vary as a function of the emotional status of the animal. Thus, we studied in anatomical detail how the amygdala microcircuit involves different oxytocinergic neurons, and precisely characterized their functional involvement in the regulation of contextual fear learning.

In a first attempt, using a combination of neuroanatomical fluorescent tracing, ex vivo patch-clamp recording of CeM neurons and genetic tools, we performed the structural and functional analysis of the oxytocinergic amygdala innervation in both naive and fear conditioned animals. In a second attempt, using the newly developed virus-delivered activity-induced tagging (vGATE) tool, we targeted the specific oxytocin neurons activated upon a contextual fear conditioning (OTFear+). This allowed us to express anatomical tracer Venus or the opsin channel-rhodopsin 2 (ChR2) in this specific subset of oxytocin neurons. We eventually deciphered the precise anatomical and functional involvement of OTFear+ neurons in the regulation of amygdala microcircuits.

As a result, we observed i) a complexification of the axonal PVN-CeL oxytocin projections as a consequence of the contextual fear conditioning; ii) the appearance of recurrent bursting activity, illustrating a modification of the amygdala network function as a consequence of the contextual fear conditioning; iii) a pharmacological switch from oxytocin-based release to glutamate-based release from OTFear+ PVN-CeL projections, fitting with our anatomical observations; iv) light-driven stimulation of OTFear+ PVN-CeL projections is sufficient to dramatically release the freezing response upon a fearful context.

In conclusion, we show with our combined approaches, an unexpected level of plasticity of the oxytocinergic system highlighting the importance of segregated oxytocinergic neuronal populations acting upon different emotional events.
Mécanismes neuronaux sous-tendant la perturbation du comportement de cour suite à l’exposition adulte au di-(2-éthylexy1) phthalate chez la souris.

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Enfin, j’ai cherché à déterminer les mécanismes d’action du DEHP. Pour cela, des études moléculaires ont été menées et révélées par l’analyse des altérations comportementales de l’axe gonadotrope ou les niveaux circulants de testostérone. Une analyse protéomique par séparation des protéines par 2D-DIGE et identification par LC-MS-MS de la région préoptique, région clé dans la motivation à accoupler et à produire des vocalisations, a retrouvé un effet neural au DEHP. En effet, l’analyse du réseau de protéines par Ingenuity Pathway Analysis a mis en évidence un effet neural au DEHP. En effet, l’analyse du réseau de protéines par Ingenuity Pathway Analysis a mis en évidence un effet neural au DEHP. En effet, l’analyse du réseau de protéines par Ingenuity Pathway Analysis a mis en évidence un effet neural au DEHP. En effet, l’analyse du réseau de protéines par Ingenuity Pathway Analysis a mis en évidence un effet neural au DEHP. En effet, l’analyse du réseau de protéines par Ingenuity Pathway Analysis a mis en évidence un effet neural au DEHP. En effet, l’analyse du réseau de protéines par Ingenuity Pathway Analysis a mis en évidence un effet neural au DEHP. En effet, l’analyse du réseau de protéines par Ingenuity Pathway Analysis a mis en évidence un effet neural au DEHP. En effet, l’analyse du réseau de protéines par Ingenuity Pathway Analysis a mis en évidence un effet neural au DEHP. En effet, l’analyse du réseau de protéines par Ingenuity Pathway Analysis a mis en évidence un effet neural au DEHP. En effet, l’analyse du réseau de protéines par Ingenuity Pathway Analysis a mis en évidence un effet neural au DEHP. En effet, l’analyse du réseau de protéines par Ingenuity Pathway Analysis a mis en évidence un effet neural au DEHP.
Female mammals display ovarian and daily rhythms that ensure the right timing of reproduction. A hypothalamic population of neurons that release GnRH controls the synthesis and secretion of the lutenizing (LH) and follicle stimulating (FSH) hormones, which in turn control the gonadal activity. Two hypothalamic neuropeptides, kisspeptin and RF (Arg-Phe) amide-related peptide (RFRP), have been showed to act upstream and control the activity of the GnRH neurons (1, 2). Kisspeptin is already established as a potent stimulator of the GnRH neurons (3), while RFRP has been showed to either stimulate or inhibit the gonadotropic axis (4). Originally RFRP was found to inhibit gonadotropin secretion in birds and other species (6, 7, 8) but consequently it was showed that it has a stimulatory effect on Syrian and Siberian hamsters (9, 10). Moreover, it has been showed that the effect of the RFRP on the gonadotropic axis is sex-dependent, being stimulatory in males, while inhibitory in females (11, 12). Along this line, it was found that female Syrian hamsters have more RFRP neurons compared to males (11). Altogether these findings point towards an important role of the RFRP system in regulating reproductive activity. However, based on all those deviations, it is difficult to determine the functional role of the RFRP system.

Therefore, the aim of this study is to take advantage of RFRP-CRE mice to explore the role of RFRP on the gonadotropic axis of adult male and female mice. To this end, we perform cell-attached electrical recordings on coronal brain slices. Preliminary results show that RFRP neurons exhibit mainly silent or irregular firing patterns and partly bursting and tonic firing patterns. Furthermore, the firing pattern of the RFRP neurons appears to show fluctuation across the stages of the estrous cycle, being predominantly irregular during the estrous stage. Finally, other preliminary data point towards a small-scale response of the RFRP neurons to vasopressin, indicating a potential circadian control of the RFRP system.
#4. Kisspeptin and RFP3 modulate lean and fat mass in the Phodopus Sungorus via two different hypothalamic neuronal pathways

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The Phodopus Sungorus has adapted its reproduction according to the seasons, being sexually active under long days (LD) and inactive under short days (SD). Moreover, the body weight and food intake, two metabolic parameters, are also under photoperiodic regulation, with decreased bodyweight (BW) and food intake (FI) in SD. In parallel, kisspeptin (Kp) and RFRP3, two hypothalamic neuropeptides involved in the central control of reproduction, show strong photoperiodic variations.

Since the hypothalamus is a key structure for the central regulation of both reproduction and metabolism, we wondered whether the variation in RFRP3 and Kp could also be involved in the photoperiodic regulation of the hamster’s BW and FI. To test this hypothesis, we challenged the SD-adapted hamster’s metabolism with a chronic infusion of Kp or RFRP-3. Our data indicated that Kp displays significant metabolic effects inducing an increase in FI, BW and leptin. In addition, our data also showed a synergistic effect of chronic Kp, which was sex hormone–related because castration blocked the effects of Kp on BW and FI. In order to investigate the central targets of Kp and RFRP3, we performed acute injections of RFRP3. Our results by in situ hybridization analysis confirmed the acute orexigenic effect of RFRP3 and indicated that it is associated with an increased mRNA expression of NPY and Orexin. On the other hand, chronic infusion of Kp caused an upregulation of POMC mRNA.

Altogether, our data indicate that Kisspeptin and RFRP3 modulate the seasonal energy metabolism via two different mechanisms: Kisspeptin increases lean body mass through the POMC neurons in a sex steroids-dependent manner, whereas RFRP3 increases body weight by modulating food intake and lipid metabolism via the NPY and Orexin system.
#5. A role for tanyocyte exocytosis in the central control of energy homeostasis?
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Introduction: The control of food intake and energy expenditure that allows for the maintenance of body mass, requires a continued dialogue between the periphery and the hypothalamic region of the central nervous system. 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 highly specialized ependymoglial cells called tanyocytes. The tanyocytes have recently been shown to shuttle metabolic signals such as leptin into the cerebrospinal fluid, via transcytosis.

Aim: Identifying the molecular mechanisms involved in this transport seems essential to our understanding of the phenomenon of central hormone resistance, found in obese and type 2 diabetes patients.

Experimental approach: After an infusion of a recombinant fusion protein (TAT-Cre) in iBot mice (from F W Pfrieger lab) in the 3rd ventricle, we investigate the effect of the expression in tanyocytes of the type B botulinum neurotoxin BoNTB on the central control of energy homeostasis. BoNTB inactivates synaptobrevins 1-3 by proteolytic cleavage and thus alters synaptobrevin-mediated exocytosis.

Results: Our results show that selectively impairing synaptobrevin function in tanyocytes alters basal food intake in male mice but also progressively the glucose tolerance. In order to verify whether BoNTB expressing tanyocytes alters the transport of leptin into the brain, quantification of hypothalamic P-STAT3 activation and a study of the blood-brain barrier were performed on this mouse model.
Hypothalamic astrocytes and tanyocytes are prominent producers of lactate compared to cortical astrocytes but seem to involve different precursors: A potential distinct role in the regulation of energy balance and reproductive function

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The ability of the hypothalamus to detect changes in plasma glucose concentration is critical for the regulation of energy balance and has an impact on the regulation of the reproductive function. Among the mechanisms involved in hypothalamic glucosensing, a metabolic interaction between glial cells and neurons via lactate transfer has been proposed. The hypothalamus has the particularity to possess two types of macroglial cells astrocytes, and tanyocytes. Unlike for the cortex, little is known about their cellular metabolism and their function in supporting neuronal metabolism. Knowing that the hypothalamus and the cortex have different functions, one may wonder whether the degree of glycolytic metabolism and the metabolic response of hypothalamic glial cells are the same than cortical astrocytes. Indeed, previous studies revealed differences for other metabolic pathways between hypothalamic and cortical astrocytes. So, we generated primary cultures of tanyocytes, hypothalamic astrocytes and cortical astrocytes from mice to study and compare their cellular metabolism.

By biochemical analysis, we highlighted that, for example, hypothalamic astrocytes consume more glucose and release more lactate than cortical astrocytes under basal condition. This difference of metabolic phenotype is explained by the different expression of key actors involved in the glucose and monocarboxylate transporter isoforms, as well as the hexokinase and pyruvate kinase isoforms which has been characterized by RT-qPCR and western-blot. Our results showed that the basal rate of lactate for hypothalamic astrocytes would be dependent on the expression of a heterodimer of PKM2/Pyruvate kinase isozymes. In an interesting manner, our results also showed that the regulation of the metabolism of these two types of astrocytes differs. In contrast to cortical astrocytes, exposure to glutamate did not enhance glucose utilization and lactate production in hypothalamic astrocytes.

Regarding tanyocytes, we also observed that these glial cells express high levels of MCT4, allowing them to release as much lactate as hypothalamic astrocytes. Surprisingly, exposure to glutamate did not increase glucose compared to hypothalamic astrocytes, despite a higher expression of the key glucose-sensing element GLUT2.

This study showed that hypothalamic astrocytes, tanyocytes and cortical astrocytes possess different metabolic phenotypes. The release of high levels of lactate via MCT4 seems to be a characteristic of hypothalamic glial cells, which may take part in hypothalamic fuel sensing. This study also showed that unlike hypothalamic astrocytes, tanyocytes seem to use other substrates besides glucose to produce lactate, suggesting a distinct role for these glial cells in hypothalamic fuel sensing.
The influence of maternal diet on progeny’s health has been thoroughly investigated regarding metabolic and cardiovascular diseases. However, the impact of a deleterious maternal diet on sensory systems is still unexplored. Olfaction is of great importance for numerous behaviors like avoiding predators and feeding. In childhood especially, olfaction might be involved in establishing food preferences, which partly determines adult eating habits. The olfactory system is made of sensory neurons that develop during the embryonic life, pursue their maturation after birth and are continuously regenerated over life. These neurons are known to be under metabolic influences, and patients with metabolic disorders present impaired olfactory sensitivity. But whether or not olfactory abilities are affected by the perinatal nutritional environment remains unknown.

