In non-breeding ewes the kisspeptin analog c6 triggers ovulation without progestogen priming

Massimiliano Beltramo, Caroline Decourt, Vincent Robert, Didier Lomet, Vincent Aucagne

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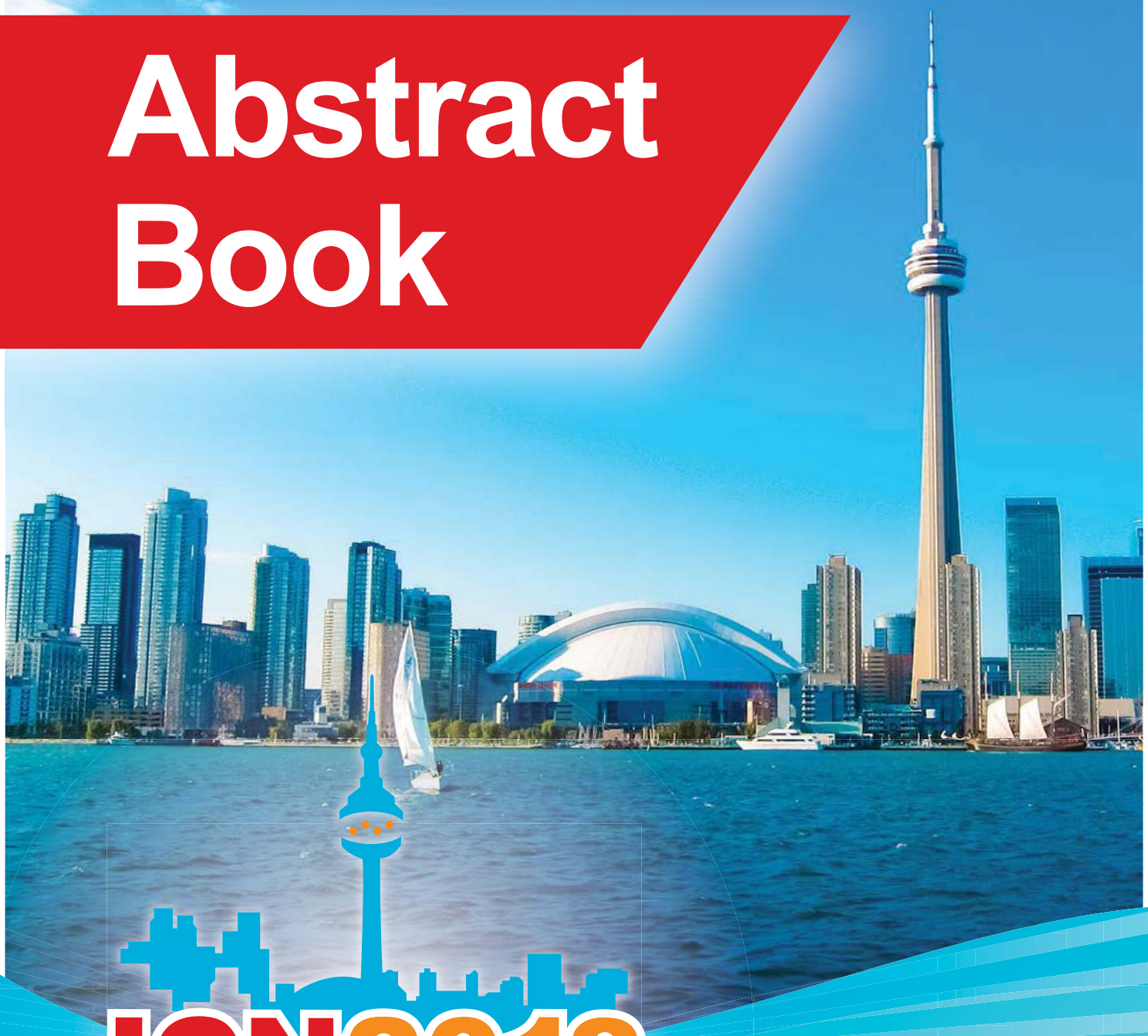
Abstract Book



ICN2018

INTERNATIONAL CONGRESS OF
NEUROENDOCRINOLOGY

July 15 - 18 • Toronto, Canada

ICN2018 is a Joint Meeting of the Affiliated Societies of the International Neuroendocrine Federation with the:
22nd Annual Meeting of the Society for Behavioral Neuroendocrinology | British Society for Neuroendocrinology | Société de Neuroendocrinologie | Hypothalamic Neuroscience and Neuroendocrinology Australasia | Pan American Neuroendocrine Society | Japan Neuroendocrine Society

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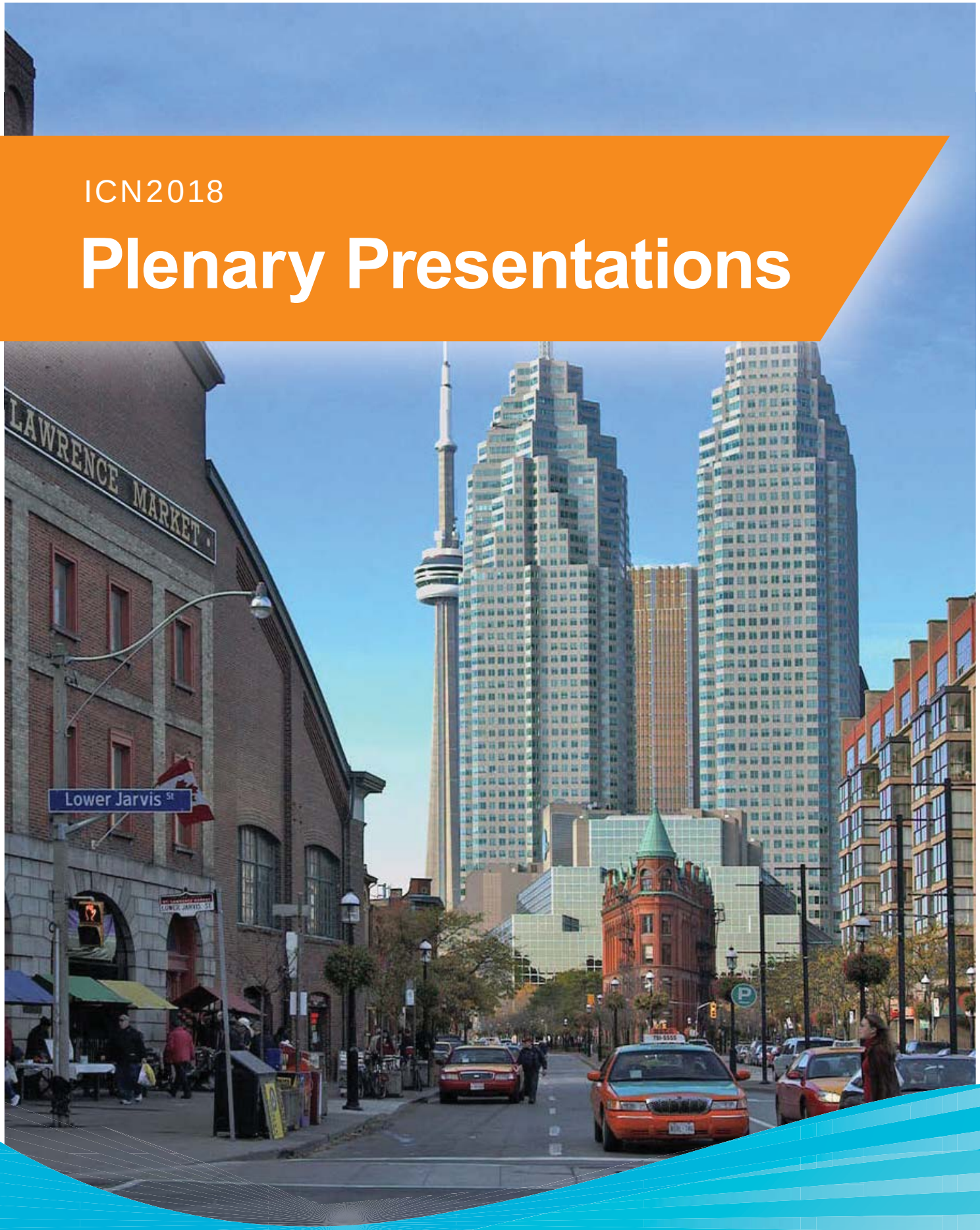

Plenary Presentations – Saturday, July 14, 2018

PL01.001 THE NEUROGENOMIC ARCHITECTURE OF STRESS SUSCEPTIBILITY

Michael Meaney
McGill University, Montreal, QC, Canada

Stress is a well-established 'trigger' for multiple forms of chronic illness including mental disorders. Despite the compelling epidemiological evidence, most individuals exposed to chronic, severe stress during development or adulthood are largely unaffected. These findings reflect the remarkable individual variation in susceptibility to stress. Animal models of differential susceptibility have identified transcriptional pathways and neural circuits associated with susceptibility. In the studies reported here we attempted to integrate this knowledge into an analysis of the genetic architecture of stress susceptibility in humans using a publicly-available genome-wide association study of addictions in individuals who were or were not exposed to stressful conditions known to predict an increased risk for chronic illness, including addictions. We developed a genomic profile score for stress susceptibility (Gypsums) by comparing stress-exposed individuals with (susceptible) or without (resilient) a history of addiction. The resulting Gypsums predicted stress-related mental disorders in an independent sample of individuals exposed to childhood abuse. We also found highly significant overlap between the genes that comprised our Gypsums and differentially expressed genes in a rodent model of stress susceptibility (chronic social defeat) using Ranse data from the ventral hippocampus. The overlapping genes are highly enriched for mithqal's, suggesting epigenetic mediation through DNA methylation. Informatic analyses of both human and rodent data sets implicate estrogen- and glucocorticoid-signaling pathways.
Direct manipulation of ESR1 expression resulted in significant alterations in stress susceptibility in the murine chronic social defeat model. These findings suggest a role for brain region-specific, steroid-sensitive transcriptional signaling in defining individual differences in susceptibility to stress.

Plenary Presentations – Sunday, July 15, 2018

PL02.001 A NEURAL CIRCUIT THAT RESPONDS TO THREATS AND CONTROLS APPETITE

Richard Palmiter
University of Washington, Seattle, United States of America

Both visceral and somatosensory neuronal information is relayed to higher brain regions by glutamatergic neurons that reside in the parabrachial nucleus (PBN) that express calcitonin gene-related peptide (CGRP) and several other neuropeptides. Activation of these CGRP-expressing neurons either by photo-activation of channel rhodopsin (ChR2) or CNO activation of

hM3Dq DREADD receptors inhibits feeding and chronic activation of these neurons would lead to starvation. Additional experiments demonstrate that a relevant output of these CGRP-expressing neurons that mediates anorexia is a projection to the lateral capsule region of the central nucleus of the amygdala onto neurons that express the receptor for CGRP. Pairing artificial activation of CGRP-expressing neurons with either a novel taste or a novel tone/context is sufficient to establish conditioned taste aversion or fear behaviors (freezing), respectively. Inactivation of CGRP neurons ameliorates taste or fear conditioning, indicating that they transmit the classical unconditioned stimulus for both learning paradigms. Calcium imaging reveals that virtually CGRP neurons are activated by all threats that have been examined; thus, they serve as a general alarm system. These neurons respond to potential threats and cues that have been associated with harm in the past. Different threats are presumably distinguished by the intensity of the stimulations and where conditioned stimuli (sensory cues) and unconditioned stimuli (pain) converge in the brain.

PL03.001 HYPOTHALAMIC PULSE GENERATION FOR FERTILITY

Allan E. Herbison
Centre For Neuroendocrinology and The Department of Physiology, Otago School of Medical Sciences, University of Otago, Dunedin, New Zealand

Several hypothalamic circuits can generate a pulsatile pattern of hormone secretion. The gonadotropin-releasing hormone (GnRH) neuronal network is one such circuitry that releases GnRH in an episodic manner to drive pulsatile gonadotropin hormone secretion. The GnRH neurons migrate from the nose into the brain during development and have many peculiar properties including a scattered distribution and the projection of a blended dendrite/axon ("dendron") to the median eminence secretory zone. Studies in a variety of genetic mouse models using tract-tracing, brain slice and *in vivo*optogenetic approaches have been able to demonstrate the that kisspeptin neurons located in the arcuate nucleus are the GnRH pulse generator. These kisspeptin neurons appear to drive episodic GnRH secretion by regulating the distal dendrons of the GnRH neuron as they funnel together to enter the median eminence. This unusual mechanism likely provides an efficient manner for synchronizing secretion from a population of neurons with widely scattered cell bodies.

PL04.001 NEUROBIOLOGY OF SOCIAL BEHAVIOR CIRCUITS

Catherine Dulac
Molecular And Cellular Biology, Howard Hughes Medical Institute/Harvard University, Cambridge, MA, United States of America

Social interactions are essential for animals to reproduce, defend their territory, and raise their young. The conserved nature of social behaviors across animal species suggests that the neural pathways underlying the motivation for, and the execution of, specific social responses are also

maintained. Modern tools of neuroscience have offered new opportunities for dissecting the molecular and neural mechanisms controlling specific social responses. This lecture will describe data from our lab aimed at deciphering the identity and principles of functioning of neural circuits underlying specific types of social behaviors, and in particular, I will present our recent progress in understanding control mechanisms of a particular important form of social behavior, that of parental care. We will discuss how these findings open new avenues to deconstruct maternal and paternal behaviors, and to help understand the neural basis of parenting in health and disease.

Plenary Presentations – Monday, July 16, 2018

PL05.001 THE ROLES OF HOMEODOMAIN PROTEINS IN GNRH NEURONAL DEVELOPMENT AND FERTILITY

Pamela L. Mellon[1], Erica C. Pandolfi[2], Hanne M. Hoffmann[3]
[1]Reproductive Medicine, UC San Diego, La Jolla, CA, United States of America, [2]Reproductive Medicine, UC San Diego, La Jolla, United States of America, [3]Animal Science, Michigan State University, East Lansing, MI, United States of America

During development, GnRH neurons are born in the olfactory placode then migrate along the olfactory nerves to the olfactory bulb and into the hypothalamus where they are required for fertility. We have identified a number of homeodomain proteins crucial for *Gnrh1* gene expression, as well as GnRH neuron migration and/or survival. Several of these genes are haploinsufficient, revealing strong reproductive phenotypes as heterozygous alleles. Interestingly, some also have opposite phenotypes in full body knock–out animals versus when deleted only in GnRH neurons, causing increased numbers of GnRH neurons instead of decreased, indicating strong effects on the pathway cues that are required for migration and different roles within GnRH neurons. We find not only that proteins such as Vax1, Otx2, Six6, and Six3 have differing roles in GnRH neurons, but that they also participate in development of the olfactory system and the suprachiasmatic nucleus, and thus can influence mating behavior and circadian rhythms to affect fertility.

PL06.001 MELATONIN: OF SWIMMING GHOSTS, FLYING SAUCERS AND CONTEXT SPECIFIC ACTIONS

Russel J. Reiter
Cell Systems And Anatomy, UT Health Science Center, San Antonio, TX, United States of America

Melatonin is an unusual molecule, doing unusual things, in unusual ways. When Aaron Lerner *et al* discovered this molecule in 1958, there was no way in their wildest imaginings they could have predicted what it would be shown to do. Moreover, they used the pineal from which to extract and identify melatonin, which was totally unnecessary. Since melatonin is now known to

be synthesized in many, perhaps all, cells, he and his colleagues could have, with much less effort, extracted melatonin from the skin (a much larger organ than the pineal). Making matters more difficult, they used the pineal gland of cows that were killed in the light, when melatonin levels are at their nadir. Finally, the discovery of melatonin in plants, where the concentrations are much higher than in animals and where melatonin is inducible, would have also been a better choice to use as starting material. The pineal gland was associated with reproduction well before melatonin was even discovered and the gland's role in the control of seasonal changes in reproductive competence in photoperiod-sensitive mammals are well documented, but context specific. Whereas the pineal gland was clearly linked to seasonal reproduction, proving that melatonin was the endocrine mediator of this action proved to be more difficult. Whereas melatonin's intervention in the hypothalamo-pituitary axis has been confirmed, these discoveries also highlighted the significance of the pars tuberalis in these actions; this portion of the pituitary had long been overlooked. The last two decades has witnessed a mushrooming of reports linking melatonin to almost every function of every plant and animal species. This is consistent with its proposed three billion year evolutionary history; it surely has had ample time to fill functional niches and to hone its multiple actions; however, many discoveries regarding melatonin have yet to be made.

Plenary Presentations – Tuesday, July 17, 2018

PL07.001 GEOFFREY HARRIS LECTURE

Jeffrey Friedman
Rockefeller University, New York, United States of America

Leptin is an adipose tissue hormone that maintains homeostatic control of adipose tissue mass. This endocrine system thus serves a critical evolutionary function by protecting individuals from the risks associated with being too thin (starvation) or too obese (predation). Mutations in leptin or its receptor cause massive obesity in mice and humans, and leptin can effectively treat obesity in leptin deficient patients. The identification of leptin has thus provided a framework for studying the regulation of feeding behavior and the pathogenesis of obesity.
While most obese patients have high endogenous levels of leptin indicating that they are leptin resistant , obese patients with low endogenous levels show robust weight loss with leptin treatment. Leptin also links changes in nutrition to adaptive responses in other physiologic systems with effects on insulin sensitivity, fertility, immune function and neuroendocrine function (among others). Leptin is an approved treatment for generalized lipodystrophy, a condition associated with severe diabetes, and has also shown promise for the treatment of other types of diabetes and for hypothalamic amenorrhea, an infertility syndrome in females. Studies of leptin gene regulation also suggest that leptin would be an effective treatment for the subset of obese patients with low endogenous levels of the hormone.
The identification of leptin has also advanced our understanding of the neural mechanisms that control feeding. Current research focuses on the function of specific neural populations in the

hypothalamus and other brain regions. The role of these neural subtypes is being evaluated by identifying molecular markers for specific subpopulations modulating their activity.

PL08.001 DISCOVERIES OF NOVEL PEPTIDES INVOLVED IN ENDOCRINE FUNCTIONS

Masamitsu Nakazato
University of Miyazaki, Miyazaki, Japan

Discoveries of peptides have led to our better understanding of the variety of regulatory systems in the body. After the ghrelin discovery focused on deorphanization of G protein-coupled receptor (GPCR) in 1999, we have developed novel techniques for comprehensive analyses of peptides secreted from endocrine cells. The change in the intracellular Ca^{2+} concentration is one of the most conveniently and frequently measured physiological responses downstream of cell surface receptors such as GPCR. We have recently identified four peptides to regulate vasopression release, insulin secretion or feeding behavior by increasing intracellular Ca^{2+} elevation. I will present our peptide identification and their physiological roles.

PL09.001 THE VERSATILE TANYCYTE: A HYPOTHALAMIC INTEGRATOR OF REPRODUCTION AND ENERGY METABOLISM

Vincent Prevot
Inserm U1172 - Jean-Pierre Aubert Research Center - University of Lille, Lille, France

The fertility and survival of an individual rely on the ability of the organism to promptly, effectively and reproducibly communicate with brain neural networks that control reproduction, food intake and energy homeostasis. Tanycytes, a specialized glial cell type lining the floor of the third ventricle in the median eminence of the hypothalamus, appear to act as the linchpin of these processes by dynamically controlling the secretion of neuropeptides into the portal vasculature by hypothalamic neurons and regulating blood-brain and blood-cerebrospinal fluid exchanges, both processes that depend on the ability of these cells to adapt their morphology to the physiological state of the individual. In addition to their barrier properties, they possess the ability to sense blood glucose levels, and play a fundamental and active role in shuttling circulating metabolic signals to hypothalamic neurons that control food intake. In this talk, we will examine recent advances in the understanding of tanycytic plasticity and function in the hypothalamus, and the underlying molecular mechanisms. We will also discuss the putative involvement and therapeutic potential of hypothalamic tanycytes in the pathophysiology of metabolic and fertility disorders.

Plenary Presentations – Wednesday, July 18, 2018

PL10.001 ENDOCRINE DISRUPTORS AND DEVELOPMENTAL PROGRAMMING OF BRAIN AND BEHAVIOR

Andrea C. Gore
College Of Pharmacy, University of Texas at Austin, Austin, TX, United States of America

Environmental endocrine-disrupting chemicals (EDCs) perturb hormones and their actions, especially when exposures occur during critical developmental periods. Even transient disruptions of endogenous hormones by EDCs can reorganize the brain in a sexually dimorphic manner, manifesting later as a disease or dysfunction. We have been using a rat model of prenatal exposure to EDCs: a polychlorinated biphenyl (PCB) mixture used previously in industry, the fungicide vinclozolin, in current agricultural use, or the vehicle (6% DMSO in sesame oil), administered during the period of brain sexual differentiation. When pups are born, we monitor birth outcomes and postnatal development, and in adulthood, animals are run through a battery of behavioral tests to assess functional neurobiological changes. Protein and gene expression in the brains, and serum hormone concentrations are assayed. Our results show that social, sociosexual and anxiety-like behaviors are changed by EDCs in a sexually-dimorphic manner. Gene-expression profiling of brains from these animals has identified suites of genes differentially affected by EDCs compared to vehicle rats, with sex-, age-, and brain-region specific differences. The lab has extended work to determine whether effects of EDCs may be transmitted through context-dependent or germline epigenetic changes across generations, with initial data supporting perturbations in behaviors up to the F6 generation, and evidence that the sperm epigenome is hyper-methylated. This body of work indicates that gestational exposure to PCBs has life-long effects on the developing brain, neuroendocrine systems, and reproductive and social behaviors in exposed individuals, and that transgenerational epigenetic effects propagate the behaviors to unexposed offspring.

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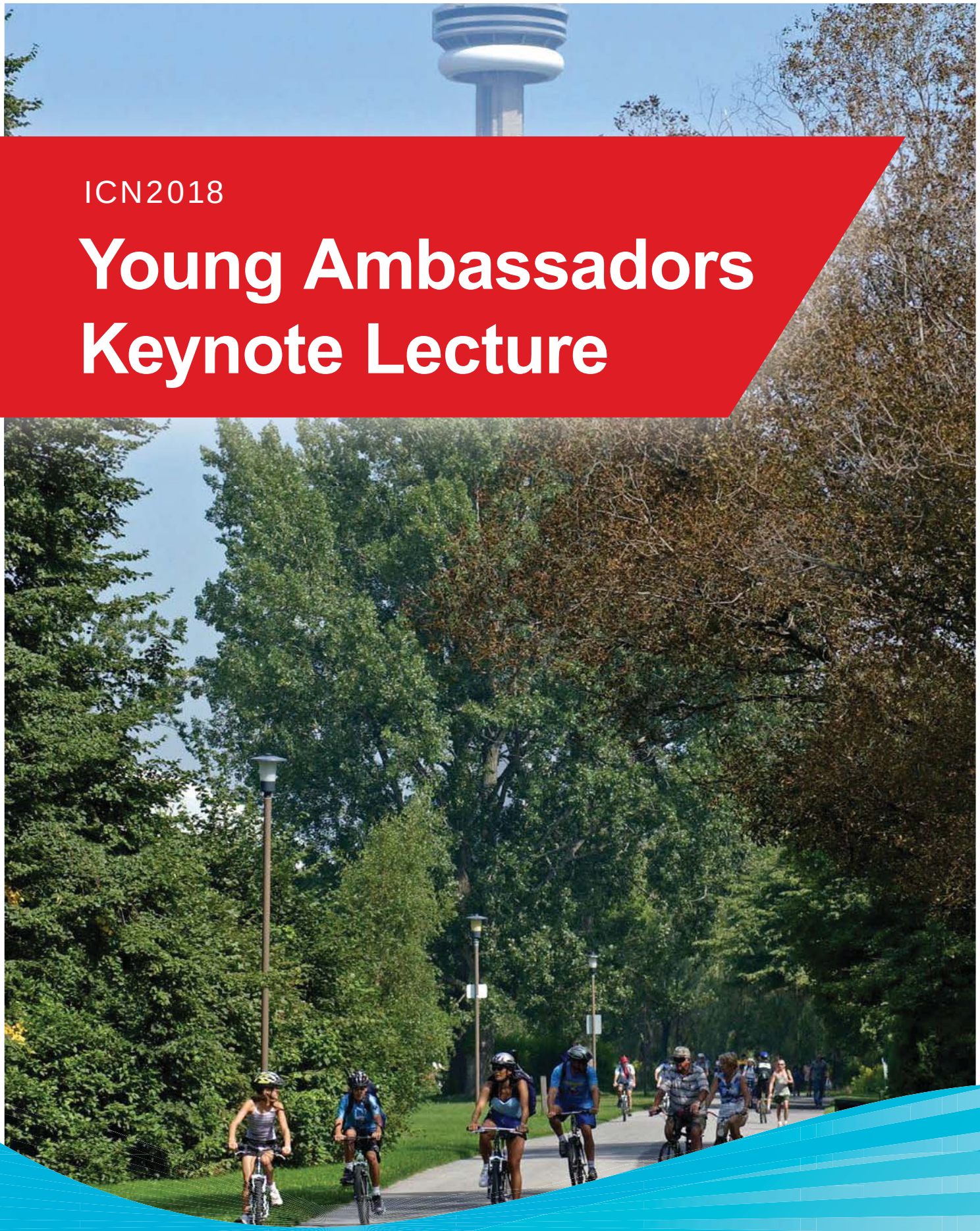
Young Ambassadors Keynote Lecture