Here we investigated the effect of a high fat high sucrose (HFHS) maternal diet on the olfactory system of the progeny, focusing on their behavior and the physiology of their olfactory epithelium.

Female mice were fed either with a control or HFHS diet during pregnancy and lactation. In the three-week-old male offspring, olfactory function was assessed by investigating their sniffing behavior. Using a plethysmograph, we recorded mice respiratory frequency before and during an odor presentation. Odor detection by the mouse is admitted when its respiratory frequency increases during odor presentation. The sensitivity of the olfactory epithelium was then measured using electroolfactogram (EOG) recordings.

At weaning, male mice from maternal diet fed dams exhibited a similar weight compared to mice from control diet fed ones, but presented increased visceral fat mass. The progeny of HFHS diet fed dams showed reduced olfactory detection ability for the odor of phenylethanol. Meanwhile, there was a slight change in the level of EOG responses. Interestingly, the sensitivity of the olfactory epithelium of progeny of HFHS diet fed dams tended to be increased. This non-intuitive finding needs further explanation.

Our preliminary results demonstrate that a HFHS maternal diet during pregnancy and lactation affects olfactory abilities in the offspring. Leptin produced by the adipose tissue is known to decrease olfactory abilities. Since we found a higher adiposity in the HFHS group, leptin signaling might be affected. It could be involved in the reduced odor detection ability. The central process of olfactory information will also be explored in order to explain the behavioral observations.

#7. Impact of a high fat high sucrose maternal diet on the progeny’s olfactory system
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Obesity is defined by the excessive accumulation of body fat and accompanied by chronic low-grade inflammation of peripheral metabolic tissues, especially of adipose tissue. Adipocytes secrete inflammatory mediators such as cytokines and chemokines, which can act at the cerebral level and modulate neuronal activity. The hypothalamus is an important region of the brain, which contains neural networks involved in the control of energy metabolism and feeding behaviour. Emerging evidence indicates that inflammation occurs also at the level of the hypothalamus.

We were interested in the inflammatory response of the hypothalamus and different adipose tissues to high fat diet (HFD) and its role in the development of diet-induced obesity (DIO). In particular, we are focusing on the role of the previously identified chemokine CCL5, in the central inflammation associated with the deregulation of energy metabolism and the pathogenesis of obesity. I addressed this question by comparing wild type and knockout CCL5 mice, which were fed either a standard diet or HFD. After 16 weeks of feeding, animals were sacrificed and serum, peripheral and cerebral tissues collected. Metabolic parameters, locomotor activity, expression levels of pro-inflammatory mediators and peptides involved in feeding behaviour were measured. Our results suggest that the absence of CCL5 seems to have a protective, age-dependent effect on the development of obesity and the associated metabolic impairment. Furthermore, knockout mice of CCL5 show a different expression pattern of inflammatory markers compared to control mice. Thus, CCL5 seems to be involved in the maintenance of overfeeding, through indirect action on neuronal hypothalamic systems involved in the control of food intake found in obesity.

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#9. Changes in neuroglial interactions on POMC neurons at the meal scale

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The melanocortin system of the arcuate nucleus is one of the best characterized neuronal feeding circuits. This includes POMC neurons which express proopiomelanocortin a precursor of the anorectic peptide α-MSH. In the adult brain, this system can undergo synaptic remodeling in response to change in blood hormones. However this phenomenon has been evidenced during extreme metabolic situations only, such as 24H-fasting or high-fat induced overfeeding, two situations characterized by large hormonal variations. Thus, the physiological role of this phenomenon remains to be clarified. In particular, we still do not know whether this synaptic plasticity is recapitulated in response to moderate hormonal fluctuations, as it happens at the meal scale.

To examine this possibility, we investigated neuronal and glial interactions onto POMC neurons in the arcuate nucleus in preprandial state (PRE) and postprandial state which correspond to 1H-Standard Diet (1H-SD) and 1H-High Fat Diet (1H-HFD) in adult mouse. These three distinct feeding situations are characterized by few, if any, variations in hormone levels. Morphometric analysis after electron microscopy and confocal imaging reveals changes in glial interactions on POMC perikarya in 1H-SD fed mice and changes in synaptic configuration in 1H-HFD fed mice. In 1H-SD fed mice, glial changes correlate with an increase in basal activity of POMC neurons.

These results show that synaptic and glial plasticity on POMC neurons activity are elicited at the meal scale in adult mouse. Because of their plasticity, these mechanisms might contribute to the regulation of satiety.
Apart from participating in lipid digestion, bile acids (BA) can promote energy expenditure (EE), participate in glycemic control and decrease body weight (BW) by binding to their specific receptor, the Takeda G protein-coupled receptor 5 (TGR5). These outcomes have increased importance in the context of obesity, but have only been studied in the periphery. We have found that TGR5 is expressed in anorexic POMC neurons of the arcuate nucleus (ARC), a hypothalamic structure that regulates energy homeostasis. What is more, when mouse brain slices are exposed to a selective TGR5 agonist, there is an increase in the activity of POMC cells. To test this in vivo, we used an obese rodent model that received the selective TGR5 agonist both acutely and chronically in the lateral ventricle. Acutely, central TGR5 agonism decreased food intake (FI), BW and feed efficiency. These effects were prevented by the central co-administration of a melanocortin receptor antagonist. Chronic central TGR5 agonism decreased FI, BW and fat mass, while increasing EE independently of locomotor activity. Thus, we propose that BA are among the lipid-related signals integrated in the ARC that provide information on energy availability. Once in the ARC, BA can activate TGR5 on POMC neurons, modulating FI and EE. Our findings contribute to the understanding of novel molecular targets against obesity.
High glucocorticoid signaling as a marker of depression in obese patients
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Neuroendocrine dysregulations, notably in the form of hypothalamus-pituitary-adrenal (HPA) axis hyper-reactivity, have been described as fundamental characteristics of neuropsychiatric disorders including depression. In this study we have assessed the relationship between mood symptoms and glucocorticoid receptor (GR) signaling before and after bariatric surgery-induced weight loss. Thirty-three obese patients (body mass index (BMI>35-50 kg/m2) seeking bariatric surgery were included. Twenty-five of them were followed up 4-12 months after surgery. Montgomery and Asberg Depression Scale (MADRS) was used to assess depressive symptoms before and after surgery. Blood samples were collected from the patients before and after surgery to assess GR signaling through a transcriptomic analysis. RNA was extracted from whole blood and gene expression profiling was assessed by pangenomic microarrays. GR signaling was then evaluated by calculating a composite z score of the expression of glucocorticoid target genes. Before surgery, patients with a MADRS score equal or above 15 (i.e. depressive patients) presented a significantly higher GR signaling than non-depressive patients (MADRS score < 15) (p<0.0005) but GR signaling was unrelated to body mass index. After surgery there were no longer differences in GR signaling between these same patients. Interestingly GR signaling decreased in the depressive patients together with depressive symptoms (p<0.0002). In conclusion these data suggest that GR signaling, estimated by transcriptomic analysis, is a good marker of depressive symptoms in obese patients.
#1. Effets cytoprotecteurs et différenciateurs de l'ODN sur des neurones murins N2a
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In male rodents, testosterone released by fetal and neonatal testicles permanently masculinizes and defeminizes the neural circuits involved in behavioral and neuroendocrine responses related to reproduction. In adulthood, it is necessary for their initiation and maintenance. In the nervous system, testosterone can act directly via the androgen receptor (AR) or can be metabolized into oestradiol, via stimulating the estrogen receptors (ERα and ERβ).

In order to study the contribution of each of these neural signaling pathways corresponding to the effects of testosterone, without altering their peripheral functions, my team generated a mouse line selectively invalidated for the genes encoding these receptors in the nervous system. The results obtained show that the AR signaling pathway, not ERb, is involved in the regulation of the neural circuitry involved in the expression of sexual behavior. In particular, AR plays a role in the postnatal maturation of spinal centers involved in erection and ejaculation, as well as adult activation of brain structures. Indeed, mutant males are sexually deficient and emit fewer ultrasonic vocalizations in the presence of receptive females, despite high testosterone levels, and normal olfactory preference and neuroanatomic organization of sexually dimorphic populations. ERα receptors appears to be involved in the perinatal organization of nerve structures by testosterone.

This study aims to identify the AR signaling pathway in the preoptic area, the key hypothalamic area involved in the expression of male sexual behavior, which was shown to be disrupted in the nervous system. Moreover, such signaling pathway is of a particular interest as it shows a great vulnerability following adult exposure to endocrine disruptors.

In order to identify the neural targets of AR, my team recently conducted a comparative study of the proteome of the preoptic area between control and mutant males by using 2D-DIGE technique followed by the identification of the proteins by LC-MS/MS (Salpêtrière platform). This revealed a number of cellular actors whose protein amount level was differentially expressed between these two genotypes. The obtained data and their validation will be presented in my poster.
Neuroendocrine cells store neurohormones through the biogenesis of dense core-secretory granules. These organelles bud from the TGN compartment after the interaction of neurohormone-chromogranin aggregates with the TGN membrane. Chromogranins are a family of soluble glycoproteins involved in secretory granule biogenesis. Indeed, chromogranin A (CgA) is the first member of this family which has been demonstrated to act as an on/off switch regulating the formation of secretory granules. Then, we decided to study the interaction between CgA and membrane lipids to highlight the molecular mechanisms controlling this process. Using lipid-protein overlay assays, we observed that recombinant CgA specifically binds to phosphatidic acid. Phosphatidic acid (PA) being known as a crucial actor in the formation of secretory granules and in the process of membrane curvature, we are currently studying the interaction between CgA and PA in neuroendocrine cells and its role in the regulation of hormone secretion. The quantitative and comparative analysis by LC-MS/MS of the lipidome of purified CgA granules and Golgi apparatus revealed an enrichment of PA in the CgA granules with the predominance of PA36:1, PA38:2 and PA40:6 species. Moreover, using a pull-down assay with liposomes enriched with various phospholipids including phosphatidylserine, phosphatidylcholine or phosphatidic acid species, we showed that CgA from cell lysate specifically interacts with the predominant PA species identified by the lipidome study. We postulate that CgA interacts with PA at the level of the TGN membrane, thus contributing to the formation of secretory granules and the recruitment of cytosolic proteins involved in the DCSG trafficking crucial to neurohormone secretion. To study these phenomena in living cells, we are currently working with organic chemists on the synthesis of biocompatible and photoactivatable PA analogues, and with biophysicists on the analysis of CgA/PA interaction-induced microdomains using Fluorescence Correlative Spectroscopy.
Mammals adapt their physiological functions like reproduction or metabolism to the seasons. The major environmental synchronizer is the light duration during 24h called photoperiod. Reproduction in most of the mammals is activated by summer long photoperiod whereas it will be inhibited in winter short photoperiod. If inhibitory short photoperiod is maintained over 20 weeks reproduction axis will escape to the photoperiodic control and reactivate endogenously. On male Siberian hamster, an hypothetic molecular pathway linking change in photoperiod to change in reproductive capability has been proposed. The melatonin is synthetized by the pineal gland only during the night. The summer short melatonin peaks remove the inhibition of the melatonin on pars tuberalis TSH production. This TSH will stimulate tanyctes and induce overexpression of Deiodinase 3 and inhibition of Deiodinase 3. This lead to a global increase of active thyroid hormone T3 in the mediobasal hypothalamus. By an unknown mechanism, T3 would increase neuropeptides Kisspeptin and RFRP3 expression (Revel et al., 2006, 2008), two well known regulators of the hypophyso-gonadotropic axis (HPG axis).

However, nothing is known about the endogenous reactivation of the reproduction. My goal was to confirm the hypothetic pathway and study its role in the endogenous reactivation. My results suggest the presence of an activation mechanism using 2 pathways, a fast one Dio3 dependent and a slower one TSH, Dio2 and neuropeptides dependent. Dio3 catabolize thyroid hormones that are able to stimulate the reproduction, the next part of my project is to confirm that Dio3 inhibition is enough to induce hypothalamic T3 peak able by itself to produce HPG axis activation. To finish we will analyze potential easy regulators suggested by bibliography, and we will research other candidates by RNA-seq.
Les neurones à kisspeptine du noyau arqué expriment plus fréquemment le récepteur à somatostatine SSTR1 que le SSTR2A chez le rat mâle.
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La somatostatine (SST) est un neuropeptide impliqué dans plusieurs fonctions au niveau central parmi lesquelles la modulation de la sécrétion de GH. Au laboratoire, nous avions montré une inhibition de la sécrétion de LH chez la brebis traitée par voie intracérébroventriculaire (icv) avec de la SST suggérant une action de ce peptide sur le contrôle central de la reproduction (Pillon et al., 2004). Comme la SST est co-distribuée dans les mêmes régions hypothalamiques que la kisspeptine (KP), le plus important stimulateur de la sécrétion de GnRH connu à ce jour, nous avions recherché les relations neuroanatomiques existant entre les deux populations chez la brebis et chez le rat. Chez cette espèce, la plupart des neurones à KP du noyau arqué hypothalamique montrent des appositions à SST chez les deux sexes. Pour déterminer si la SST peut affecter l’activité cellulaire des neurones à KP, il est maintenant nécessaire de déterminer si des récepteurs à SST, les SSTR, sont présents dans les neurones à KP. Nous avons recherché la présence de SSTR1 et de SSTR2A dans le cerveau de rats Wistar mâles adultes traités 24h avant leur mort par une injection icv de colchicine. Ces animaux ont reçu 35 min avant leur mort l’injection d’1nmol d’octreotide (Tocris) afin de favoriser leur détection (Casba et al., 2003). Après perfusion, les cerveaux ont été traités pour permettre une double immunocytochimie séquentielle permettant de détecter soit KP et SSTR1 ou KP et SSTR2A. Nous avons constaté une distribution superposable des neurones IR pour KP et SSTR1 et des neurones IR pour KP et SSTR2A. Nous avons constaté qu’un peu moins d’un tiers des neurones à KP et de SSTR1 sont IR pour KP et SSTR2A dans les neurones à KP. Ces résultats demandent à être confirmés et élargis. Ils suggèrent que l’action de la SOM sur KP passerait par le SSTR1 qui est plutôt associé à un rôle inhibiteur de la SOM.
Polycystic ovarian syndrome (PCOS), is the most common endocrinopathy among women of reproductive age and the main cause of female infertility worldwide. PCOS is characterized by excessive ovarian androgen secretion and chronic oligo-anovulation. Most PCOS women exhibit elevated luteinizing hormone (LH) levels, suggestive of rapid gonadotropin-releasing hormone (GnRH) surge and high levels of Anti-Müllerian Hormone (AMH) as compared to control women. While the exact origin of PCOS is unknown, recent clinical and animal studies suggest that it may originate in utero and that environmental influences, such as hormonal changes during fetal life, may be important biological factors in PCOS.

To study the effects of in utero AMH exposure on the hypothalamic–pituitary–gonadal (HPG) axis function of the offspring, we treated pregnant mice with AMH and followed the neuroendocrine phenotype of their female progeny as adults.

Prenatal AMH-treated (PAMH) female offspring recapitulated the major PCOS cardinal neuroendocrine reproductive features, namely hyperandrogenism, elevation in LH pulse frequency and oligo-anovulation. PAMH female offspring also exhibited masculinization of the sexually dimorphic brain nuclei that regulate reproduction, an increase in the excitatory drive to GnRH neurons and a persistent elevation of GnRH neuronal firing activity in adulthood.

These results show that fetal exposure to excess AMH induces permanent changes in the hypothalamic–pituitary–gonadal (HPG) axis of the offspring and identify AMH as a fetal programming factor of PCOS.
In Alzheimer’s disease (AD), cognitive deficits and psychological symptoms are associated with an early deregulation of the hypothalamic-pituitary-adrenal (HPA) axis associated with elevated glucocorticoids (GC) in plasma and CSF. Through their interaction with glucocorticoid receptors (GR), GC are crucial to maintain homeostasis and control numerous brain processes such as memory and learning.

Here, in an acute model of AD obtained after a single ICV injection of an oligomeric solution of an Aβ fragment (oAβ25-35), we analyzed the impact of Ab toxicity in the prefrontal cortex (PFC), a structure particularly involved in the control of the HPA axis, cognitive and affective processing, and prematurely altered in AD. The particular trait of this region is that GR levels are 4- to 5-fold higher than mineralocorticoid receptor levels, suggesting that PFC could be particularly sensitive to a deregulation of the HPA axis activity or a modification of GR functioning.

Based on our previous reports, this in vivo study aims to evaluate in the PFC (1) the time-course impact of 2 doses of oAβ25-35, (2) the effects of a new selective modulator (Corcept Therapeutics) on the principal hallmarks of AD and (3) on the principal intracellular pathways involved in the activation of GR. We showed that the dose of 10 µg oAβ25-35/rat was the more efficient to alter GC levels and PFC oxidative stress. Treatments with selective GR modulator CORT113176 reversed the PFC amyloidogenic pathway induced by oAβ25-35 by inhibiting BACE1 and increasing one enzyme involved in Aβ clearance, IDE. The modulator restored the PFC levels of synaptic markers, reversed neuroinflammation and apoptotic processes, re-established plasma levels of GC and restored cognitive functions. In addition, in the PFC, CORT113176 reversed the over-activation of GR induced by oAβ25-35 through a decrease in GR phosphorylation.

In conclusion, the PFC appears to be particularly sensitive to the deregulation of the HPA axis induced by Ab. This study confirms that the selective GR modulator is very attractive tool for AD treatment and thus places GR as a central player in AD. (This project is sustained by France Alzheimer, FRC & the LabEx LipSTIC)
26RFa: a new actor of the central regulation of glucose homeostasis

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26RFa, a neuropeptide expressed in the hypothalamus and at the periphery, was identified as the endogenous ligand of the orphan human receptor, GPR103. This neuropeptide is known to strongly stimulate food intake and this orexigenic activity is accentuated in obese-diabetic animal models. Recent studies revealed that neuropeptides regulating hypothalamic feeding behavior may also be involved in the regulation of glucose homeostasis. Indeed, we recently showed that 26RFa acts as an incretin to regulate glucose homeostasis. It is now well accepted that the hypothalamus plays a crucial role in the regulation of glucose homeostasis, acting in coordination with the pancreatic islets.

Altogether, these observations prompted us to investigate whether 26RFa may play a role in the hypothalamic regulation of glycaemia. For this, we examined whether central administration of 26RFa may modulate the response to a glucose challenge and to insulin in C57BL6/J mice. In contrast to what was observed peripherally, central administration of 26RFa exerts an anti-hyperglycemic effect similar to that observed peripherally, which is associated with an insulinotropic activity of the neuropeptide. However, in contrast to the insulin-sensitizing effect observed peripherally, central administration of 26RFa induces an insulinoresistance. In order to determine whether the hypothalamic 26RFa/GPR103 system is involved in the regulation of glucose homeostasis, we examined whether the expression and the secretion of 26RFa by hypothalamic neurons can be regulated by factors known to be involved in the regulation of glycaemia. To address this issue, we used two complementary models i.e. culture of hypothalamic neurons (the mHypoA-59 cell-line) and mouse hypothalamic explants. In our experimental conditions, glucose does not impact neither the expression nor the release of 26RFa in both models. In contrast, we found that insulin strongly stimulates 26RFa secretion by hypothalamic neurons whereas leptin inhibits 26RFa expression without affecting production of the neuropeptide. In conclusion, our data reveal for the first time, that hypothalamic 26RFa-expressing neurons are involved in the central regulation of glucose homeostasis.
#9. Non-obese type 2 diabetes impairs emotional behavior and leads to medial prefrontal cortex alterations

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An expanding body of evidence documents an increased co-occurrence of type 2 diabetes (T2D) with psychiatric disorders such as anxiety and depression (Roy and Lloyd, 2012); however the nature of the underlying co-morbidity is unclear. In animals, emotional disturbances have been recurrently reported in obese models of T2D (André et al., 2014; Dinel et al., 2011; Zemdegs et al., 2016). Since T2D is not always associated with obesity, our study aims to explore emotional behaviors in Goto Kakizaki (GK) rats, a non-obese model of T2D.

GK rats were generated by repeated inbreeding of Wistar rats selected at the upper limit of the normal glucose tolerance (Portha et al., 2012). Emotional behaviors, hypothalamic-pituitary-adrenal (HPA) axis response to stress and inflammatory markers in emotion-related brain areas were assessed in 2 month-old GK and control Wistar rats (n=6-8 per group). GK rats exhibited hyper-anxiety, reduced social behavior and hyper-response of the HPA axis to stress compared with Wistar rats. The mPFC was the most affected brain area showing increased expression of 5HT-R1a (serotonin receptor 1a), 5HTT (serotonin transporter) and a 4-fold increase of interleukin-6 (IL-6). GK rats also exhibited a decrease of astrocytes density and an increase of microglia process length in the prelimbic cortex.

Our study reveals that type 2 diabetes, independently of obesity, impairs emotional behavior. Behavioral changes were associated with hyper-reactivity of the HPA axis and inflammation in the medial prefrontal cortex. Further studies are needed to determine the precise role of these alterations in the GK emotional phenotype.
The release of Gonadotropin releasing hormone (GnRH) from GnRH neurons in the pre-optic area of the hypothalamus is central to reproductive function in vertebrates. This release of GnRH requires a co-ordinated increase in GnRH neuron activity, which is regulated by the gaseous neurotransmitter nitric oxide (NO). The enzyme neuronal Nitric oxide synthase (nNOS) is required for the production of NO in neurons. In rodents, GnRH neurons are present in close proximity to nNOS expressing neurons of the Organ Vasculosum Laminae Terminalis (OVLT). The role of NO in regulating GnRH neuronal activity and fertility in adults has been well established by results from our lab and others. However, very little is known about the involvement of NO in the development of sexual maturity during infancy and pre-pubertal stages. Mice lacking the nNOS gene show deficits in activation of GnRH transcription during infancy and pharmacological inhibition of NOS in embryos disrupts GnRH cell migration. In order to understand the implications of knocking out nNOS on the output of the GnRH circuit, we carried out an electrophysiological study to compare GnRH neuron activity in wild type (WT) and nNOS knockout (nNOS KO) mice, both in adults and in prepubertal animals. Our results suggest that knocking out nNOS results in an increase in GnRH neuronal activity in both adults and neonates. This is in agreement with previous data from our lab where activation of NO release was shown to inhibit GnRH neuron firing. In order to further dissect the circuit dynamics of NO and GnRH neuron interactions, we also recorded the activity of nNOS expressing OVLT neurons in neonates in the presence of superfused GnRH peptide to understand whether GnRH was capable of regulating nNOS neuron activity. These experiments help further our knowledge about the interactions between nNOS and GnRH neuron activity and thus how these factors regulate sexual development and fertility.
Recent studies have uncovered an unexpected role for Anti-Müllerian hormone (AMH) in the central control of reproduction. In the pituitary, we showed that AMH stimulates secretion and pituitary gene expression of FSH in vivo in rats, specifically in females before puberty. Furthermore, we showed that GnRH lowers AMH receptivity in vivo and in vitro, identifying GnRH as a new regulator of AMH specific receptor (AMHR2) expression (Garrel et al, Sci Rep, 2016). This study aimed to elucidate the underlying mechanisms of GnRH regulation.