Young Ambassadors Keynote Lecture – Wednesday, July 18, 2018

YA1.001 AMH IN THE REGULATION OF GNRH NEURONAL FUNCTION AND IN THE AETIOLOGY OF POLYCYSTIC OVARY SYNDROME

Paolo Giacobini
Research Center Jean-Pierre Aubert, Inserm U1172, Lille, France

Reproduction in mammals is dependent on specific neurons secreting the Gonadotropin Releasing Hormone (GnRH). Indeed, many reproductive disorders in humans are associated with abnormal or deficient GnRH secretion. Among reproductive dysfunctions, polycystic ovary syndrome (PCOS) is the most common form of female infertility with a prevalence of up to 10%. The syndrome is underpinned by excessive ovarian and/or adrenal androgen secretion, oligo-anovulation and insulin resistance. The pathophysiology of PCOS also extends to hypothalamic neuronal dysregulation and increased risks for metabolic derangements. Intriguingly, many women with PCOS exhibit increased luteinizing hormone (LH) levels, suggestive of rapid GnRH release, and higher serum levels of ovarian Anti-Müllerian Hormone (AMH) as compared to healthy women. The origin of this dysregulation and the possible cross-talk between AMH and GnRH have just started to be elucidated in recent years. This lecture will focus on recent advances on the extra-gonadal functions of AMH in the central nervous system, namely related to novel findings showing that AMH increases GnRH neuronal activation and neuro-hormone secretion in rodents. A large discussion will be given to new integrative, functional and mechanistic *in vivo* strategies, which, combined with clinical human investigations, support the hypothesis that AMH-dependent deregulation of GnRH release could be involved in the aetiology of PCOS. Finally, PCOS-like animal models will be described to support the concept that PCOS may originate *in utero* and that fetal environmental factors play important roles in the onset of this syndrome.

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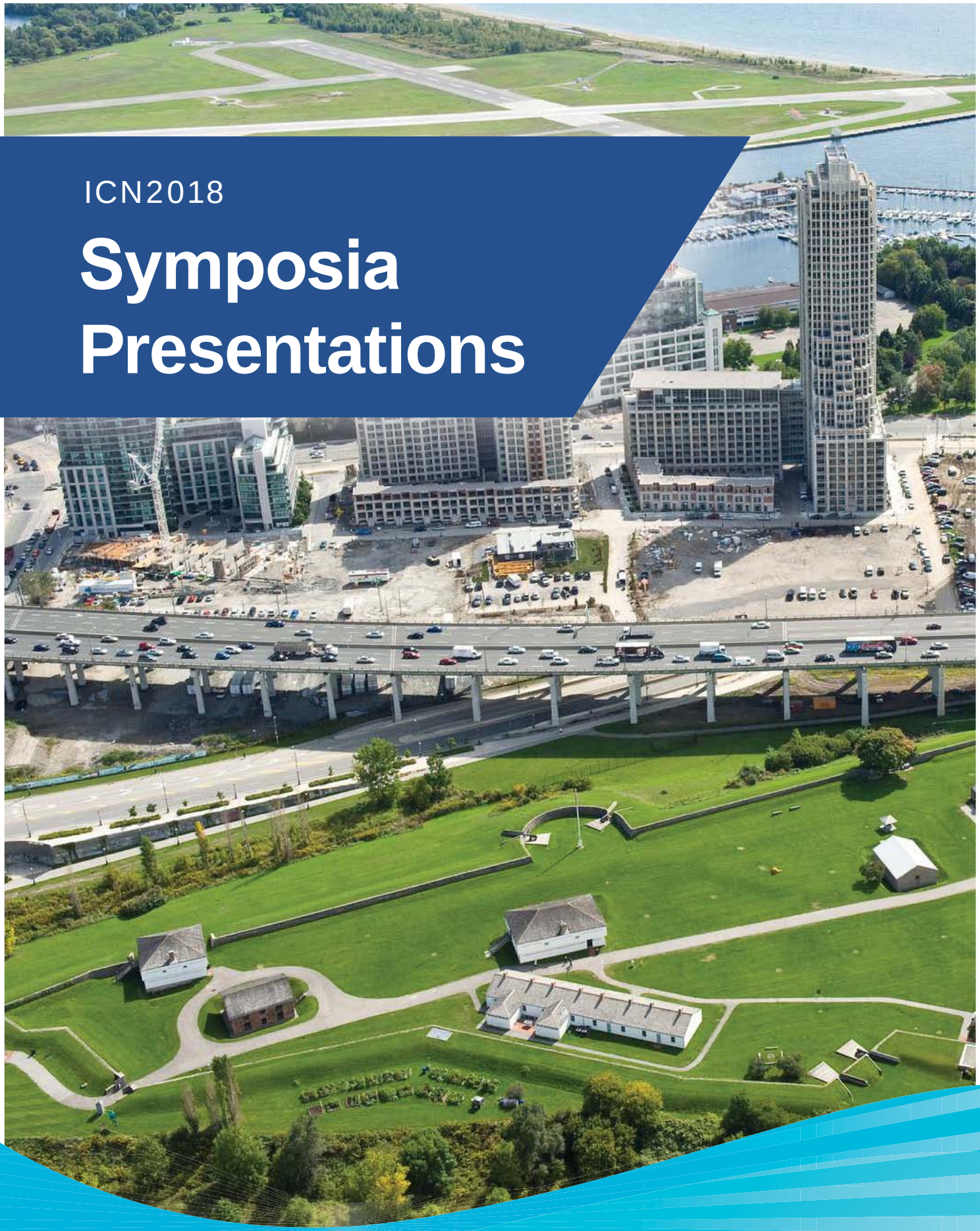
Symposia Presentations



CS1B.001 SEX DIFFERENCES IN DOPAMINE-MEDIATED SOCIAL LEARNING

Elena Choleris, Richard Matta, Angela N. Tiessen, Emily A. Underwood, Noah Bass, Cheryl L. Limebeer, Linda A. Parker
Department Of Psychology And Neuroscience Program, University of Guelph, Guelph, ON, Canada

Social learning, the acquisition of novel information from others, is widespread in the animal kingdom and is adaptive in that it allows bypassing learning through direct experience. The neurotransmitter Dopamine has been implicated in various social and cognitive behaviors including the social transmission of information. Initial systemic studies found that D1-type receptors mediate the social transmission of food preferences in female mice. Subsequent studies investigated the involvement of the dorsal hippocampus and discovered intriguing sex differences. Infusion of a D1-type antagonist 15 minutes prior to a 30 minutes social interaction with a conspecific recently fed a novel diet, blocked the development of a socially acquired food preference. This effect was stronger in males than females, the latter requiring a much higher dose of the D1 antagonist for the impairment in social learning to become apparent. Conversely, infusion of a range of doses of a D2-type antagonist in the dorsal hippocampus 10 minutes prior to a social interaction blocked social learning only in females. In addition, dorsal hippocampal D1-type and D2-type antagonists both reduced sex-typical social behavior: agonistic interactions in males, social investigation in females and dominance in both sexes. With microdialysis we found that in response to novel food and social odors dorsal hippocampal dopamine was increased in males and decreased in the females. This sex difference might partly explain the differences in dorsal hippocampal D1-type and D2-type receptors mediation of social learning and social behavior in males and females. Currently, we are investigating the possible role of gonadal hormones.

CS1B.002 SEX DIFFERENCES IN MEMORY IN MEMORY CONSOLIDATION

Karyn Frick
Psychology, University of Wisconsin-Milwaukee, Milwaukee, United States of America

Much has been learned in recent years about the molecular mechanisms through which estradiol regulates memory consolidation in females. However, much less is known about estrogenic regulation of memory consolidation in males. Estradiol can rapidly enhance spatial memory consolidation and increase hippocampal dendritic spines in gonadectomized rats, but the mechanisms governing these changes *in vivo* are largely unknown. This talk will first summarize our laboratory's work in female mice identifying cell signaling and receptor mechanisms in the dorsal hippocampus necessary for estradiol to enhance memory consolidation and increase hippocampal and prefrontal dendritic spine density. Next, our recent work examining how estradiol regulates memory consolidation in gonadally-intact and

castrated male mice will be described, including data suggesting sex differences in the cell-signaling mechanisms underlying estradiol-induced memory enhancement. Finally, the influence of the testes in mediating the role of hippocampally-synthesized estradiol on memory consolidation in males will be discussed.

CS1B.003 SEX DIFFERENCES IN AFFECTIVE BEHAVIOURS AND NEUROGENESIS

Liisa A.M. Galea, Wansu Qiu
Psychology, University of British Columbia, Vancouver, BC, Canada

Sex differences exist in the prevalence and timing of psychiatric disease. For example, men are more likely to develop schizophrenia in adolescence, while women are more likely to develop depression in adulthood (particularly during the perinatal period). Many psychiatric diseases are exacerbated with stress, and stress differentially influences neuroplasticity and behavior in males and females. For example, exposure to restraint stress across adolescence reduced hippocampal neurogenesis and increased basal corticosterone (CORT) levels in adult female, but not male, rats. In a series of studies, we have explored the influence of high CORT administered to the dams (during postpartum as an animal model of postpartum depression) on both the dams and her male and female offspring. Maternal postpartum CORT treatment increased depressive-like behavior, reduced hippocampal plasticity (neurogenesis, dendritic complexity), and disrupted maternal care in the dams. Maternal fluoxetine only reversed the disrupted maternal care, while maternal exercise reversed increased depressive behaviour and reduced neuroplasticity seen with maternal CORT postpartum. Male adolescent or and adult offspring were more likely to exhibit perturbations in anxiety-like behavior after exposure to maternal postpartum CORT or fluoxetine. Maternal fluoxetine reduced neuroinflammation (IL-1β, IL-13, IFNγ, TNFα) in both sexes, but maternal CORT increased neuroinflammation in males only (IL-1β. IL-5, IL-4, IL-6) in the hippocampus. Together these studies show that perturbations during different developmental periods (postpartum, adolescence, adulthood) lead to sex differences in psychiatric endophenotypes that may explain sex differences in vulnerability to psychiatric diseases during different points of across the lifespan in men and women.

CS1B.004 SEX DIFFERENCES IN ISCHEMIC INJURY AND NEUROPROTECTION

Farida Sohrabji
Texas A&M University College of Medicine, Bryan, TX, United States of America

Stroke is the 5th leading cause of death and the leading cause of acquired disability in the US. Women are disproportionately affected by stroke, having a higher incidence and worse outcomes than men. Several preclinical studies have identified novel therapies for the

treatment of stroke, but almost all of these were found to be unsuccessful in clinical trials. Despite known sex differences in occurrence and severity of stroke, few studies, both preclinical or clinical, take into account possible sex differences in treatment. However, a growing body of evidence shows that novel therapies for stroke effective in one sex over the other, or effective in one sex but not the other. Our studies using small non-coding (micro)RNA as stroke neuroprotectants show an age and sex-specific impact on stroke outcomes. Specifically, (a) Let7f antagomirs were effective in reducing infarct volume and improving impaired sensory-motor performance due to stroke in young females, but paradoxically, had toxic effects on stroke outcomes in middle-aged females. (b) mir363-3p, identified from a large profiling study of circulating mirRNA, was effective in reducing stroke induced cell loss in both young and middle-aged females, but not in males at any age. Finally, (c) mir20, identified by profiling of astrocytic miRNA, was effective in adult and middle-age males and females. These data underscore the important of sex-specific neuroprotective pathways and suggest that one way to increase translational success of stroke therapies would be to stratify by biological sex.
Supported by AG042189 and NS074895 to FS

CS1M.001 STRESS AND OBESITY: THE GHRELIN CONNECTION

Alfonso Abizaid
Neuroscience, Carleton University, Ottawa, ON, Canada

The stress response is associated with metabolic challenges that require adaptations including increases in caloric intake and changes in energy expenditure that promote carbohydrate metabolism and the accumulation of fat. Ghrelin, a hormone secreted by the stomach during periods of negative energy state, produces similar effects. Interestingly, chronic social defeat stress (CSDS) induces a state of hyperghrelinemia that is accompanied by increased caloric intake and weight gain and that can ultimately lead to obesity and insulin resistance in mice. These effects are not seen in mice that lack ghrelin receptors (GHSR KO) or mice treated with CF801 (a drug that reduces plasma ghrelin levels) show lower caloric intake and weight gain in the face of stress. These effects are partially mediated by GHSR in the brain, as central blockade of these receptors also attenuates CSDS induced hyperphagia and weight gain. One potential site for the hypephagic action of ghrelin is the ventral tegmental area (VTA) in the midbrain, a region that is associated with reward seeking behaviors and the stress response. This region not only expresses the GHSR, but also shows an increase in the expression of these receptors following CSDS. Moreover, blockade of GHSR in the VTA attenuates caloric intake. In contrast, blocking GHSR in the hypothalamic paraventricular nucleus (PVN) or in the dorsomedial hypothalamic nucleus result in changes associated with heat production and increased sympathetic tone. These results suggest that ghrelin is a stress related hormone that acts at different brain sites as a means to attain energy homeostasis.