In a first step, we studied the regulation of human AMHR2 (hAMHR2) promoter by GnRH. This was done by transfection of the two mouse gonadotropes cell lines, αT3-1 and LBT2, with a reporter construct containing 2252 bp (-2197, +54) of the hAMHR2 promoter. We demonstrated in LBT2 cells that GnRH as well as the GnRH agonist Triptorelin (GnRHa) stimulate, in a dose-dependent manner, hAMHR2 promoter activity. A similar effect was observed with a shorter construct (428 bp (-374, +54) of hAMHR2 promoter) suggesting that the regulatory sequences targeted by GnRH are restrained to this fragment. Moreover, no GnRH effect was observed in the less differentiated αT3-1 cells, indicating that appropriate transcription factors are probably missing in this cell line. Although the transcription factor SF1 has been involved in the expression of hAMHR2 in human or in rodent testis cell lines, mutation of the two SF1-binding sites or overexpression of the SF1 did not affect AMHR2 expression. We demonstrated by pharmacological approaches that Jun N-terminal Kinase and beta-catenin signaling pathways are involved in GnRH effect. Complementary strategies (dominant negative TCF4 and TCF sites mutation) are currently developed.

Contrasting with human promoter, no effect of GnRHa was observed on a transiently transfected mouse Amhr2 promoter (-1600, +50). However, using RT-qPCR, we found a decrease (30±4%) in endogenous Amhr2 mRNA levels after a continuous treatment of LBT2 cells with GnRH that was not associated with reduced transcript stability. Interestingly, when GnRH was delivered in a pulsatile fashion (2 pulses/h), it significantly increased Amhr2 mRNA levels (133±11%) demonstrating that Amhr2 is differentially regulated according to GnRH pulse frequency.

Altogether, our study identifies GnRH as a new regulator of AMHR2 in human and mouse and highlights a crosstalk between AMH and GnRH to regulate gonadotrope function.
#12. Pattern of LH secretion after repeated treatment with kisspeptin analog C6 reveals differential sensitivity between mice and ewes

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Modulation of kisspeptin system could be beneficial to treat human infertility as well as to manage reproduction in domestic animals. We developed a series of kisspeptin analogs with improved pharmacological features. The lead compound, C6, triggers fertile ovulation in ewes in both breeding and non-breeding seasons. In addition daily injection of C6 (5X0.15 nmol) in prepubertal female mice from post-natal day 26 to 30 significantly advanced puberty. In this experimental setting following 5 daily injection, C6 effect on LH secretion was reduced suggesting a possible desensitization. Desensitization of gonadotrophe cells by continuous stimulation is a feature utilized to treat some hormone dependent cancers such as prostate cancer. Therefore, for field and therapeutic applications it is of paramount importance to identify the dosing regimen necessary to obtained continuous stimulation versus inhibition.

We performed an initial experiment in adult mice ovariectomized (OVX) or OVX and replaced with estradiol (+E) to control for E feedback. In OVX+E mice daily injection of C6 (0.15 or 0.4 nmol/mouse ip) for 4 days produced no evident tachyphylaxis on LH plasma concentration increase. Similarly, in the basal LH level was already high and C6 injection had minimal if any effect on LH plasma concentration. In OVX and OVX+E mice, administration of GnRH at the end of the experiment caused a notable increase in LH. The possible difference in species sensitivity to repeated treatment we performed a similar experiment in OVX+E ewes. Intramuscular C6 injections were performed at 12 or 24-hour intervals (3X0.4 nmol/ewe/injection; 6 injections/ewe for the 12 hours dosing regimen). Regardless of dosing regimen, the first injection induced an LH increase. However, subsequent injections had modest or no effect on LH plasma concentration. Injection of GnRH (5 µg) at the end of the 12 hours dosing regimen significantly increased LH. Ongoing experiments aim at evaluating the effect of injections every 36 or 48 hours to establish the minimal interval required to obtain repeated stimulation. These results suggest the following conclusions: i) under our experimental conditions the capacity for increased GnRH secretion is the potential limiting requirement for continuous repeated C6 treatments; ii) a differential sensitivity to repeated treatment exists between species. Identification of the limiting conditions for sustained LH elevation or desensitization will provide relevant information for developing field and therapeutic applications but caution should be used in translating information from one species to another.
#13. Do endogenous kisspeptins and synthetic agonist C6 trigger different intracellular responses?
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Several forms of the endogenous neuropeptide kisspeptin (KP) have been described and named KP10, 13, 14, 16 and 54 based on their amino acids’ number. In mammals, acute KP administration invariably increase LH plasma concentration. Conversely, repeated administration or continuous infusion of KP could either result in desensitization with reduced LH secretion, or stimulation over an extended time window. Doses’ difference, peptide pharmacokinetics, species-specific sensibility and difference in receptor responses to different KP forms could account for these conflicting results. Surprisingly, no thorough in vitro study on this last aspect is available. Furthermore, the question about possible dissimilar intracellular mechanisms triggered by endogenous versus synthetic agonists has not been addressed. Considering the relevance of this topic for potential therapeutic applications, we initiated a study comparing the effect at cellular level of the human (h) endogenous ligands (hKP10, hKP13, hKP14, hKP16 and hKP54) and the kisspeptin analog C6. Experiments were performed in HEK-293 cells expressing hKISS1R and receptor activation recorded using a Ca²⁺-sensitive dye. All compounds showed similar potency and efficacy. Hence, we focused our attention on the hKP10 and hKP54, which are the most used for in vivo studies, and on C6. To explore potential difference in intracellular Ca²⁺ dynamic, Ca²⁺ mobilization was monitored for 30 minutes after stimulation with either of the drugs at 1, 10 and 100 nM concentration, hKP10 and hKP54 profiles were similar with a sharp initial increase of Ca²⁺ followed by a biphasic decrease including an initial rapid decline and a later more gentle one. However, at 100 nM, Ca²⁺ remained higher than basal level until experiment end. At 100 nM basal level was reached experiment end and at 1 nM Ca²⁺ returned to basal after about 50 minutes after stimulation. Conversely, after stimulation irrespective of drug concentration, Ca²⁺ levels did not return to basal. We start exploring the mechanisms underpinning the different phases of Ca²⁺ mobilization and their possible involvement in shaping differences in agonists’ response. Initial experiment were performed using hKP10 in the presence of caffeine (to block Ca²⁺ release from calcium store) or the absence of extracellular calcium (EGTA) did not block initial Ca²⁺ rise. However, induced Ca²⁺ decline to basal level completely abrogated the slow declining phase. Further studies are ongoing to clarify if this response pattern would be shared for all three compounds and to evaluate receptor expression after repeated stimulation.
The adult human hypothalamus contains putative neural stem/progenitor cell populations not found in the mouse, rat and grey mouse lemur (Microcebus murinus)

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The adult brain contains niches of neural stem cells that contribute to the addition of new neurons to selected circuits throughout life. Besides the subventricular zone of the lateral ventricles and the subgranular zone of the hippocampal dentate gyrus, which have been extensively studied, a third neurogenic niche has recently been identified in the adult hypothalamus of several animal models, mostly rodents. In order to evaluate whether a neural stem cell niche also exists in the adult hypothalamus in humans, we performed multiple immunofluorescent stainings to assess the expression of a panel of neural stem/progenitor cell (NPC) markers (Sox2, nestin, vimentin, GFAP, GLAST) in the human hypothalamus in comparison with the mouse, rat and non-human primate species: the grey mouse lemur (Microcebus murinus). Our results show that the adult human hypothalamus contains three populations of cells co-expressing the NPC markers: i) a ribbon of small stellate cells that lines the third ventricular wall behind a hypocellular gap and is similar to that found along the lateral ventricles, ii) tanyocytes, which line the floor of the third ventricle in the tuberal region, and iii) a population of small stellate cells in the suprachiasmatic nucleus. In the mouse, rat and mouse lemur hypothalamus, co-expression of NPC markers is found in tanyocytes in the suprachiasmatic nucleus, and these species lack a ventricular ribbon. Altogether, we identify in the adult human hypothalamus three distinctive cell populations harboring an antigenic profile of neural stem cells, two of which appear specific to humans.
The urotensin-II receptor is a new target for imaging neuroendocrine tumors with radiolabeled DOTA-peptide ligands

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Overexpression of G protein-coupled receptors (GPCR) in tumor has been exploited to develop GPCR-targeting radioligands for diagnosis approaches. In particular, somatostatin analogs labeled with 111In (111In-OctreoScan) are used to image neuroendocrine tumors. The vasoactive neuropeptide urotensin-II (UII), which shares structural analogies with somatostatin, interacts with a single high affinity GPCR named UT. We previously established that UII exhibits potent chemotactic properties to promote cell migration and angiogenesis in glioblastomas. High expression of UT in human solid tumors including lung, colorectal, prostate or breast carcinomas, suggests that UT constitutes an interesting target to design radiolabeled UII analogs for diagnostic or therapeutic approaches. In a first step, two urotensinergic ligands (UII and a biased analog utantide) containing the chelating group DOTA, able to bind radioactive isotopes, have been successfully synthesized.

A radiolabeling protocol of DOTA-UII and DOTA-urantide was first developed and validated. After a 3h period incubation in human plasma, only 30% of the radioligand showed degradation. Graded concentrations of both DOTA-UII and DOTA-urantide induced a dose-dependent increase in cytosolic calcium concentration in HEK293 cells expressing UT with a similar potency and efficacy to that obtained with UII (EC50 : 1.26 x 10-8 M and 2.09 x 10-8 M for Ull and DOTA-UII, respectively) or urantide (EC50 : 1.82 x 10-8 M and 1.52 x 10-8 M, urantide and DOTA-urantide, respectively). DOTA-UII was also able to bind to UT internalization (EUSA and immunocytochemistry) in HEK293 cells expressing UT, whereas DOTA-urantide was ineffective, together suggesting that DOTA analogs kept ability to bind and activate human UT.

To characterize the most appropriate cell lines for imaging tumors with DOTA-UII radioluclide, we have first characterized a series of human tumoral cell lines. Among 12 cell lines tested from brain, breast, lung, colorectal and prostate origins, only the four cell lines exhibited a strong expression of UT. Moreover, UT stimulation with UII stimulated cell proliferation (7/12) and/or cell migration (5/12). The most responsive cell lines are currently tested in vivo in xenografted mice. To control first the biodistribution of 111In-DOTA-UII, we performed injection in C57Bl/6J mice (mUTS2R+/+, mUTS2R-/-, or hUTS2R+/+). We observed a slight signal of 111In-DOTA-UII exactly at a similar level in all animals and without fast clearance of the tracer, indicating a binding of UT in physiological conditions. Although UII radioligands constitute a very promising candidate for the diagnosis and/or therapy of solid tumors.
High fat diet induces differential inflammatory and mitochondrial responses in C57BL/6J and WSB/EiJ mice

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Many social parameters can explain the obesity pandemic observed worldwide, as sedentary lifestyle, stress, shift work, and unhealthy food. Notably, fat rich food can disrupt energy balance and lead to obesity, which is often associated with pathologies grouped under the term “metabolic syndrome”. In rodents, high fat diet (HFD) prompts not only the development of diet-induced obesity (DIO), but also inflammation and mitochondrial dysfunction. In the brain, the hypothalamus controls many aspects of metabolic homeostasis, by regulating energy intake and energy expenditure. Among the hypothalamic nuclei, the paraventricular nucleus (PVN) and the arcuate nucleus (ARC) play a central role in metabolic regulations. The thyroid hormones (TH) are intimately linked to both inflammation and metabolism, with TH modulating inflammatory responses and energy expenditure. The wild-derived mouse strain WSB/EiJ is characterized by low levels of circulating TH, increased longevity and a striking resistance to DIO, when compared with the more common C57BL/6J. To identify factors underlying obesity resistance, we compared metabolic and inflammatory responses to short vs long term HFD treatment (for three days, 3d or eight weeks, 8w) in WSB/EiJ and C57BL/6J mice.

After 3d and 8w HFD, C57BL/6J mice, in contrast to WSB/EiJ mice, displayed significantly increased body weight, paralleled by increased circulating levels of leptin, cholesterol, HDL and LDL. WSB/EiJ mice displayed a more severe inflammatory status, both centrally (less activated microglia in the hypothalamus) and peripherally (lower levels of circulating cytokines). In order to identify genes potentially involved in these differential regulations, we selected 85 genes related to inflammation and mitochondria and analyzed their expression levels by high throughput microfluidic qPCR on RNA extracted from laser micro-dissected ARC and PVN hypothalamic nuclei of the two strains of mice under the different diets. We observed a differential expression of several of the selected genes between the strains and noted a differential effect of HFD on between both strains. Using bioinformatics tools, first we showed by PCA analysis that the differences in the gene expression data were for the segregation of the two datasets according to the strain. Second, we identified the biological pathways involved in the differential expression of these genes.

Our results shed light on the importance of inflammatory and mitochondrial pathways in the control of energy homeostasis.
Is lactate a new player in the central regulation of the Hypothalamic-Pituitary-Gonadal (HPG) axis?
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Regulation of the HPG axis involves, among others, hormones (i.e. steroids, leptin and insulin) and metabolic factors (i.e. glucose) which act directly, or indirectly on GnRH neurons via other neuronal populations or glial cells. Lactate is a novel factor, produced from glucose both by peripheral tissues (i.e. muscle) and by the brain (i.e. glial cells), that could play a key role in the regulation of the HPG axis. Dysregulation of lactate levels is described in numerous pathophysiological conditions associated with hypofertility such as excessive muscle activity, diabetic ketoacidosis, anorexia nervosa, and obesity. Recently, it has been shown that lactate infusion, in the 4th ventricle of the caudal hindbrain, restores LH secretion under hypoglycemic condition. We hypothesize that lactate could regulate directly GnRH neuron activity.

The presence of lactate transporters has been characterized in GnRH neurons by immunohistochemistry using GnRH-GFP male mice. GnRH cell bodies and GnRH neuronal projections are immunopositive for the neuronal lactate transporter MCT2, but also for 60% of them for MCT1, suggesting an action of lactate at both subcellular levels.

The direct effect of lactate on GnRH neuron activity has been studied using the Gnv-3 cell line, a GnRH neuronal cell line that possesses the capacity to stop its proliferation and mature. In condition of low glucose level (0.1mM), the presence of 0.5 mM of lactate restores the extracellular electrical activity of the GnRH network, comparable to what is observed in the 2.5mM glucose condition. GnRH secretion is also restored with lactate. In presence of the MCT1/2 inhibitor ARC-C155858, 14C-Lactate uptake by these neurones was decreased and their extracellular activity was not restored after adding Lactate. Although phosphorylation of AMPK has been observed in the low glucose condition, adding lactate does not decrease AMPK phosphorylation, suggesting that the effect of lactate on GnRH neuron activity does not appear to be AMPK-dependent.

This study shows that GnRH neurons are lactate sensing neurons via MCT1/2. Knowing the correlation between GnRH and LH secretion suggests that lactate could regulate fertility by acting directly in the brain.
Recently, our group suggested the existence of a pre-mammillary lateral hypothalamic (LHA) region characterized by an absence of glutamic acid decarboxylase expression, but the expression of the tachykinine1 gene as in medial pre-mammillary nuclei. Several cell groups are identified in this region: the paraventricular nucleus (Pvfox), the calbindin nucleus (Cbn), the parvafox nucleus (Pvfox) and the nucleus gemini (NG). Initial anatomical data suggest that networks involving these nuclei resemble to that of the laterally adjacent subthalamic nucleus (STN). Therefore, our investigations concerned the cortico-striato-pallidal circuitry converging in the direction of the PSTN and the Cbn as to understand their organizations. Tract tracing data show that both nuclei display strong anatomical links with the central nucleus of the amygdala (CEA) and the anterior part of the basomedial nucleus (BMAa). After fluorogold injections into the PSTN/Cbn, the medial part of the CEA (CEAm) appears to be the main supplier of projections from the CEA. Injections of the anterograde tracer, Phaseolus vulgaris leucoagglutinin (PHAL), into the CEAm and BMAa revealed that projections from the CEAm follow two pathways into the LHA. Moreover, PHAL injections into different parts of the insular cortex revealed micro-circuitries between the insular areas, the striatum and the pallidum with a distinct innervation of the different parts of the CEA and a convergence on the PSTN/Cbn. Five circuits from distinct agranular, gustatory or visceral areas of the insular cortex, most probably involved in distinct functions, were identified. These results help to better characterize the topographic organization of connections involving the insular cortex, the amygdala and the STN/CEA complex. This circuitry resemble to that described for the motor cortex and the STN.
Does the sodium leak channel NALCN contribute to tune action potential firing of adrenal mouse chromaffin cells?

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Adrenal chromaffin cells are neuroendocrine cells responsible for the secretion of catecholamines (mainly epinephrine, and norepinephrine). Once delivered into the blood circulation, epinephrine and norepinephrine exert multiple actions, leading to physiological adjustments enabling the organism to cope with a threat for its survival. Catecholamine secretory pattern is critically shaped by the electrical activity of chromaffin cells and elucidating the mechanisms regulating the firing discharge is therefore of a great interest.

In situ, chromaffin cell excitability is chiefly regulated by the splanchnic nerve cholinergic inputs but also depends on intrinsic ionic conductances expressed at the chromaffin cell plasma membrane. The background conductances operating near the resting membrane potential are crucial in the cell competence to spontaneously fire. In particular, the background current flowing through the sodium leak channel NALCN (a TTX-resistant and gadolinium-sensitive channel) has been recently reported to tune the resting potential of neuronal cells. The expression of the NALCN transcript in rodent chromaffin cells prompted us to investigate the possible contribution of this background channel to chromaffin cell excitability in mouse acute adrenal slices. Because NALCN channels conduct Na⁺ ions, we first decreased the external Na⁺ concentration. Reducing extracellular NaCl from 125 to 15 mM (N-Methyl-D-Glucamine or Tris substitution) leads to a robust membrane hyperpolarization (mean value of -8.7 ± 1.5 mV, n = 47), abrogating thus action potential discharge. We next investigated the role of NALCN in the development of this hyperpolarization through pharmacological experiments. Extracellular application of tetrodotoxin (0.5 µM) does not hyperpolarize the cell, ruling out the involvement of a voltage-gated Na⁺ conductance. Although eliciting a modest membrane hyperpolarization (mean value of -3.5 mV), bath-applied gadolinium (Gd³⁺, 10 µM) significantly reduced the firing discharge (9-fold decrease). Voltage clamp protocols indicate that decreasing external Na⁺ blocks a current, in a linear manner, between -130 and -50 mV. The calculation of reversal potential of the current argue for a non-selective conductance. Collectively, our results describe the presence of a voltage-independent, Na⁺-permeable conductance operating near the resting membrane potential of mouse chromaffin cells. Although additional experiments are required to unequivocally identify the nature of the channel(s) underlying this conductance, a TTX resistance and Gd³⁺ sensitivity match well two NALCN attributes.
#20. Maternal photoperiod programmes hypothalamic thyroid status via the fetal pituitary gland

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In wild mammals, offspring development must anticipate forthcoming metabolic demands and opportunities. Within species, different developmental strategies may be employed, dependent on when in the year conception takes place. This phenotypic flexibility is initiated before birth, and is linked to the pattern of day length (photoperiod) exposure experienced by the mother during pregnancy. This depends on transplacental communication via the pineal hormone melatonin. Here, we show that, in the Siberian hamster (Phodopus sungorus), the programming effect of melatonin is mediated by the pars tuberalis (PT) of the fetal pituitary gland, before the fetal circadian system and autonomous melatonin production is established. Maternal melatonin acts on the fetal PT to control expression of thyroid hormone deiodinases in ependymal cells (tanycytes) of the fetal hypothalamus, and hence neuroendocrine output. This sets the trajectory of reproductive and metabolic development in pups, and has a persistent effect on their subsequent sensitivity to photoperiod. This programming effect depends on tanycyte sensitivity to TSH, which is dramatically and persistently increased by short photoperiod exposure in utero. Our results define the role of the fetal PT in developmental programming of brain function, maternal melatonin, and establish TSH signal transduction as a key substrate for the encoding of internal calendar time from birth to puberty.
Selenoprotein T (SelT) is a recently characterized thioredoxin-like protein whose expression is very high during development, but is confined to endocrine tissues in adulthood where its function is unknown. We report here that SelT is required for adaptation to the stressful conditions of high hormone level production in endocrine cells. Using immunofluorescence and TEM immunogold approaches, we found that SelT is expressed at the endoplasmic reticulum membrane in all hormone-producing pituitary cell types. SelT knockdown in corticotrope cells promoted unfolded protein response (UPR) and ER stress, and lowered endoplasmic reticulum-associated protein degradation (ERAD) and hormone production. Using a screen in yeast for SelT membrane protein interactions, we sorted keratinocyte associated protein 2 (KCP2), a subunit of the protein complex oligosaccharyltransferase (OST). In fact, SelT interacts not only with KCP2 but also with other subunits of the A-type OST complex which are depleted after SelT knockdown leading to POMC N-glycosylation defects. This study identifies SelT as a novel subunit of the A-type OST complex, indispensable for its integrity and for ER homeostasis, exerting a pivotal adaptive function that allows endocrine cells to properly achieve the maturation and secretion of hormones.
#22. Impaired fasting blood glucose is associated to cognitive impairment and cerebral atrophy in middle-aged non-human primates

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Age-associated cognitive impairment is a major health and social issue because of increasing aged population. Cognitive decline is not homogeneous in humans and the determinants leading to differences between subjects are not fully understood. In middle-aged healthy humans, fasting blood glucose levels in the upper normal range are associated with memory impairment and cerebral atrophy.

Due to a close evolutionary similarity to humans, non-human primates may be useful to investigate the relationships between glucose homeostasis, cognitive deficits and structural brain alterations. In the grey mouse lemur, Microcebus murinus, spatial memory deficits have been associated with age and cerebral atrophy but the origin of these alterations have not been clearly identified.

Herein, we showed that, in 28 female grey mouse lemurs (age range 2.4-6.1 years-old), age correlated with impaired fasting blood glucose (rs=0.37) but not with impaired glucose tolerance or insulin resistance. In middle-aged animals (4-6 years-old), fasting blood glucose was inversely and closely linked with spatial memory performance (rs=−0.56) and hippocampus (rs=−0.62) or septum (rs=−0.55) volumes.