CS1M.002 HUNGER-SENSING AGRP NEURONS ENGAGE THE HPA AXIS TO MEDIATE ADAPTIVE RESPONSES TO STRESS

Zane B. Andrews
Physiology, Monash University, Clayton, Australia

Hunger-sensing Agouti-related peptide (AgRP) neurons not only increase food intake but also facilitate adaptive behaviors to cope with hunger by reducing anxiety and increasing motivation to optimise food seeking behavior. A fundamental question remains; what are the physiological mechanisms through which AgRP neurons regulate adaptive behaviors. We examined the hypothesis that activation of AgRP neurons in the absence of food engages the Hypothalamic Pituitary Adrenal (HPA) axis to mitigate anxiety associated with acute stress. Using a hM3Dq DREADD approach, prior activation of AgRP neurons 3-hours before acute restraint stress significantly increased plasma corticosterone 15, 30 and 60 minutes and ACTH 30 minutes after stress onset. The plasma corticosterone response was significantly greater when AgRP neurons were activated 3 hours, compared to 10 minutes, prior to stress onset. Anterograde tracing of AgRP neurons using the cre-dependent herpes simplex virus H129 ΔTK-TT confirmed that AgRP neurons target ~30% of CRH neurons in the PVN. In behavioral experiments, prior activation of AgRP neurons reduced anxiety-like behavior, increased memory recall and promoted food intake after acute stress. Prior activation of AgRP neurons also promoted food-seeking and food consumption in a food-baited novel environment, used to evoke acute stress. We show that AgRP neurons potentiate the HPA axis response to acute stress, which regulates anxiety-like behavior and memory recall, and promotes food seeking and consumption. We argue AgRP neurons integrate metabolic information with mood and memory function to optimize current food-seeking and future food-seeking opportunities in an acutely stressful dangerous environment.

CS1M.003 EPIGENETIC MECHANISMS IN MATERNAL STRESS PROGRAMMING OF METABOLIC OUTCOMES

Tracy Bale, Bridget Nugent
Pharmacology, University of Maryland, School of Medicine, Baltimore, MD, United States of America

Background: Prenatal stress is a risk factor for neurodevelopmental and comorbid metabolic disorders. In our mouse model of early prenatal stress (EPS), stress exposure during the first week of gestation imparts long-term neurodevelopmental programming deficits in male offspring resulting in hypersensitivity to stress, cognitive impairments, and alterations in metabolic programming. The placenta, a fetally-derived organ reflecting fetal sex, acts as an arbitrator between the mother and fetus, providing necessary factors for early fetal neurodevelopment. Thus, sex differences in placental function may dramatically influence sex bias in vulnerability to prenatal insults. Methods and Results: We previously identified the X-linked, stress-sensitive, nutrient sensor O-linked-N-acetylglucosamine (OGT) as a placental biomarker of prenatal stress. OGT escapes X-inactivation in the placenta, providing females

with increased expression. Placental-specific reduction of OGT recapitulates our EPS phenotype. Using ChIP-Seq, biochemistry, and RNA-Seq in mouse placentas with trophoblast-specific OGT reduction, we found that OGT determines genome-wide sex differences in H3K27me3 and gene expression in placental trophoblasts. Further, RNA-Seq of the embryonic hypothalamus revealed that reducing OGT in the female placenta masculinized the expression of key genes associated with hypothalamic development, suggesting that placental OGT contributes to sex differences in brain development. Conclusions: We hypothesize that female-biased epigenetic repression protects females from prenatal insults. These studies, aimed at elucidating the basic biological differences between male and female developmental programs, will bring us closer to fully understanding the etiology of sex-biased neurodevelopmental disorders.

CS1M.004 HYPOTHALAMIC AUTOPHAGY IN RESPONSE TO LIPID OVERLOAD

Marciane Milanski
Health, Unicamp, Limeira, Brazil

Protein degradations systems are critical process related to the maintenance of cellular proteostasis. In central nervous system impairment in ubiquitin-proteasome system and autophagy are linked to neuronal dysfunction in many neurodegenerative diseases. The hypothalamus is one of the main area of central nervous system responsible for the control of food intake and neuronal dysfunction in this area is one of the hallmarks of obesity pathophysiology. In this talk I will present our results showing that dysregulation of autophagy in hypothalamus could be connected to hypothalamic dysfunction in diet-induced obese mice.

CS1R.001 DEVELOPMENTAL REGULATION OF REPRODUCTIVE NEURONS AND PUBERTY IN MICE

Alexander S. Kauffman
Obgyn and Reproductive Sciences, University of California, San Diego, La Jolla, CA, United States of America

Puberty reflects the developmental awakening of GnRH and LH secretion, which typically occurs earlier in females than males. Although the molecular and cellular mechanisms underlying the onset of puberty remain mysterious, the gating processes likely include changes in the sensitivity of neuronal circuits to the actions of gonadal sex steroids, as well as gonad-independent changes in either restraining or activating networks in the brain. Kisspeptin, synthesized in key hypothalamic brain nuclei, is thought to play an important role in puberty based on observations that *Kiss1* gene expression in the brain increases at the time of puberty and administration of exogenous kisspeptin to juvenile animals induces precocious puberty.

Moreover, disabling and activating mutations in the kisspeptin receptor have been linked to delayed and precocious puberty, respectively. Data from monkeys has established that the secretion of GnRH and LH during postnatal development are under non-gonadal regulation before the pubertal period, but whether this also occurs in rodents has remained controversial. This talk will discuss whether gonad-independent central restraint on GnRH and LH secretion operates in prepubertal mice. I will also discuss whether or not such putative central restraint might involve kisspeptin neurons and how this might relate to sex differences in pubertal timing.

CS1R.002 THE EMERGING ROLE OF CHROMATIN REMODELING FACTORS IN FEMALE PUBERTAL DEVELOPMENT

Alejandro Lomniczi, Carlos A. Toro, Carlos F. Aylwin
Neuroscience, OHSU/ONPRC, Beaverton, OR, United States of America

During the infantile-pubertal transition, a diversity of behavioral, physiological, morphological and molecular changes are required in order to the attain fertility. An essential step in this process is the reactivation of the pituitary-gonadal axis by increased hypothalamic secretion of Gonadotropin Releasing Hormone (GnRH). The current dogma postulates that diminishing transsynaptic inhibition jointly with increased excitatory inputs is responsible for the reactivation of GnRH release. With the advent of new high throughput genomic technologies today we can interrogate the hypothalamus for changes in whole-genome mRNA changes as well as histone posttranslational modifications (PTMs) associated with gene regulatory regions. Over the last several years, a plethora of new transcriptional complexes with epigenetic capabilities have been found to be involved in the hypothalamic control of pubertal development. We will review the contribution of several families of epigenetic writers, readers, and erasers in the shift from a repressive to an activated chromatin state at promoter and enhancer regions of the Kiss1 gene during the infantile-pubertal transition. In addition, we will discuss the tantalizing possibility that epigenetics serves as a relay of environmental signals known for many years to modulate pubertal development.

CS1R.003 MENOPAUSE AND THE HUMAN HYPOTHALAMUS: FROM LH PULSES TO HOT FLUSHES

Naomi Rance
Pathology, University of Arizona College of Medicine, Tucson, AZ, United States of America

Degeneration of ovarian follicles in postmenopausal women results in serum estradiol declining to castrate levels and increased LH secretion. Estrogen withdrawal also leads to hot flushes, the primary reason that women seek treatment for menopause. In postmenopausal women,

hypothalamic KNDy neurons hypertrophy and express increased levels of kisspeptin and neurokinin B gene transcripts. These changes are due to estrogen withdrawal because they are duplicated by ovariectomy of young monkeys and reversed by estrogen replacement. Studies using a variety of animal models suggest that KNDy neurons modulate GnRH pulses and mediate estrogen negative feedback. There is also substantial evidence that KNDy neurons are involved in the generation of hot flushes. In rodents, KNDy neurons project to the median preoptic nucleus (MnPO), a major CNS site that controls heat dissipation effectors such as cutaneous vasodilation. Importantly, neurons in the MnPO express NK_3R (neurokinin 3 receptors), the primary receptor for NKB. Pharmacological stimulation of NK_3R in the MnPO reduces core temperature, consistent with the activation of heat dissipation effectors. Moreover, ablation of NK_3R neurons in the MnPO reduces tail skin vasodilation and blocks the effects of senktide. Ablation of KNDy neurons reduces tail skin vasodilation and interferes with the estrogen modulation of body temperature. Notably, infusion of NKB in women induces hot flushes and treatment with NK_3R antagonists reduces their number and severity (Prague et al. Lancet 2017). A dual function for KNDy neurons in modulating LH pulses and thermoregulatory vasodilation explains why in humans, LH pulses are timed with hot flushes.

CS1R.004 KISSPEPTIN IN THE AGING HUMAN BRAIN

Erik Hrabovszky
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No Abstract Submitted

CS1S.001 PRENATAL PROGRAMMING OF OFFSPRING DEVELOPMENT

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Exposure to prenatal stress can have a lasting impact on neurodevelopmental and behavioral outcomes. Stress-associated alterations in DNA methylation globally and within the regulatory region of target genes may account for these long-term effects and may occur within the developing brain and the placenta to shape neurobehavioral outcomes. Using a rodent model of chronic variable stress (CVS) exposure and analyses of the impact of perceived psychosocial stress (PSS) in human mothers, we have explored the epigenetic effects of prenatal stress in both the brain and placenta. In humans, we find an association between PSS and global alterations in placental DNA methylation as well as increased DNA methylation within the *11BHSD-2* gene. Moreover, PSS-associated increases in DNA methylation of the *11BHSD-*

2 gene mediate the association between PSS and fetal neurodevelopment. In laboratory rats, CVS exposure is similarly associated with increased DNA methylation of the *11BHSD-2* gene in the placenta. We have also determined that the *de novo* DNA methyltransferase *DNMT3a* is reduced in expression in the placenta, fetal brain and postnatal brain following CVS. These effects vary dependent on sex and by tissue sub-region. In contrast, the maintenance DNA methyltransferase *DNMT1* is decreased in expression in the placenta and increased in the fetal and postnatal brain following CVS. Collectively, these data indicate that while are sex-specific and tissue-specific effects of prenatal stress, there is a generalized dysregulation of enzymes involved in both *de novo* and maintenance DNA methylation patterns that can account for long-term epigenetic and neurobehavioral outcomes.

CS1S.002 STRESS, EPIGENETICS AND THE DEEP GENOME

Richard G. Hunter
Psychology, University of Massachusetts Boston, Boston, MA, United States of America

Stress is a general term for a unpredictable range of potential threats to an organism's homeostasis. How organisms are able to adapt to a wide variety of unknown stressors with a fixed genome is a fundamental biological challenge. Epigenetic mechanisms have been proposed as an explanation for trait *adaptability* in response to stress and a large literature now supports this idea. Our lab has shown that stress dynamically modulates histone methylation levels in the hippocampus and that this modulation has as a principal target, not protein coding genes, but transposons. Transposons are mobile genetic elements, which comprise an order of magnitude larger fraction of the genome than protein coding genes. Further, we have shown that a number of transposons are directly regulated by the glucocorticoid receptor. The rapid regulation of transposon expression by stress suggests a functional role for these elements in stress adaptation in the nervous system, and a role for the deeper genome in the adaptability of organisms to their environment.

CS1S.003 STRESS-INDUCED GENOMIC ACTION OF GLUCOCORTICOIDS IN THE BRAIN

Johannes M.H.M. Reul[1], Clare L.M. Kennedy[2], Silvia Salatino[3], Eshita Sharma[3], Simon Engledow[3], Emily M. Price[4], Hannah M. Goss[1], Polina Panchenko[5], Helen Lockstone[3], Karen R. Mifsud[6]
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Glucocorticoid hormones (GCs) play a critical role in coping responses and the consolidation of (contextual) memories after a stressful challenge. GCs act through mineralocorticoid (MRs) and glucocorticoid receptors (GRs) – ligand-dependent transcription factors colocalized in hippocampal neurons – but it is still unclear how these receptors interact with the genome and alter gene transcription *in vivo*. Recently, we investigated the interaction of MRs and GRs with GC response elements (GREs) within the rat hippocampal GC-responsive genes *Fkbp5*, *Per1* and *Sgk1* under baseline and stress conditions (Mifsud and Reul (2016) Proc Natl Acad Sci USA). Acute stressors like forced swimming evoked substantial, transient increases in MR and GR binding to GREs within these genes peaking at 30min post-stress. Responses were highly gene- and GRE-dependent but, surprisingly, rather consistent between different stressors. MR and GR binding following stress occurred mainly as GR/GR homodimers and MR/GR heterodimers. Chromatin immunoprecipitation in combination with DNA sequencing (ChIP-seq) revealed that GRs mainly bind to GREs within the genome whereas MR ChIP-seq produced a more complex picture: MRs bound to GREs as well as non-GRE sites within the genome. At present, we are conducting extensive bioinformatics and pathway analyses on these ChIP-seq as well as RNA-seq data. These aim to reveal the putative transcription factor binding motifs, the intracellular pathways, and the (patho-)physiological functions involved. Our data on the genomic action of GCs on the brain paint a picture of growing complexity. They may hold the key to elucidating the true role of GCs in brain function.

CS1S.004 DYSREGULATION OF NON-CG METHYLATION BY CHILD ABUSE

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Background: A growing number of studies suggest a relationship between child abuse and lifetime morphological and functional changes in the amygdala, a brain structure critically involved in emotional regulation, and these changes are likely mediated by epigenetic regulation. **Methods:** Using postmortem brain tissue, we conducted whole genome bisulfite sequencing (WGBS-Seq), RNA-Seq and Chip-Seq (H3K4me1, H3K4me3, H3K27ac, H3K36me3, H3K9me9 and H3K27me3) to obtain a comprehensive map of epigenetic changes in the amygdala associated with child abuse. **Results:** Non-CG methylation is strongly enriched in brain tissue and progressively accumulates during the first few years of life. Therefore, we conducted parallel analyses of DNA methylation differences in the CG context and in the CAC context, which exhibited the highest genome-levels of non-CG DNA methylation. Surprisingly, a history of child abuse associates with epigenetic adaptations that are as frequent in the CAC as in the reference CG context. By incorporating information on histone modification, we observed that the cross-talk between these 2 epigenetic layers strikingly differs among CG and CAC contexts. Importantly, we also found that differentially methylated regions associated with child abuse occur in distinct chromatin states in the CG and CAC contexts. We then further investigated the most significant differentially methylated regions that showed evidence of

functional impact at transcriptional level. **Conclusions:** Our results unravel a previously uncharacterized source of epigenetic plasticity in the brain, which may help us explain the lifelong impact of early-life adversity on brain function, and contribute to the negative mental health outcomes that are strongly associated with child abuse.

CS1T.001 ENOUGH ENERGY FOR REPRODUCTION? IT'S TIME TO GROW-UP.

Carol F. Elias
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Nutrition and growth are important signals to pubertal development. However, how three complex systems are integrated in brain circuits is not well defined. Both signals recruit the PI3K pathways but whether they act in the same neuronal population is also not known. In this symposium, we will discuss recent findings from our laboratory showing the role of PI3K subunits in cells directly responsive to leptin in the coordination of growth and pubertal development. Disruption of p110α and p110β subunits in leptin receptor cells (LRΔα+β) produces a lean phenotype associated with increased energy expenditure, locomotor activity, and thermogenesis. LRΔα+β mice have deficient growth and delayed puberty. Single subunit deletion (i.e., p110α in LRΔα) resulted in similarly increased energy expenditure, deficient growth, and pubertal development, but LRΔα mice have normal locomotor activity and thermogenesis. Blunted PI3K in leptin receptor (LR) cells enhanced leptin sensitivity in metabolic regulation due to increased basal hypothalamic pAKT, leptin-induced pSTAT3, and decreased PTEN levels. However, these mice are unresponsive to leptin's effects on growth and puberty. We further assessed if these phenotypes were associated with disruption of insulin signaling. LRΔInsR mice have no metabolic or growth deficit and show only mild delay in pubertal completion. Our findings demonstrate that PI3K in LR cells plays an essential role in energy expenditure, growth, and reproduction. These actions are independent from insulin signaling. We will also discuss potential neuronal pathways underlying these effects.

CS1T.002 ROLE OF PITUICYTES, THE RESIDENT ASTROGLIA OF THE NEUROHYPOPHYSIS IN NEURO-VASCULAR DEVELOPMENT.

Gil Levkowitz
Weizmann Institute of Science, Rehovot, Israel

The hypothalamo-neurohypophyseal system (HNS) is an interface through which the brain regulates body homeostasis by means of releasing the hypothalamic neurohormones oxytocin and arginine-vasopressin to the general circulation. The basic components of the HNS are the hypothalamic axonal projections, endothelial blood vessels and astroglial-like cells, termed pituicytes. These three tissue types converge and interact at the ventral forebrain to establish an efficient neuro-vascular interface, which allows the release of neurohormones from the

brain to the periphery. However, the mechanism underlying this process and in particular the role of the pituicytes is unknown. In the adult animal, pituicytes facilitate hormone secretion from neurohypophyseal axons to the perivascular space but their exact role is still not clear. Using zebrafish as model organism, we have been studying the role of pituicytes in HNS development and function. I will present new data concerning the molecular identity and role of pituicytes in establishing a major neuroendocrine system which regulates body homeostasis in vertebrates.

CS1T.003 DEVELOPMENT OF THE CHICK HYPOTHALAMUS

Marysia Placzek[1], Travis Fu[2], Matthew Towers[2]

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An important research goal is to understand how the vertebrate hypothalamus is built and maintained. Our studies in the embryonic chick suggest that the hypothalamus self-assembles from a multipotent FGF-responsive *Fgf10*$^{+}$ stem-like cell that is induced in response to signals from the underlying prechordal mesoderm. Our work in chick and zebrafish suggest that hypothalamic stem-like cells generate hypothalamic progenitors that grow anisotropically in a highly-ordered manner in space-and-time, to generate anterior, then posterior parts of the hypothalamus, and the ventrally-protruding infundibulum. *Fgf10*$^{+}$ stem-like cells appear to be retained in the centre of the hypothalamus - potentially even into adulthood. The ligands Fgf and Shh appear to form key components of a highly-regulated network that ensures the correct balance of stem-like and progenitor cells, promotes the self-assembly and maintenance of the hypothalamus and underpins development of the infundibulum – key to formation of the median eminence and hypothalamo-pituitary axis. 1. Fu T, Towers M and Placzek M. (2017) Fgf10+ progenitors give rise to the chick hypothalamus by rostral and caudal growth and differentiation. Development 10.1242/dev.153379 2. Muthu, V., Eachus, H., Ellis, P., Brown, S and Placzek, M. (2016) Rx3 and Shh direct anisotropic growth and specification in the zebrafish tuberal/anterior hypothalamus. Development pii: dev.138305. 3. Robins S, Stewart I, McNay DE, Taylor V, Giachino C, Goetz M, Ninkovic J, Briancon N, Maratos-Flier E, Flier JS, Kokoeva MV and Placzek M (2013) Alpha-tanycytes of the adult hypothalamic third ventricle include distinct populations of FGF-responsive neural progenitors. Nat. Commun. 4 2049

CS1T.004 MATERNAL DIET INFLUENCES ON OFFSPRING DEVELOPMENT AND HEALTH

Kellie L. Tamashiro

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Metabolic dysregulation, including obesity and diabetes, has been associated with a range of cognitive deficits, from mild cognitive impairment to dementia. Even prodromal metabolic dysregulation is associated with and predictive of cognitive decline, with impairment arising as early as adolescence. Exposure to a high-fat (HF) diet has also been suggested to directly impair cognition in the absence of apparent peripheral metabolic disturbance. Given the current acceleration of maternal obesity and overnutrition rates and parallel globalization of the HF "Western" diet, understanding the consequences of HF diet exposure early in development and the potential for cognition-protecting interventions is crucial. We found that offspring of rats dams that consumed a HF diet (60% kcal from fat) during pregnancy and lactation show hallmark signs of metabolic dysregulation, including increased adiposity, impaired glucose tolerance, and leptin resistance, despite being weaned onto a standard low-fat chow diet (CH). HF offspring also have cognitive deficits, exhibiting compromised performance as adults in tasks of learning and memory and object recognition. Exercise has been shown to be a promising intervention in targeting both metabolic and cognitive deficit in adults. However, few studies have assessed the effects of gestational exercise exposure on offspring development, especially against the setting of maternal HF diet. Exercise introduced during gestation to pregnant dams had beneficial effects against metabolic and cognitive deficits in HF offspring that persisted into adulthood. Exercise during gestation could be an effective early intervention against the adverse effects of maternal overnutrition and metabolic state on offspring development and long-term health.

Symposia Presentations – Monday, July 16, 2018

CS2B.001 SEASONAL CHANGES IN THE BRAIN AND SOCIAL BEHAVIOR OF VOLES

Annaliese K. Beery
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Social relationships between peers are central to the ability to live in groups. Our understanding of the neurobiology of affiliative behavior has been greatly advanced by studies of maternal attachments and reproductive pair-bonds, but little is known about the pathways supporting non-reproductive relationships. Peer relationships may be mediated through prosocial reinforcement pathways, such as are present in reproductive affiliation, or through other means such as the absence of antisocial tendencies including territoriality, aggressiveness, and fear of other individuals. To determine how neural pathways supporting group living are similar to and different from those supporting reproductive relationships, we study same-sex affiliative behavior in a seasonally group-living species. Meadow voles (*Microtus pennsylvanicus*) transition from being solitary and aggressive in summer months to living in social groups during winter. This transition is mirrored in the lab by changes in social tolerance and partner huddling between long and short day lengths. I will discuss the contributions of multiple neuropeptides

and their receptors to natural variation in social selectivity in meadow voles, with comparisons to other rodent species. These studies indicate that neuroanatomical substrates of non-sexual social behavior differ from those implicated in sexual bond formation, while sharing some common elements.

CS2B.002 FOOD, ESTROUS CYCLES, AND THE CIRCADIAN CLOCK

Eric M. Mintz
Biological Sciences, Kent State University, Kent, OH, United States of America

Circadian clocks drive daily rhythms of physiology and behavior. In most organisms, these rhythms are synchronized to the daily light/dark cycle, however, both the timing and the amplitude of expressed rhythms are modulated by a wide range of factors. Two important factors are the timing of feeding and the phase of the estrous cycle. Both feeding and estrous cycle timing alter circadian locomotor activity rhythms without necessarily altering the phase of the underlying circadian pacemaker. In mice, restricting feeding to a 4-hour window during the day induces a period of food anticipatory activity prior to feeding, however, the amplitude of this activity is higher in males than in females. Estrous cycle-dependent variations in locomotor activity are also eliminated during temporally-restricted feeding. To determine the source of the sex differences in food anticipatory activity, a series of experiments were performed to identify when during development this phenomenon first appears. Surprisingly, when juveniles are exposed to food restriction this eliminates adult sex differences in food anticipatory activity. Estrous cycle-dependent variations in locomotor activity, however, are quickly restored when animals are returned to ad/lib feeding. Ongoing experiments will evaluate whether the timing of restricted feeding alters the functionality of the female reproductive system in a circadian rhythm-dependent manner. These data will be important in understanding how disruption of normal activity cycles can have a negative effect on fertility.

CS2B.003 LIGHT AT NIGHT, CLOCKS, AND HEALTH

Randy J. Nelson
Behavioral Health and Psychiatry, West Virginia University, Morgantown, WV, United States of America

Life on earth has evolved under relatively bright days and dark night conditions. The widespread adoption of electric lights during the past century exposed animals, including humans, to significant light at night for the first time in their evolutionary history. Endogenous circadian clocks depend on light to entrain to the external daily environment, and seasonal rhythms depend on clear nightly melatonin signals to assess time of year. Thus, light at night can derange temporal adaptations. Indeed, disruption of naturally evolved light-dark cycles

results in several physiological and behavioral changes with potentially serious implications for health, including deranged metabolism and cancer. In this talk, data from our lab will address the role of dim light at night on clock genes, metabolic hormones, and pancreatic cancer. The association among light at night, dysregulation of clock gene expression, and neuroinflammation in the context of pancreatic function will be presented.