These findings corroborate observations in humans and further support the grey mouse lemur as a natural model to unravel mechanisms linking impaired glucose homeostasis, brain atrophy and cognitive impairment.
#23. Blood-borne liraglutide transport into the hypothalamus: a tanycytic route?
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Not available yet.
Ghrelin is a gut hormone processed from the preproghrelin gene and acting as the endogenous ligand of the Growth Hormone Secretagogue Receptor (GHS-R). Using preproghrelin-deficient mice, we previously demonstrated that endogenous ghrelin is a modulator of growth hormone (GH) peak amplitude in rodents. However, in this mouse model, both acyl and desacyl ghrelin are absent but the receptor is still functional and may maintain basal constitutive activity even in the absence of endogenous ligand. The specific contribution of acyl ghrelin through a GHS-R dependent mechanism of action in the control of growth in relationship with pulsatile secretion, metabolic parameters, meal pattern and activity has never been characterized.

In the present study, the effect of GHS-R invalidation on body weight and composition, meal pattern and the activity of the GH/IGF-1 axis were investigated in male and female C57BL/6 J littermates (WT) of both sexes. Body weight and growth length was monitored weekly from weaning. Blood samples were collected by tail bleeding every 10 h for a period of 6 h and parameters of GH secretion were analyzed by deconvolution. Meal frequency and ambulatory activity were measured in a LabMaster System and meal patterns were determined based on the following definition of a meal: at least 0.03 g of food separated from the next feeding episode by at least 10 min. Differences across sex and genotypes were identified by 2-way ANOVA and between genotypes (within same sex) by multiple comparisons, using Fisher LSD post-hoc analysis.

Young GHS-R KO mice displayed a deficit in weight and linear growth and decreased lean mass that were more pronounced in males than females but had intact fat mass and plasma leptin levels. Ambulatory activity was similar in both genotypes. Although daily food consumption was identical in WT and KO mice, growth deficit was accompanied by reduced meal frequency and increased intermeal intervals in KO male mice only. Interestingly, modifications in meal pattern in GHS-R KO mice was associated with significant reductions in hypothalamic arcuate expression of NPY and POMC. In females, total and pulsatile GH secretion and GH secretogogue-stimulated GH release were decreased in GHS-R KO pituitary explants, which may reflect the lower hypothalamic levels of NPY and POMC. In males, neither mass nor pattern of GH secretion was modified, suggesting that growth deficits may occur independently of altered GH secretion.

These results demonstrate that GHS-R modulates GH secretion and meal pattern independently, and that there is a sexual dimorphic effect of GHS-R invalidation on the regulation of growth and food intake in mice.

*GHS-R1a deficient mice were provided by AstraZeneca.

This work was supported by ANR JCJC ISO-GHRELIN (ANR-12-JSV1-0013-01) to VT.
Restrictive anorexia nervosa (AN) is an eating disorder that mainly affects young women and is characterized by a severe chronic food restriction and excessive physical activity. This disorder is currently the third largest cause of chronic illness in teenagers. Amongst the metabolic signals of negative energy balance that influence the activity of the GH/IGF-1 axis and are dysregulated in AN patients, ghrelin seems to play a pivotal role. Ghrelin is a 28-amino acid acylated peptide hormone predominantly secreted by the stomach. It is a potent GH secretagogue and orexigenic peptide discovered as the endogenous ligand of the GH secretagogue receptor (GHS-R) type 1a.

In order to decipher the role of ghrelin in the metabolic and neuroendocrine adaptations to chronic undernutrition, we submitted GHS-R KO mice to a protocol that combines caloric restriction to physical activity. In this model that mimics numerous behavioral, metabolic and neuroendocrine alterations observed in AN, we aimed to establish a link between changes in GH secretion and ghrelin and metabolic impairments.

Young adult (6-7 weeks old) female Ghsr-/- and Ghsr+/+ were submitted to a 3-weeks protocol that combines a 50% caloric restriction and voluntary activity in a running wheel (FRA) and compared to an ad libitum fed group with a free access to wheel activity (ALA). Wheel revolution was monitored thanks to a computer system linked to their wheel-equipped homecages. Blood glucose was measured every other day using a Glucometer 30 minutes prior to food distribution. At the end of the protocol, GH ultradian secretion was assessed by collecting blood samples by tail bleeding every 10 min over a period of 6 h. Body composition was determined at autopsy and plasma hormonal parameters were measured using selective immunoassays.

In both Ghs-r+/+ and Ghs-r-/- mice, body weight loss under FRA conditions was mainly due to a decrease in adipose mass, Ghs-r-/- mice exhibited a greater decrease in fat than Ghs-r+/+ mice whereas a similar decrease in muscle mass was observed in both genotypes. During chronic undernutrition, Ghs-r-/- mice exhibited a 33% decrease in blood glucose versus a mean decline of 16% in Ghs-r+/+ mice. While plasma IGF-1, insulin and ghrelin levels were higher between the genotypes in ALA conditions, high plasma ghrelin concentrations and low plasma IGF-1 and insulin concentrations were associated with elevated GH secretion in Ghs-r-/- mice, supporting a dysregulation of the ghrelin/GH/IGF-1 axis under chronic undernutrition as observed in AN patients.

These data indicate that ghrelin signaling is involved in the metabolic and neuroendocrine adaptations to chronic undernutrition in order to maintain sufficient body fat and blood glucose levels. Some of these effects may depend on ghrelin’s ability to promote GH secretion. The central mechanisms involved in coping with undernutrition remain to be elucidated.

*GHS-R1a deficient mice were provided by AstraZeneca. This work was supported by ANR JCJC ISO-GHRELIN (ANR-12-JSV1-0013-01) to VT.
A cluster of metabolic disturbances like hyperglycemia, dyslipidemia, central adiposity, and hypertension defines metabolic syndrome. The prevalence of metabolic syndrome is around 35% of the American population and is increasing because our food behavior and our way of life have changed. It is the main risk factor of diabetes mellitus, which is associated to micro and macro vascular changes. However, the early consequences of metabolic syndrome in the retina are not well described. Thus, we tested the hypothesis that feeding rats with high fructose and high fat would contribute to the development of MetS and retinal complications.

Male Brown Norway rats (6 weeks of age) were fed for 8 days, 4 weeks and 12 weeks with 60% fructose+10% lipid rich diet (HFHF), or a standard chow. At each time point, intraperitoneal insulin tolerance test (ITT-0.5 U/ml) and intraperitoneal glucose tolerance test (GTT-2g/kg body weight) were carried out. Blood was collected to measure insulin during GTT. Flicker (8Hz) electroretinograms (ERG) were recorded to evaluate the sensitivity of the retina. The rate of retinal neovascularization was evaluated by laser-induced choroidal angiography after laser rupture of Bruch’s membrane. Body fat was quantified using Echo MRI. At the time of euthanasia, blood was collected to measure lipemia, glycemia and plasma cytokine levels.

At the three time points, HFHF diet increased fasting glycemia (+20% for 8 days, +12% for 4 weeks, +6.5% for 12 weeks) as compared to standard chow diet (p<0.01). Moreover, HFHF feeding induced a significant increase in plasma glucose in ITT (p<0.05) and in GTT (p<0.05), with an elevated insulin response at 12 weeks compared to control group. Our data highlighted a partial loss of the sensitivity to light, which led to 4 weeks with HFHF as revealed by 8Hz Flicker ERG (Δ =0.5 log unit). Moreover, HFHF triggered a significant increase of the neovascularization two (+72%, p<0.05) and three weeks (+67%, p=0.05) after laser induction of neovascularization, compared to rats fed the standard chow diet. After 4 weeks of diet, the data of body composition indicated a 30% increase of body fat in HFHF fed rats (p<0.01). Dyslipidemia or cytokine dysregulation were not revealed by plasma analyses.

The consumption of high fructose and high fat diet triggered dysregulation of glucose metabolism, loss of cone sensitivity and emphasized choroidal neovascularization in our model. Our data suggest that the altered retina might be related to sugar metabolism disturbances and not to lipid disturbances.

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Structural sex differences in the human brain have been consistently described. These include sex differences in overall brain structure and in specific brain regions. Delineating the mechanisms underlying the sexual differentiation of the human brain is important for understanding the origins of brain development and the etiology of neuropsychiatric disorders with a sex difference in prevalence, such as depression, anxiety disorders, autism and attention-deficit hyperactivity disorder. Animal studies have consistently shown that male-typical neural characteristics develop under the influence of testosterone produced by the testes during perinatal development. Available clinical evidence in humans also suggest an important role for androgens in the development of a male-typical brain. There is however, increasing evidence that genes on the sex chromosomes might also play an important role in the sexual differentiation of the brain, but it has been shown difficult to disentangle the effects of sex chromosome genes from hormonal effects. Women with complete androgen insensitivity syndrome (CAIS) provides a unique opportunity to study the relative importance of sex hormones and sex chromosome genes in the sexual differentiation of human brain structure and function. Women with CAIS have a 46,XY karyotype and normally functioning testes that secrete normal or exaggerated amounts of testosterone while in situ. Mutation(s) in the androgen receptor (AR) gene lead to absent or dysfunctional androgen receptors resulting in androgen resistance, resulting in a female phenotype regardless of testosterone concentrations within the normal range.

In the present study, we compared gray matter (GM) volumes in 21 women diagnosed with CAIS, 33 control men, 33 control women taking hormonal contraceptives, 31 control women with regular menstrual cycles. Voxel-based morphometry analyses (SPM12) were conducted using high-resolution T1-weighted anatomical images. Images were segmented, modulated, normalized into a study-specific Dartel template and smoothed with an 8 mm FWHM Gaussian kernel. A full factorial analysis was then used to assess whole-brain potential differences in GM volumes between the groups and accounted for total intracranial volume. Sex differences were observed with control women showing greater GM volumes in the parietal, temporal, olfactory gyri, middle temporal pole, dorsal areas, thalamus, hypothalamus and cingulate regions compared to control men. Furthermore, women with CAIS had greater GM volumes in the cuneus, superior medial frontal gyri, supplementary motor area, parietal lobule, and olfactory areas compared to control men. All other contrasts (CAIS vs control women, control women vs control men, CAIS vs control men) did not show significant differences at p<0.05 FWE. These results of female-typical GM volumes in women with CAIS suggest a more important role for androgens than sex chromosome genes in the sexual differentiation of gray matter.

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#28. Une alimentation maternelle enrichie en gras pendant la gestation ou l’allaitement oriente les préférences du souriceau vers un aliment gras à la naissance et au sevrage.
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Les modèles murins montrent que les préférences et le contrôle de la prise alimentaire à l’âge adulte peuvent être perturbés par un environnement nutritionnel enrichi en gras pendant la gestation et l’allaitement, avec un accroissement de la vulnérabilité à l’obésité nutritionnelle. Par ailleurs, les préférences chimiosensorielles et alimentaires s’expriment bien avant l’âge adulte : des souriceaux nouveau-nés expriment une préférence pour l’odeur d’un lait ou pour la saisie d’une tétine d’une femelle de même fond génétique ou dont le stade de lactation correspond à leur âge. Pour autant, les effets d’un régime maternel obésogène sur le comportement alimentaire du nouveau-né et du jeune au sevrage sont encore largement méconnus.

Nous avons donc analysé les préférences sensorielles et alimentaires de souris C57Bl/6, à la naissance (P2) et au sevrage (P17), suite à une exposition à un environnement nutritionnel enrichi en gras pendant la gestation et/ou l’allaitement. Pour cela, nous avons réalisé, avant toute ingestion lactée, des adoptions croisées de portées de femelles exposées ou non à un régime gras. Quatre groupes de souriceaux, exposés 1) pendant la période fœtale, 2) pendant l’allaitement, 3) pendant ces 2 périodes, ou 4) non exposés à un environnement nutritionnel enrichi en gras, ont ainsi été testés à P2 pour leur préférence olfactive entre 2 laits produits ou non sous régime gras et à P17 pour leur préférence alimentaire entre 2 types de croquettes enrichis ou non en gras.