CS2B.004 LIGHT, EMOTION AND COGNITION

Lily Yan
Psychology, Michigan State University, East Lansing, United States of America

Light has profound effects on behavior and physiology in mammalian species. The level of illumination during the day has been found to be positively associated with emotional well-being and optimal cognitive functions in humans, although the underlying mechanisms are poorly understood. One of the barriers in this research area is due to the fact that commonly used laboratory animal models are often nocturnal species. There are substantial differences in how light affects nocturnal and diurnal species, *e.g.*, light induces sleep in nocturnal mammals and wakefulness in diurnal ones, like us. Therefore, the mechanisms through which light modulates emotion and cognition must be unique for each of these chronotypes. Our recent work has developed a diurnal rodent model, the *Nile* grass rat (*Arvichantis niloticus*), to explore the neural pathways mediating the effects of light on emotion and cognition. Animals were housed under the same 12:12hr light/dark cycle but different daytime light intensity to simulate bright sunny days vs. dim cloudy days. The results revealed increased depression- and anxiety-like behaviors as well as impaired hippocampal functions in grass rats housed in dim light during the day. Utilizing this unique diurnal model, we have identified a few neural substrates that likely mediate the effects of ambient light on emotion and cognition. A better understanding of how light modulates emotion and cognition will contribute to novel preventive and therapeutic strategies for mood disorders as well as cognitive decline associated with aging and dementia. Supported by NIH grants R01MH111276 and R21NS098173.

CS2M.001 PHYSIOLOGICAL ROLE OF ASTROCYTES IN METABOLIC CONTROL

Julie Chowen
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The role of glial cells in metabolic control has received increasing attention during the past decade, primarily due to the observation that hypothalamic inflammation/gliosis in response to high fat diet intake is involved in the pathophysiological responses to poor dietary habits and weight gain. However, glial cells play a very important role in maintaining metabolic homeostasis throughout life. Astrocytes are the most abundant glial cell in the brain and are involved in numerous physiological processes including neuroprotection, synaptogenesis,

synaptic plasticity, synaptic transmission, glucose up-take and transport, glycogen storage and fatty acid metabolism. They express receptors for metabolic hormones such as leptin, ghrelin and insulin, as well as for other factors involved in metabolic control such as endocannabinoids and insulin-like growth factors (IGFs) 1 and 2. Astrocytes communicate with surrounding cells, including neurons, through the release of gliotransmissors, growth factors, metabolites, cytokines and other messengers that are currently under investigation. These glial cells also undergo morphological changes that modify their physical interactions with neighboring neurons and the synaptic inputs to specific neuronal populations. This conference will concentrate on our advances in the understanding of how hypothalamic astrocytes modulate physiological metabolic control and the possible mechanisms and signals involved. In addition, possible differences between hypothalamic astrocytes from males and females will be addressed.

CS2M.002 ASTROCYTE INFLAMMATION AND ENERGY HOMEOSTASIS

Kate L.J. Ellacott
Medical School, University of Exeter, Exeter, United Kingdom

Astrocytes, abundant glial cells in the central nervous system, are critical mediators of tissue homeostasis and synaptic plasticity. Our lab and others have shown that in rodents acute and chronic high-fat feeding cause hypothalamic astrocytes to undergo gliosis, a process of morphological change and activation of inflammatory signalling; however, the physiological significance of high-fat diet-induced hypothalamic astrogliosis is not fully understood. Our work has contributed to understanding how astrocytes regulate the acute homeostatic response to high-fat feeding. We hypothesized that rapid activation of inflammatory signalling cascades in astrocytes following high-fat feeding is part of a homeostatic compensatory response and inhibiting inflammation in astrocytes should acutely increase food intake during the initial hyperphagic response to the high-fat diet. To test this hypothesis we bred transgenic mice with doxycycline inducible inhibition of NF-κB signalling specifically in astrocytes. These mice did not show the rapid onset astrogliosis in response to acute high-fat feeding and ate significantly more in the first hyperphagic phase (24h) compared with control littermates. These data demonstrate that inflammatory activation of astrocytes is part of a novel homeostatic mechanism regulating feeding. Current work in our lab is focused on understanding how astrocytes sense nutritional change and modulate neural networks regulating energy homeostasis. This work was supported by grants from the Medical Research Council (UK) and the British Society for Neuroendocrinology

CS2M.003 HYPOTHALAMIC GLIOSIS AND OBESITY

Joshua P. Thaler
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Obesity and high fat diet (HFD) consumption in rodents is associated with hypothalamic inflammation and activation of microglia, the resident CNS immune cells. Recently, we provided evidence that microglia are critical intermediary cells that transmit the inflammatory signal induced by HFD exposure. Mice with reduced inflammatory capacity in microglia are protected from HFD-associated hyperphagia and DIO while those with excess activation show diet-independent weight gain. Using these and other genetic models, we now describe a novel aspect of CNS regulation of metabolism in which microglial inflammatory activation promotes HFD overconsumption and weight gain but offsets obesity-associated dysregulation of glucose homeostasis. These results indicate the need for pathway-specific targeting to develop glial-based therapeutics for obesity and diabetes.

CS2M.004 HYPOTHALAMIC NEURON-MICROGLIAL INTERACTION IN OBESITY AND DIABETES

Chun-Xia Yi
Department of Endocrinology And Metabolism, Academic Medical Center (AMC), University of Amsterdam (UvA), Amsterdam, Netherlands

Microglia are the innate immune cells of the brain that act as the key homeostatic keeper in the microenvironment for neural function. In obesogenic diet-fed animals, hypothalamic microglia enter a proinflammatory state, in association with a selective loss of anorexigenic pro-opiomelanocortin (POMC) neurons. We have explored how the hypothalamic neuron-microglial interactions that control energy metabolism are interrupted in obesity and type 2 diabetes. We have proven that in a lipids-enriched obesogenic diet also dietary sugar is required to drive the hypothalamic microglial activation, mediated by the advanced glycation end products. In obesogenic diet-activated microglia, the lipoprotein lipase (LPL) gated-fatty acid influx is essential for maintaining microglial immune function. Microglia lacking LPL have defective phagocytosis and immune response to cytokine. On an obesogenic diet, mice lacking lipoprotein lipase from their microglia show an accelerated loss of POMC neurons with exacerbated weight-gain and glucose intolerance. Importantly, in post-mortem human hypothalamus of type 2 diabetic patients, we also observed a loss of POMC neurons Whether this is linked to microglial dysfunction as well as the impact of anti-diabetic treatments are being investigated.

CS2R.001 DISSECTING THE NEUROENDOCRINE DYSFUNCTION OF POLYCYSTIC OVARY SYNDROME

Rebecca E. Campbell
Centre For Neuroendocrinology and Department Of Physiology, University of Otago, Dunedin, New Zealand

Polycystic ovarian syndrome (PCOS), characterised by hyperandrogenism, menstrual irregularities and poly-follicular ovaries, is the leading cause of anovulatory infertility in premenopausal women. High frequency luteinizing hormone (LH) secretion and impaired gonadal steroid hormone negative feedback responses are also evident in women with PCOS, suggesting that neuroendocrine dysfunction is likely to be central to PCOS pathology. We have employed a preclinical mouse model mimicking the impaired steroid hormone feedback of PCOS to identify the specific circuit abnormalities underpinning the development and pathophysiology of PCOS. We have identified that prenatally androgenised, PCOS-like mice have dramatically increased GABAergic innervation to gonadotropin-releasing hormone (GnRH) neurons, originating largely from steroid hormone-sensitive GABAergic neurons in the arcuate nucleus. This lecture will highlight our most recent work investigating the functional relevance of arcuate GABA neuron activation, and the development and maintenance of aberrant GABA innervation to GnRH neurons in the PCOS phenotype. Opto- and chemo-genetic activation of arcuate GABA neurons supports the stimulatory role of this population in mediating increased GnRH/LH secretion in PCOS. We have found that increased GABAergic innervation to GnRH neurons develops early, prior to pubertal onset and the development of hyperandrogenism, suggesting that changes in the brain precede disease development. Despite this early programming, we found that long-term androgen receptor blockade in adulthood is sufficient to completely rescue normal GABA innervation and this is coincident with improved ovarian morphology and a restoration of estrous cyclicity. These findings are expanding our current understanding how GnRH/LH pulsatility is regulated and revealing potential PCOS treatment targets.

CS2R.002 SEMAPHORIN MUTATIONS IN KALLMANN'S SYNDROME

Roberto Oleari[1], Valentina Andre[1], Sophia Tahir[2], Lise Roth[3], Antonella Lettieri[1], Ivano Eberini[1], Valeria Scagliotti[4], Hellmut Augustin[3], Carles Gaston-Massuet[4], Khalid Hussein[5], Anna Cariboni[6]
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Gonadotropin releasing hormone neurons are a small group of scattered hypothalamic neuroendocrine cells that control reproductive functions in all mammals and many vertebrates. Despite their position in the adult hypothalamus, during development they originate in the nasal placode and migrate along the vomeronasal nerve to reach the forebrain and attain their final position in the hypothalamus. Failure of GnRH neurons to migrate lead to

Hypogonadotropic Hypogonadism (HH) or Kallmann Syndrome(KS), genetic disorders characterised by GnRH deficiency and absent or delayed puberty. The genes underlying HH/KS are largely unknown but the combination of genetically modified mouse models with exome sequencing may help to identify the unknown genes. We have previously demonstrated that class 3 semaphorin (SEMA) 3A controls the positioning of the vomeronasal nerve and therefore the migration of GnRH neurons via Neuropilin (NRP1-2) receptors. Mice lacking SEMA3A display typical KS features including hypogonadism and mutations of the SEMA3A gene have been subsequently identified in patients with KS. In the search for additional SEMA3-mediated signalling pathways involved in this developmental process, by applying exome sequencing and bionformatic approaches we identified mutations in other genes belonging to the class 3 semaphorins. Thus, during my talk I will present published and unpublished work showing the different roles of these genes during the development of GnRH neurons.

CS2R.003 PUBERTAL DEVELOPMENT AND REGULATION IN HUMANS: FROM BEDSIDE TO BENCH

Ursula B. Kaiser

Department of Medicine, Endocrinology, Diabetes And Hypertension, Brigham and Women's Hospital/Harvard Medical School, Boston, MA, United States of America

The hypothalamic-pituitary-gonadal (HPG) axis controls puberty and reproduction and is tightly regulated by a complex network of excitatory and inhibitory factors. Delayed or absent activation of the HPG axis results in delayed puberty or hypogonadotropic hypogonadism, whereas early activation results in central precocious puberty (CPP). In recent years, many genes have been identified in this complex network, from genetic studies of human subjects with pubertal disorders, providing insight into the regulation of GnRH secretion. These advances were heralded by the discovery of the kisspeptin system as a critical component for the activation of GnRH secretion, and followed by the discovery of the tachykinin, neurokinin B, and its role in puberty initiation, in turn, through regulation of kisspeptin secretion. More recently, we identified loss-of-function mutations in the *MKRN3* gene, encoding makorin ring finger protein 3, in patients with CPP. MKRN3 is an imprinted gene on chromosome 15q11.2 in the Prader-Willi Syndrome critical region, with expression only from the paternally inherited allele. *Mkrn3* is expressed at high levels in the mouse hypothalamus prepubertally and decreases prior to puberty onset, suggesting that it acts as an inhibitor of GnRH secretion. Studies in cellular and animal models will help to elucidate the mechanisms by which MKRN3 regulates GnRH secretion and bring new insights into reproductive physiology.

CS2R.004 GENETIC DETERMINISM OF PUBERTAL ONSET DISORDERS

Nicolas De Roux
INSERM U1141, Paris, France

Pubertal onset disorders are conditions defined by an abnormal timing of the neuroendocrine reactivation of the gonadotropic axis after birth. An early reactivation during childhood causes central precocious puberty whereas the absence of reactivation causes a gonadotropic deficiency and an absence of puberty. For the last 20 years, genetics of rare neuroendocrine disorders have led to characterize several new mechanisms of congenital hypogonadotropic hypogonadism (CHH). Initially, studies were focused on Kallmann syndrome and isolated CHH by linkage analysis and candidate gene approaches. New proteins playing a fundamental role in the GnRH neuron development or function have thus been discovered. More recently, an interest has been developed on very rare complex neurodevelopmental or neurodegenerative disorders which include congenital gonadotropic deficiency. These phenotypes represent an interesting opportunity to discover unexpected proteins in the post-natal maturation of the GnRH neuronal network. At the opposite of the phenotypic spectrum of pubertal disorders, central precocious puberty represents an unique opportunity to discover cellular and molecular pathways which control the post-natal maturation of the function of reproduction. Although less investigate than CHH, due probably to the more complexity of the genetic model, this strategy looks very promising.

CS2S.001 THE EFFECTS OF ALTERING THE PATTERN OF GLUCOCORTICOIDS ON COGNITIVE FUNCTION.

Becky L. Conway-Campbell
Henry Wellcome Laboratories for Integrative Neuroscience and Endocrinology, Bristol University, Bristol, United Kingdom

Adrenal glucocorticoids (GCs) cortisol and corticosterone are secreted in a characteristic pulsatile manner establishing an ultradian pattern. We have demonstrated, both in cell models and *in vivo,* that ultradian GC exposure induces a functional output in individual target cells. The intracellular GC receptor (GR) is activated in distinct pulses and transmits this signal to the chromatin template, inducing a 'gene pulsing' effect. Notably, dysregulated GC pulse characteristics are reported in variety of chronic pathophysiological conditions, including Cushing's Disease and Obstructive Sleep Apnea. Symptoms including cognitive and affective dysfunction are often reported in these patients, therefore we have assessed the effect of altering the endogenous ultradian GC pattern on transcriptional output in the hippocampus, a brain region involved in cognitive processing and affective state. RNA-Seq expression profiling of hippocampus from adrenalectomised rats replaced with pulsatile or constant corticosterone revealed specific pattern-dependent regulation of GC target genes.Furthermore, chronic treatment with synthetic GCs (sGCs) resulted in even greater dysregulation of the endogenous GC profile. sGCs such as dexamethasone and prednisolone are potent anti-inflammatory

agents, but have well-documented adverse side effects including memory impairment. Therefore we have characterised the molecular, physiological and cognitive impairments arising from chronic sGC treatment. Notably, we report that sGCs can induce prolonged GR activation, disruption of circadian gene regulation in the brain, and impaired memory. Pathophysiological or pharmacological alteration to GR dynamics can therefore result in profound functional changes in target tissue function, adversely affecting circadian physiological processes and function of discrete brain regions including the hippocampus.

CS2S.002 NON-CANONICAL GR PHOSPHORYLATION PATHWAY ON DENDRITIC SPINE PLASTICITY

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Stress causes reversible remodeling of brain circuits involved in cognitive, executive and hedonic behaviors in part through glucocorticoid hormones. While small amounts of glucocorticoids support cognition and spine synapse turnover, excess stimulation by glucocorticoids predisposes to stress disorders via aberrant structural remodeling of dendritic spines. Which spines survive, which spines are eliminated may encode for the structural basis of allostasis. To influence spine formation and elimination, glucocorticoids use distinct signaling pathways and temporal domains. Spine formation is rapid, does not require gene transcription whereas, spine elimination is a slow process that requires new gene products. Both are required to forge connectivity networks within neuronal ensembles activated at the time of learning. One hypothesis to account for is that glucocorticoids have permissive effects on spine turnover that coincidence detection signals exploit for persisting structural and behavioral adaptation. Learning objective The learner will be able: To distinguish canonical from non-canonical glucocorticoid signaling To understand the utility of context-dependent glucocorticoid signaling by the example of interaction between the glucocorticoid and the neurotrophin pathways. To describe spine turnover in normal and stress conditions.

CS2S.003 GLUCOCORTICOIDS AND GENE TRANSCRIPTION IN THE BRAIN: BEYOND THE RECEPTORS

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Glucocorticoid hormones alter emotional and behavioural reactivity by activating neuronal mineralocorticoid (MR) and glucocorticoid receptors (GR). Many MR and GR-mediated effects

depend on regulation of gene transcription. MR and GR target genes differ, but there is also a marked diversity in GR-mediated effects in different brain regions. Neuronal excitability may be stimulated or suppressed, dendritic complexity increased or reduced, *Crh* gene transcription activated (amygdala) or repressed (hypothalamus). The cell and gene-specificity of MR and GR effects depends on 1) cellular chromatin structure, 2) different interacting transcription factors, and 3) different *coregulators,* the genomic signal transduction partners of the receptors. Using genome-wide analysis of MR/GR chromatin binding in the rat hippocampus, we identified NeuroD transcription factors as likely signalling partners that are specific for MR mediated effects. Using both candidate-driven and genome wide co-expression analysis, we found that there can be strikingly cell type specific co-expression of GRs with particular coregulators. Functionally, we have shown that manipulation of expression of single coregulator can induce gene-selective GR resistance (that is: Steroid Receptor Coactivator 1 splice variant expression in the Central Amygdala determines regulation of the mouse *Crh* gene). Thus, the dual receptor MR/GR system depends on an as yet unidentified number of downstream signalling pathways. An interesting development is the availability of Selective Receptor Modulators, that can target subsets of downstream pathways. These compounds can help us to dissect the signalling mechanism underlying particular glucocorticoid effects, *and* may constitute an alternative strategy for treatment of stress-related disease.

CS2S.004 STRESS DESENSITIZATION OF THE EXCITATORY ADRENERGIC DRIVE TO THE HPA AXIS

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Noradrenergic innervation of corticotropin releasing hormone (CRH) neurons of the hypothalamic paraventricular nucleus (PVN) provides an excitatory drive to the HPA axis in response to physiological stress. Glucocorticoids secreted in response to HPA activation feed back to arrest HPA activation in a negative feedback manner. Slice-patch recordings of CRH neurons revealed a novel norepinephrine (NE) regulation of PVN CRH neurons, involving a retrograde neuronal-glial activation of local excitatory and inhibitory circuits. The NE response is triggered by activation of postsynaptic alpha1 adrenoreceptors (AR1a's) in the CRH neurons. Prior restraint stress caused desensitization of the CRH neurons to the NE excitatory effect, which was prevented by inhibiting glucocorticoid synthesis. Preincubation of brain slices in corticosterone (5-10 min) also caused AR1a desensitization in the CRH neurons, which was prevented by an endocytosis inhibitor, suggesting that rapid glucocorticoid-induced NE desensitization is mediated by AR1a internalization. Live-cell imaging of AR1a's in a hypothalamic cell line revealed that corticosterone, without effect alone, rapidly facilitated NE-induced AR1a internalization. Corticosterone increased AR1a interaction with the late endosomal marker Rab 11, but had no effect on interaction with the early endosomal marker Rab 5, suggesting that corticosterone prevents AR1a recycling to the membrane. These studies demonstrate a stress desensitization of CRH neurons to noradrenergic activation by the rapid

glucocorticoid-induced inhibition of AR1a trafficking to the membrane. This stress desensitization of the excitatory adrenergic drive to the CRH neurons may contribute to the rapid negative feedback regulation of the HPA axis by glucocorticoids. Supported by NIH 2R01 MH066958.

CS2T.001 NEURAL CIRCUITS INVOLVED IN THE CLOCK CONTROL OF FEEDING

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Carolina Escobar, RM Buijs, M. Angeles-Castellanos, E Espitia Dept. of Anatomy, Faculty of Medicine UNAM, Mexico Meal schedules have proven to be relevant time signals for the circadian system. The moment of food access can eleicit anticipatory responses at the behavioral and physiological level, eliciting arousal, foraging and increased locomotion, as well as promoting changes at the level of the digestive system and brain areas related with food intake and metabolic balance. In the brain hypothalamic structures as well as corticolimbic areas exhibit increased activity (measured with c-.Fos) in anticipation to food access. In the same areas the clock protein PER1 acquires daily oscillations driven by the meal schedule. This food entrained activity indicates the powerful influence that feeding schedules have on the circadian function. In this talk I will present evidence obtained with a scheduled food access paradigm using regular food and with palatable food and discuss the emergence of neural circuits that may account for food driven circuits and for compulsive ingestive behavior. Support by PAPIIT-UNAM IG-200417 and CONACyT 239403

CS2T.002 INFLUENCE OF PERIPHERAL AND CENTRAL CLOCKS ON METABOLISM AND BEHAVIOUR

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Circadian rhythms are important regulators of physiology and behaviour, adapting the organism to the 24-hour day cycle. In mammals, these rhythms are coordinated by interplay of central and peripheral circadian clocks through tissue-specific transcriptional programs. Circadian clock dysfunction and circadian rhythm disruption promote the development of metabolic diseases such as obesity, type-2 diabetes and the metabolic syndrome. Recent studies have started to dissect the differential input of central and peripheral tissue clocks to metabolic homeostasis. We and others have shown that adipose tissue clocks are involved in the regulation of fatty acid release into the blood and the regulation of body weight. Genetic ablation of adipose clocks in

mice leads to hyperphagy and obesity. In another mouse model, we have shown that gene therapeutic restoration of liver clock function may restore body weight regulation in *Clock* mutant animals. The central circadian pacemaker in the hypothalamic suprachiasmatic nucleus (SCN) coordinates peripheral clocks with each other and with the external light-dark cycle. Lesion of the SCN in rodents results in overweight and insulin resistance, highlighting the pivotal role of the SCN clock in metabolic homeostasis. We have recently developed a mouse model with genetic ablation of SCN clock function, leaving peripheral tissue clocks functionally intact and conserving light input into the circadian system. In these mice, we found that body weight regulation and metabolic function depends on external lighting conditions, highlighting the interplay of SCN and peripheral clock function in metabolic homeostasis.

CS2T.003 TIME-RESTRICTED EATING FOR THE PREVENTION AND TREATMENT OF METABOLIC DISEASES

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Circadian timing system is a dynamic integrator of genome-environment interaction. Chronic circadian rhythm disruption increases risk for numerous chronic diseases. Therefore, approaches to sustain robust circadian rhythm hold untapped potential to prevent or reverse chronic diseases. Time-restricted eating (TRE; 8-12 h food access in the active phase) without changing nutrient quantity or quantity improves circadian rhythms and aligns them appropriately to the period of fasting or feeding. Subjecting rodents to TRE prevents excessive weight gain, adiposity, glucose intolerance, systemic inflammation, hepatosteatosis and hypercholesterolemia independent of diet type. Rodents on TRE also show increased endurance, motor coordination, and brown fat function. When high fat diet induced obese mice or mice with genetic predisposition to obesity are subjected to TRE, they also experience similar therapeutic benefits. TRE does not alter the major gut microbiome composition, yet it modulates gut metabolism of carbohydrates and bile acids. Unbiased assessment of the temporal changes in transcriptome, metabolome and gut microbiome revealed TRE exerts pleiotropic effect on metabolism in multiple tissue types in both rodents and insects. To test the translational potential of TRE in humans, we have begun to monitor daily eating pattern using a novel unbiased, evidence-based, and scalable method. Preliminary data shows erratic eating pattern with extended period of frequent caloric intake events that potentially maintains a post-prandial metabolic state in humans in widespread. Time-restricted feeding without overt attempt to alter nutrition quality or quantity might be a potential new lifestyle intervention to improve human health.

CS2T.004 METABOLIC AND CARDIOVASCULAR CONSEQUENCES OF CIRCADIAN DISRUPTION, HUMAN STUDIES

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Obesity and diabetes have obtained epidemic proportions and contribute significantly to cardiovascular disease and mortality. Most research and clinical attention has focused on the importance of what we eat and how much we exercise in these developments. However, in recent years it has become clear that also other modern life style changes such as the timing of food intake, of physical activity and of sleep importantly impact metabolism and cardiovascular risk factors. This presentation will focus on the role of the endogenous circadian system, and its interaction with a disturbed timing of the behavioral/environmental sleep/wake, rest/active, fasting/feeding, and dark/light cycles on cardiometabolic function. For example, the circadian system and circadian misalignment (i.e., the misalignment between the circadian system and the behavioral/environmental cycle) influence glucose metabolism, energy expenditure, food intake, weight regulation, inflammation, and cardiovascular function in humans. These new observations provide possible mechanistic evidence for the adverse cardiometabolic effects observed with shift work, late night snacking, and circadian-related gene variants. The objectives of my talk will be to (a) discuss the effects of the human circadian system and circadian misalignment on glucose control, metabolism, inflammation, and cardiovascular function; (b) present data on the effect of melatonin and its interaction with type 2 diabetes risk variant MTNR1B on glucose control; and (c) discuss the evidence for the importance of not just what you eat, but also of when you eat for health and disease.

Symposia Presentations – Tuesday, July 17, 2018

CS3B.001 THE RAT IN THE NICU: MIMICKING EARLY-LIFE STRESSORS OF PRETERM INFANTS FOR TRANSLATIONAL STUDIES

Susanne Brummelte
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Preterm infants have to undergo a lot of stressful events while they are in the Neonatal Intensive Care Unit (NICU), including exposure to painful procedures and reduced maternal contact. Recent research suggests that the number of skin-breaking procedures is related to brain maturation in preterm infants. However, there is a dearth of knowledge about what comprises an optimal NICU environment and some potentially contributing factors to negative outcome are difficult to study in humans. Thus, in our rodent model, we aim to mimic certain NICU conditions and we are currently investigating the role of corticosterone and the

modulating effect of maternal care on the negative impact of neonatal pain exposure. As most stressful and painful procedures in the NICU are mandatory for an infant's survival we need to explore mechanisms to better understand and control pain experiences at this developmental stage and thus help to prevent long-term consequences of this early adverse environment.