Les résultats montrent que les souriceaux à P2 s’orientent significativement plus longuement vers le lait qui correspond à leur environnement nutritionnel prénatal. Ils confirment l’importance des conditions physiologiques lors du développement foetal sur la mise en place des préférences sensorielles à la naissance. Au moment du sevrage, à P17, avant toute expérience directe avec les aliments solides, les résultats montrent une préférence plus importante pour l’allaitement de la part des souriceaux exposés à un environnement nutritionnel enrichi en gras avant et après leur naissance. Ces expériences confirment l’importance d’une émancipation maternelle sur le développement des préférences alimentaires, indiquant que cette influence peut être également lors de l’allaitement maternel et est plus résistante. De plus, ces résultats suggèrent que ces conduites orienteraient la vulnérabilité à l’obésité et orienteraient vers des aliments obésogènes très précocement.
Current feeding behaviors contribute to the epidemic levels of obesity and diabetes observed in Europe and worldwide. Both the quantity and the quality of ingested food are incriminated. Together with other sensory modalities, olfaction is involved in the control of food intake. Olfactory cues can influence eating behaviors, yet the nutritional status and diet can also alter olfactory abilities. Patients with metabolic disorders present impaired olfactory sensitivity which could in turn worsen their eating behaviors.

Here we examined the short-term impact of a Western diet enriched in fat and sugar (High Fat High Sugar, HFHS) on the anatomy and physiology of the olfactory epithelium of postnatal mice. We used a transgenic line of mice expressing GFP under the promoter of the SR1 odorant receptor in order to monitor the properties of a defined population of neurons. After 8 weeks of diet, HFHS fed animals were glucose intolerant without any change in basal glycaemia and insulinemia. They presented higher adiposity but no overweight compared to control mice. We measured electro-olfactogram amplitudes in response to three ligands of the SR1 olfactory receptor: amyl acetate, acetophenone and (R)-(+)carvone. Detection thresholds of amyl acetate and acetophenone estimated from the dose-response curves were higher after 8 weeks of HFHS diet. Reconstruction of the cilia of SR1 olfactory sensory neurons revealed shorter cilia in HFHS mice compared to control animals (4.5 ± 0.3 μm vs 6.0 ± 0.3 μm, p < 0.01). A buried food test revealed impaired olfactory capacities in the HFHS group.

Our results demonstrate that diet enriched in fat and sugar can rapidly alter the physiology of the olfactory epithelium. Anatomical changes of individual olfactory sensory neurons may participate to the reduced olfactory sensitivity. These olfactory dysfunctions appeared even after a short-term Western diet and led to altered olfactory behavior.
Leptin is a hormone secreted by adipose tissue that acts in the central nervous system notably as a negative feedback signal to regulate appetite. Nowadays, it is well-established that obesity is due to multiple mechanisms that alter leptin signaling which therefore amplify the extent of weight gain induced by genetic and environmental factors. We have recently hypothesized that this so-called leptin-resistance observed in most obese people, could also be the consequence of a defective leptin transport into the brain.

Tanycytes are specialized hypothalamic glial cells lining the floor of the third ventricle and contacting the fenestrated vessels in the median eminence. It has been proposed that tanycytes act as « gatekeepers » by regulating the access of blood-borne signals to the hypothalamus, and are involved in leptin transport for release into the cerebrospinal fluid from where it enters other leptin-sensitive regions. However, the cellular and molecular mechanisms controlling leptin internalization, its transcytosis as well as its release at the apex site of the tanycytes remain currently unknown. Using fluorescence and electron microscopy on primary cultures of tanycytes, we are deciphering here the endocytic route taken by leptin. Moreover, in order to maintain the topology of the cells, we are implementing tanycytes cultured in basal membrane extract gels. Leptin release from these cultures is monitored by ELISA assay and we focused on the role of synaptobrevin-2 (VAMP2), a component of the SNARE complex, which is a key component of the exocytotic machinery in glial cells. Altogether, these data provide a promising start toward the understanding of the leptin journey in tanycytes.

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#31. Role of phospholipase D-derived phosphatidic acid in neuroendocrine secretion

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The secretory pathway in neuroendocrine cells involves vesicular trafficking that leads to the release of hormones and neuropeptides in the extracellular medium. The final stage of this pathway, known as regulated exocytosis, consists of the tethering and docking of the mature vesicles or granules to the plasma membrane and ends by the fusion of secretory and plasma membrane. In addition to the important role played by proteins, recent studies have highlighted the involvement of membrane lipids to promote regulated exocytosis. Among them, phosphatidic acid (PA), the simplest glycerophospholipid, is known to generate membrane curvature and recruit key proteins. In consequence, PA appears as an attractive candidate to regulate different steps of the exocytotic process. Using adrenal chromaffin cells as a model of neuroscience, our previous studies demonstrated that PA synthesis by the enzyme phospholipase D1 (PLD1) at the plasma membrane plays a positive role during secretory granule exocytosis. More recently, using amperometric recordings and electron microscopy on PLD1-knockout mice, we found that PLD1 regulates the fusion pore dynamics and promotes docking of secretory granules in chromaffin cells.

Novel PLD1 inhibitors reproduce the effects previously observed in PLD1 knockout chromaffin cells, confirming the essential role of PLD1 activity in the regulation of regulated exocytosis. Using a pharmacological approach, we aim now to further understand the precise role of PLD1-derived PA in the tethering of secretory granules to the plasma membrane. To follow the dynamics of granules in the absence of PLD1 activity, we performed time-lapse confocal microscopy in chromaffin cells where PLD1 activity is inhibited. Tracking analysis showed reduced motility of the granules consequent to inhibition of the enzyme. Of interest, the granules that displayed a directed trajectory have their motility specifically reduced, suggesting that PLD1-mediated PA regulates transport of granules toward the plasma membrane. Furthermore, analysis of plasma membrane sheets by electron microscopy indicated that PLD1 could contribute to the formation of actin bundles, essential cortical cytoskeletal structures that link docked granules to plasma membrane. With the help of a genetically encoded PA probe expressed in chromaffin cells, we analyzed the precise distribution of this lipid near the exocytotic site and cytoskeletal structures, as well as its potential molecular partners during exocytosis.
Previous work in seasonal mammals like hamster or sheep have highlighted the role of melatonin action on the pituitary pars tuberalis to increase thyroid stimulating hormone (TSH) production under long days. TSH in turn acts on tanycytes, glial cells lining the basal part of the 3rd ventricle, to regulate the balance in deiodinase2/deiodinase3 activities leading to increased local concentration of T3. Although this melatonin-driven TSH/T3 signal is pivotal for synchronizing reproduction with the seasons, T3 cellular targets have not been established. In hamsters, two hypothalamic peptides known to regulate GnRH neurons, kisspeptin and (Arg)(Phe)related peptide (RFRP), are upregulated in long day-adapted sexually active animals but whether this seasonal regulation depends on a direct effect of T3 is unknown. In this study we hypothesised that mice, although showing no overt seasonal functions, could help us disclosing the link between hypothalamic T3 and kisspeptin/RFRP. First, we observed that melatonin-proficient CBA mice or melatonin-deficient C57 mice supplemented with melatonin in drinking water display an expected melatonin-dependant regulation of pars tuberalis TSH, tanycytic Dio2/Dio3 and hypothalamic RFRP (but not kisspeptin). Next, by comparing the effect of melatonin supplementation in wildtype C57 or C57 mutated for the T3 receptor TRα, we found that in mice lacking TRα, melatonin still regulates Dio2/Dio3 expression but do not inhibit RFRP expression anymore. Altogether our data indicate that mice, similar to seasonal mammals, integrate the seasonal melatonin signal up to the hypothalamic RFRP and that the effect of T3 on RFRP expression depends on the effect of T3 on TRα.
#33. Saisonnalité d’expression des neurones à kisspeptine et RFRP dans l’hypothalamus du dromadaire (Camelus dromedarius)

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Le dromadaire (Camelus dromedarius) est une espèce désertique qui présente une reproduction saisonnière et une ovulation provoquée. Il est sexuellement actif en jours court, ce qui permet la naissance, et donc la survie, des petits avec la période de l’année où les ressources sont abondantes et le climat est favorable. Les mécanismes impliqués dans le contrôle de la saisonnalité du dromadaire restent encore mal élucidés. Récemment, il a été montré que deux neuropeptides de la famille des RF-amides, la kisspeptine (Kp) et RFRP-3, sont impliqués dans le contrôle saisonnier de la reproduction chez plusieurs rongeurs. L’objectif de la présente étude est de déterminer si ces deux neuropeptides sont présent dans l’hypothalamus du dromadaire et s’ils prennent une variation cohérente avec leur reproduction saisonnière. Les neurones à Kp, marqués par l’anticorps AC566 ou le JLV, et les neurones à RFRP, marqués par l’anticorps QA193, ont été quantifiés manuellement sous microscope. Dans l’aire pré-optique et le noyau arqué, le nombre des neurones à Kp est augmenté pendant la période de la reproduction. Inversement, les neurones à RFRP dans l’hypothalamus dorsomédian sont plus nombreux en période de quiescence sexuelle qu’en milieu de la saison de reproduction. Les neurones à Kp au niveau de la ligne optique présentent un dimorphisme sexuel avec une densité plus élevée chez les femelles comparé aux mâles. Les neurones à Kp et RFRP présentent des expressions saisonnières d’expression opposées en fonction de l’activité sexuelle. Cela suggère que ces deux RF-amides pourraient agir dans la synchronisation de l’activité de reproduction du dromadaire avec la saison et des études complémentaires sont en cours pour comprendre les facteurs à l’origine de cette régulation ainsi que le rôle relatif de chacun de ces deux neuropeptides.
La balance énergétique (BE) est finement régulée par le système nerveux central (SNC) : celui-ci intègre les signaux périphériques reflétant le statut énergétique de l’organisme et adapte en retour la prise alimentaire et la dépense énergétique dans le but de maintenir un poids stable tout au long de la vie adulte d’un individu. L’hypothalamus (HT) est une des structures cérébrales ayant un rôle majeur dans l’intégration de ces signaux. Plusieurs études mettent en évidence que l’obésité induite par un régime riche en lipides (HFD) entraîne une inflammation au niveau de l’HT qui pourrait être à l’origine de l’obésité. De plus, les lipides contenus dans le HFD seraient directement responsables du déclenchement de la réponse inflammatoire. Au niveau cellulaire, cette inflammation se traduit, entre autres, par une activation de la microglie et des astrocytes dans l’HT. Chez le rongeur, des études récentes montrent que la prolifération de la microglie et des astrocytes est observée dès les premières 24h de consommation de HFD, bien avant la mise en place de l’obésité, et ne serait pas liée à la prise alimentaire. L’HT est donc que l’activation gliale précoce serait un mécanisme adaptatif impliqué dans la régulation de la BE et qu’une surexposition aux lipides nutritionnels pourrait déréglérer la réponse inflammatoire et l’obésité. Dans notre étude, nous observons une augmentation de l’expression du marqueur astrocytaire GFAP dans l’HT après 24h de consommation de HFD ainsi qu’une augmentation de l’expression du marqueur microgliaire Iba1 et des modifications morphologiques de la microglie dans l’HT après 72h de consommation de HFD. De plus, le remodelage des cellules gliales est associé à l’activation différentielle de certains peptides hypothalamiques. Nos résultats suggèrent donc que l’inflammation induite par la consommation de HFD est un événement précoce potentiellement impliqué dans la régulation de la BE.

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Sexual behavior ensures the perpetuation of a species. Lordosis in response to male mounting is the most prominent reproductive behavior in female rodents. We have recently demonstrated that kisspeptin neurons, already well known to play a crucial role in reproduction and implicated in controlling puberty onset and ovulation by regulating gonadotropin-releasing hormone (GnRH) neurons, are activated upon mating (lordosis) in female mice. We showed that lordosis behavior is impaired in kisspeptin-knockout mice but can be rescued by central or peripheral kisspeptin administration. Interestingly, acute ablation of kisspeptin neurons in the anteroventral periventricular area (AVPV) in adult females impaired lordosis behavior, whereas optogenetic activation of these cells triggered the behavior, suggesting a prominent role for kisspeptin in the control of lordosis behavior. In order to identify candidate neurons downstream of AVPV kisspeptin neurons, we expressed the transsynaptic tracer barley lectin (BL) exclusively in these cells to label synaptically connected cells (KissIC/R26-BIZ). We found BL+ neurons in the ventrolateral part of the ventromedial hypothalamus (VMHvl). Subsequent immunohistochemical analyses showed that the BL+ neurons predominantly express neuronal nitric oxide synthase (nNOS). The proportion of nNOS+/BL+ cells was higher in proestrus compared to diestrus, which is consistent with the period in which females are sexually receptive. These data demonstrated that kisspeptin neurons communicate with subsets of nNOS neurons in the VMHvl. To further analyze the role of NO in female sexual behavior, we used mice deficient in nNOS. We found that nNOS-KO females showed a strong decrease in lordosis behavior compared to control littermates. Overall, these data demonstrate that hypothalamic NO signaling is an essential mechanism downstream of kisspeptin neurons in a novel neural circuit governing lordosis behavior in female mice.
Identification of a novel hormonal signaling pathway regulating brain metabolic and cognitive functions

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Hormones are essential factors ensuring proper regulation of our physiological functions by mediating dialogue between organs. Their broad spectrum of actions is not limited to the peripheral organs. Some hormonal factors, such as leptin, insulin, thyroid hormones, steroid hormones reach the central nervous system (CNS) where they modulate the central regulation of whole-body metabolism. Recently, it has been shown that peripheral hormones can also influence more intrinsic functions of the CNS, such as brain development, stem neurogenesis and cognitive functions. Importantly, increasing evidence suggests that changes in their circulating levels may contribute to age-related cognitive decline, as well as to the development of neurodegenerative diseases. However, although the brain expresses receptors for most, if not all, hormones of the pituitary (of many hormones in the CNS remain unexplored.

Parathyroid hormone (PTH) is a polypeptide produced by the parathyroid gland, and is a key player in regulating calcium and phosphate homeostasis. Its functions are mediated by two G-coupled receptors: Pth1r and Pth2r. Several striking biological and clinical observations suggest that PTH may have a role in the brain: first, both PTH receptors are expressed in the CNS. Second, human patients suffering from excess PTH (hyperparathyroidism) manifest a wide array of cognitive disorders and symptoms associated with autonomic dysfunctions. Lastly, recent evidences suggest that circulating levels of PTH are significantly elevated in cases of dementia especially of the Alzheimer’s type. Indeed, these observations and data lead us to hypothesize that PTH and/or its receptors may act in the CNS.

Our preliminary results provide evidence supporting this hypothesis: (i) We have demonstrated that PTH is present in mouse and human cerebrospinal fluid. (ii) We observed that both receptors are highly expressed in the Hippocampus, Arcuate and Locus coeruleus nuclei, three brain regions essential for the regulation of cognitive functions and whole-body metabolism. (iii) Brain-specific PTH infusions induce an increase in energy expenditure, a decrease in food/drink intake, and affect memory performance. Lastly, we showed that PTH promotes Ca²⁺ flux changes in primary neurons pointing to a possible neuroactive role for this hormone in the brain.

Taken together, these data reveal a novel and unexpected role of PTH in the CNS in regulating whole-body energy balance and cognitive functions. Moreover, these results improve our understanding of the importance of hormonal homeostasis in the brain and may lead to novel therapeutics for age-related metabolic and/or cognitive disorders treatments.
How does an organism adjust its food intake? We recently showed that Drosophila perceives directly the presence of leucine, a branched amino acid, coming from the food bowl to release insulin (DILPs) from specific cells located within larval brains (insulin producing cells). We first showed that this effect requires an amino acid transporter called Minidiscs (MND), which is probably a LAT-1 homolog. MND knockdown in IPCs abolished leucine-dependent changes, including loss of DILP2 and DILP5 in IPC bodies, consistent with the idea that MND is necessary for leucine-dependent DILP release. This, in turn, leads to a strong increase in hemolymph sugar levels and reduced growth. GDH knockdown in IPCs also reduced leucine dependent DILP release, suggesting that nutrient sensing is coupled to the glutamate dehydrogenase pathway. Recently, we also found that a second leucine transporter belonging to the same transporter family, JhI-21, is necessary for proper leucine sensing in these insulin-producing cells. Drosophila uses two system-L transporters, JhI-21 and MND, to control insulin signaling from IPCs. Since system-L transporters are also expressed by mammalian β-cells our results support the idea of a unifying mechanism how insulin signaling is processed using the power of available genetic tools in Drosophila.
#2. 3D imaging of solvent-cleared brains: a new tool to study development and organization of the hypothalamic-pituitary-gonadal axis in different species

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Histology has been a golden tool for biological research for decades. Nevertheless, whole organ histology is extremely time and resource consuming and most of the time impossible due to the distortions and lack of algorithms to correctly align thousands of sections. Recently developed tissue clearing methods made it possible to explore intact organs with laser-scanning microscopy. Among those techniques, the process called 3D imaging of solvent-cleared organs (3DISCO), which achieves the highest level of transparency among all reported methods, has been proved to be a simple and inexpensive method for 3D analysis of immunolabelled transparent organs in embryonic and postnatal animals.

Herein, combining 3DISCO with light-sheet laser-scanning ultramicroscopy, we studied the development and 3D organization of the hypothalamic-pituitary-gonadal axis in several mammalian species. Our 3D data demonstrate that with thorough biochemical optimization, we can now detect morphogenetic processes, cell migration and terminal differentiation in both embryonic and postnatal development in several transgenic mouse strains. Moreover, this technique can be adapted to human tissues for volume imaging during fetal development as well as in adult post-mortem human samples. We believe this opens up a new route for high-resolution studies of brain architecture in mammals in physiological and pathological conditions.
Pro-opiomelanocortin (POMC) is a prohormone precursor for the melanocortin peptides and is produced in a distinct population of about 3,000 neurons in the hypothalamic arcuate nucleus (ARC). The mature form of POMC, α-melanocyte stimulating hormone (α-MSH), is released from a subset of these neurons upon leptin stimulation and activates downstream melanocortin-4 receptor (MC4R) neurons in paraventricular nucleus (PVN), resulting in reduced food intake and increased energy expenditure, while another subset of POMC neurons are responsive to insulin signalling. Disruptions of either the mouse Pomc or the human POMC gene results in severe obesity.

In order to further understand the role of the different POMC neuronal populations in controlling energy balance, we have isolated individual neurons from the hypothalamic arcuate nucleus of POMC-EGFP transgenic mice using fluorescence activated cell sorting (FACS) technology, and performed deep RNA sequencing to investigate the heterogeneity of ARC POMC neurons. Our data suggests that a total of 12,187 genes are expressed in POMC neurons, including over 1000 cell surface receptors (ion channels, G-protein coupled receptors). In addition to POMC, we detected the expression of other secretory molecules and peptides like Resp18, Cartpt and interestingly, in some of the neurons, Agrp and Npy. Of note, between the vast heterogeneity of POMC neurons, we observed about 25% of cells expressing Leptin receptor and about half of the population expressing Insulin receptor.

Utilizing single-cell transcriptome profiling, we have begun to unravel the complex heterogeneity of POMC neurons. The sequencing of these neurons coupled with detailed microbiomic and functional assays will help to clarify differential roles of POMC neurons within the control of energy balance.
L’horloge circadienne gouverne la rythmicité de nombreuses fonctions biologiques laquelle repose en partie sur le rythme d’expression d’ARNm que l’on sait aujourd’hui être majoritairement contrôlé au niveau post-transcriptionnel. Nous avons caractérisé un de ces mécanismes dans des cellules hypophysaires somatolactotropes, les GH4C1. Celui-ci implique des structures nucléaires, les paraspeckles, constituées d’un long ARN non-codant (Inc), Nuclear-Enriched Abundant Transcript 1 (Neat1) associé à des protéines. Tous les composants des paraspeckles ainsi que leur nombre présentent une rythmicité circadienne dans les GH4C1. Ces structures peuvent retenir dans le noyau des ARNm qui présentent dans leur région 3’UTR des séquences répétées et inversées de type Alu (IRAu) qui forment des boucles d’ARN double brin. Nous avons montré que l’insertion d’une séquence IRAu dans la région 3’UTR du gène rapporteur egfp, induit une rétention nucléaire rythmique de l’ARNm egfp associée à une expression cytoplasmique rythmique de la protéine EGFP. Pour déterminer si ce contrôle rythmique peut également s’exercer sur des ARNm endogènes, nous avons dû identifier les ARNm endogènes cibles des paraspeckles. Nous avons pour ce faire développé une technique de « Neat1 RNA pull-down » inspirée de techniques publiées permettant d’isoler la chromatine associée à un IncARN. Ce protocole adapté à l’isolement des ARNm associés au Inc Neat1 repose sur la mise au point des conditions préalables de fixation des interactions entre les différents partenaires et d’isolement des complexes par des sondes spécifiques sélectionnées sur la base de la structure secondaire de Neat1. Le couplage à du séquençage haut débit des ARN, ce protocole nous a permis de détailler le nombre des ARNm associés à Neat1 et de montrer que ces paraspeckles, grâce à leur expression circadienne, peuvent être à l’origine d’un contrôle post-transcriptionnel de la rythmicité circadienne d’expression des ARNm endogènes associés.
Recent advances in our knowledge of the complexity and specificity of neural circuits generating clear-cut behaviours suggest that the next step forward is the microdissection of the neuronal brain circuitry at a single cell/synaptic level. Standard anterograde and retrograde neuronal tracers can reveal the locations of neurons projecting to or from particular brain regions, but fail to identify the cell types that actually receive the synaptic connections. Recently, a monosynaptic circuit tracing in vivo system has been generated through a Cre-dependent targeting. The glycoprotein gene–deleted (ΔG) rabies virus is particularly useful in mapping neuronal circuits due to its ability to spread monosynaptically, exclusively in the retrograde direction. The infected cells remain viable for two weeks and ΔG-rabies virus tagged with fluorescent protein and/or functional reporter gene (e.g. channelrhodopsin-2) can be used to reveal detailed morphology and/or to manipulate activity of infected neurons. Within the last decade, kisspeptin, the potent GnRH activator, has been described playing multiple roles in many different physiological and behavioural processes from reproduction to obesity. More recently, we suggested that kisspeptin could synchronize female sexual behaviour with ovulation. We identified AVPV kisspeptin neuronal projections to the OVLT, PVN and VMHvl. All these brain regions are involved in sexual behaviour and contain GnRH neurons. With a KissCre+/− mouse model and injecting ΔG-rabies virus that can be picked up on terminals in these regions, we could determine the direct monosynaptic afferences of AVPV kisspeptin neurons on OVLT, PVN or VMHvl. Furthermore, we would be able to modulate the activity of the direct kisspeptin afferences to decipher the neuronal inputs leading to the synchronization of sexual behaviour and ovulation.
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