CS3B.002 THE IMPACT OF SOCIAL CONTEXT AND ENVIRONMENT ON EARLY ADVERSITY

Marsha Campbell-Yeo
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Current evidence suggests that repeated procedural pain contributes to long-term changes in stress regulation and brain development in vulnerable preterm infants, after accounting for associated clinical confounders. Moreover, in addition to pain, these infants experience prolonged maternal separation, and adverse environmental factors that contribute to their exposure to early adversity. Yet few studies examining long-term consequences of neonatal pain exposure have taken the potential interactions between these factors into consideration. Current evidence regarding the effectiveness of maternal-centred interventions, such as skin-to-skin contact (SSC) or breastfeeding, to diminish immediate pain reactivity and improve biobehavioral regulation will be reviewed. Findings from our recent clinical trial examining the sustained pain reducing effect of SSC, alone and in combination with a sweet tasting solution compared to a sweet tasting solution as well as the impact on neurodevelopment will be presented. The potential neuroprotective aspects of these factors will also be explored.

CS3B.003 HARNESSING THE ENVIRONMENT TO PROMOTE RESILIENCY TO EARLY LIFE ADVERSITY

Amanda Kentner
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Environmental enrichment is a protocol of enhanced stimulation; the key components of this condition appear to include novel and diverse sensory experiences that can be introduced to both animals and humans. The utility of environmental enrichment in pediatric settings has shown success in autistic children and those at risk for cerebral palsy. However, the specific components of enrichment (e.g., sensorimotor stimulation, increased opportunities for social engagement, enhanced parental care) that may lead to clinical benefits are not understood. Perinatal exposure to infection is identified as a risk factor for neurodevelopmental disorders such as autism. Moreover, adverse experiences in early life can severely and profoundly reorganize the brain, leading to changes in function and related mental health outcomes. In our work, we use animal models of early-life inflammation, and other stressful experiences, to

explore the potential for environmental enrichment to offer neuroprotection and remediation against associated neuroendocrine and behavioral detriments. Moreover, we characterize the specific enrichment components and neuromechanisms that underlie such benefits.

CS3B.004 OXYTOCIN PROMOTES RESILIENCE TO NEONATAL NEGLECT ON ADULT SOCIAL ATTACHMENT IN PRAIRIE VOLES

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Oxytocin receptors (OXTR) in the nucleus accumbens (NAcc) play a critical role in pair bond formation in adult prairie voles (*Microtus ochrogaster*). Variation in neonatal nurturing can have long-term influences on adult social behaviors. We examined the potential developmental role of neonatal NAcc OXTR signaling on resilience to early social neglect with respect to adult social bonding. Pups were socially isolated for 3-hr per day, or unmanipulated, from postnatal day 1-14. As adults, voles were tested on a partner preference test following cohabitation with an opposite sex stimulus animal. Males from both groups failed to display partner preferences excluded from further analyses. Control females displayed robust partner preferences while those socially isolation showed impaired pair bonding. In the isolated group, the density of NAcc OXTR was correlated with time spent with partner. Females with high NAcc OXTR were resilient to social isolation and formed partner preferences, while those with low NAcc OXTR failed to form bonds. Isolated pups received increased parental nurturing upon return to the neonatal nest than control pups, and simulating licking with a paint brush activated OT neurons. Evoking endogenous OT release with a melanocortin agonist upon separation eliminated the effect of isolation on adult bonding. Thus, OXTR signaling during development has an organizational effect on neural systems involved in adult social attachment and individual variation in NAcc OXTR confers resilience or susceptibility to early social neglect. The remarkable variation in NAcc OXTR density is robustly determined by a set of genetic polymorphisms in the OXTR gene.

CS3M.001 PERIPHERAL LIPID SENSING AND THE REGULATION OF REWARD

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The detection of nutrients by peripheral sensors strongly modulates feeding behavior. This is particularly the case for ingested lipids detected by the upper digestive tract. We will show how a specific subpopulation of sensory neurons of the vagus nerve convey information on gut nutrients to the midbrain reward dopamine system. We have specifically identified relay nucleus located in the viscero-sensory part of the brainstem that links vagus sensory neurons to

dopamine cells in *Substantia nigra*. Consistently, remote activation of vagus-to-brain neurons sustains self-stimulation behavior, induce both flavor and place preferences, and stimulate dopamine release by nigral neurons. Conversely, ablation of gut-innervating vagal sensory neurons disrupt nutrient-induced preferences and dopamine release.These findings establish the vagal gut-brain-axis as an integral component of the neuronal reward circuitry, and suggest novel vagus stimulation approaches to affective and eating disorders.

CS3M.002 INTEGRATION OF METABOLIC AND NUTRITIONAL SIGNALS IN REWARD CIRCUITRY

Stephanie Fulton
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The brain comprises numerous mechanisms by which it can sense the status of energy fuels so that it may adjust behavioural systems to efficiently meet energy demands. The study of the pathways and mechanisms by which hormones and nutrient signals influence feeding and food-directed behaviours has substantially advanced our knowledge of the CNS controls of energy homeostasis. Peripherally-derived energy signals not only affect hypothalamic cells but also target the midbrian and striatum. Dopamine neurons of the midbrain ventral tegmental area are an essential component of the neural circuitry regulating motivation and reward. Mesolimbic dopamine neurons respond to numerous peripheral signals including leptin, insulin and even nutrients. Fatty acids can provide a metabolic fuel source for neural cells and can act as signalling molecules. Lipids can also modulate midbrain and limbic signalling to alter feeding, reward and emotional function. Data will be presented describing the impact of leptin and lipids on reward circuits. In addition, findings showing the influence of long-term consumption of diets enriched with saturated and unsaturated fats on brain reward and neuroimmune processes. Results demonstrate that free fatty acids as well as the metabolic consequences associated with saturated fat intake can impact limbic and midbrain circuitry to affect feeding, emotions and reward function.

CS3M.003 ROLE OF NPY SIGNALLING IN THE DEVELOPMENT OF STRESS-INDUCED OBESITY

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Neuropeptide Y (NPY) is one of the most powerful orexigenic peptides known, exerting critical feeding related functions in the hypothalamus. However, NPY is also present in extra hypothalamic nuclei where it is modulated in response to metabolic and other physiological perturbations, but far less is known about these NPY populations and their influence on energy homeostasis regulation. Now we have identified that NPY neurons in the central amygdala (CeA) are responsible for an exacerbated response to a combined stress and high fat diet intervention leading to accelerated obesity development. Employing CeA NPY neuron specific AAV-NPY overexpression models we were able to replicate the obese phenotype seen in the

combined stress/HFD mouse model, which was prevented by the selective ablation of NPY from these neurons. Furthermore, by selectively activating NPY neurons of the CeA via DREADD we mapped the projections of these neurons to the Arc and PVN, known nuclei to be critical for energy homeostasis control. Moreover, using food intake and energy expenditure as the physiological readout we demonstrated that selective activation of CeA NPY neurons results in a robust increase in food intake and decrease in EE which is entirely dependent on the presence of NPY. Mechanistically it is the failure of insulin to no longer control these NPY neurons under combined stress/HFD conditions that leads to accelerated obesity. Taken together this study has uncovered a previously unknown feeding stimulatory pathway that is activated under conditions of stress in combination with calorie dense food.

CS3M.004 FAT AND SUGAR; A MATTER OF TIMING AND CHOICE

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Added sugar, often consumed in the form of sweetened beverages, is currently labeled as a big evil that increases our risk to develop obesity, cardiovascular diseases, and diabetes. Only a few decades ago, however, saturated fat received a similar negative label. Instead of singling out one of these factors we are interested to understand how fat and sugar intake interact, thus influencing brain function, metabolism and behavior. We recently showed that free choice during simultaneous fat and sugar consumption exponentially increases the risk of overeating, obesity development and occurrence of metabolic disturbances in rats. Moreover, the brain's response to this obesogenic choice diet does not promote counter regulatory mechanisms but reflects high energy demands (i.e. the brain shows a fasting-like response) pointing to aberrant nutrient signaling. During this presentation, the effects of the choice diet on timing of eating and how time restricted eating affects metabolism will be reviewed. In addition, data of human intervention studies on effects of timing and time restricted eating on brain and metabolism will be shown.

CS3R.001 SEX DIFFERENCES IN NEONATAL NEUROPROTECTIVE MECHANISMS

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Neonatal hypoxic ischemic encephalopathy (HIE) is one of the major causes of learning disabilities and memory deficits in children. Male neonatal brains are two times more vulnerable to the effects of HIE, a phenomenon that is poorly understood. We recently reported that HIE increases hippocampal estrogen receptor a (ERa) expression leading to neuroprotection only in the female mice hippocampi through crosstalk with the neurotrophin receptor, tyrosine kinase B (TrkB). Phosphorylation of the TrkB via its agonist, 7,8-dihydroxyflavone (7,8-DHF) decreases apoptosis and improves long-term neurological outcome only in female mice. Presence or absence of ERa in 7,8-DHF mediated long-term neuroprotection have not been investigated following neonatal HIE. We hypothesized that knocking down ERa will ablate the sex differences seen in 7,8-DHF mediated long-term neuroprotection following neonatal HIE. After inducing HI in P9 B6/C57 ERa wild (WT) and knock-out (KO) mice by Vannucci's HI model, at P60+ mice were assessed by novel object recognition (NOR) and location (NOL) tests for object and location memory. HI decreased the discrimination ratios for both NOR and NOL tests in ERa WT mice compared to sham WT mice ($p < 0.001$), respectively. HI induced decline in recognition and location memory were recovered by 7,8-DHF therapy only in ERa WT female mice in an ERa dependent way. TrkB agonist improves neurological outcome only in WT female mice in an ERa dependent way. Failure of hippocampal ERa upregulation in male neonate hippocampus maybe one of the mechanisms underlying the sex differences seen in TrkB-mediated neuroprotection following neonatal HIE.

CS3R.002 DOES MEMBRANE ESTROGEN SIGNALING PLAY A ROLE IN BRAIN SEXUAL DIFFERENTIATION?

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Brain circuits underlying reproduction differ between sexes explaining differences in reproductive physiology and sexual behaviors. These differences are largely organized by a differential exposure to sex steroids, estrogens in particular. Estrogens exert their effects through a combination of nuclear- and membrane-initiated actions. The relative contribution of these two modes of action to brain sexual differentiation has never been investigated systematically. Using the C451A-ERalpha mouse invalidated for membrane signaling of estrogen receptor alpha, we investigated the role of membrane ERalpha (mERalpha) in brain programming looking at the expression profile of markers of sexual differentiation in adult gonadectomized males and females treated with estrogens. In the anteroventral periventricular nucleus (AVPv), while no effect of genotype was found in the number of neurons expressing tyrosine hydroxylase, Kp-immunoreactive (Kp-ir) neurons are more abundant in mutant than in wild-type males but females are unaffected by genotype, suggesting that mERalpha mediates the organizational of Kp-ir neurons by estrogens produced during the perinatal period. To

confirm this hypothesis, pups were treated at birth with estrogens and Kp-ir neurons were counted in gonadectomized adults treated with estrogens. Control mice showed the same expression pattern as previously. However, EB treated females showed the same number of kp-ir neurons as males with an increase in mutant compared to wild-type mice. Together, these data demonstrate that membrane estrogen signaling is involved the organization of the brain. Other sexually differentiated markers are under investigation to evaluate the extent of this conclusion.

CS3R.003 FEMALE REPRODUCTION IS MEDIATED BY ESTRADIOL AND PROGESTERONE MEMBRANE SIGNALING

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In many species, reproduction is dependent on the appropriate steroid stimulation of brain circuits that coordinate behaviors with the production of gametes. This is especially critical for females, where ovarian estradiol synchronizes positive feedback, needed for ovulation, with sexual receptivity, necessary for fertilization. In addition to classical action, membrane-initiated steroid signaling is central to the induction of sexual receptivity and luteinizing hormone (LH) positive feedback. Estradiol membrane-initiated signaling (EMS) in the arcuate nucleus of the hypothalamus (ARH) initiates transient opioid inhibition and spinogenesis – both needed for the sexual receptive behavior, lordosis. Progesterone, acting through Src activation in proopiomelanocortin (POMC) ARH neurons relieves the inhibition, which allows for the lordotic response when the female is mated with a male. During this time, direct nuclear action of estradiol induces PGRs, while EMS induces kisspeptin in neurons of the rostroperiventricular neurons of the third ventricle (RP3V) – neurons that integrate estradiol and progesterone input to GnRH neurons. Astrocyte-derived neuroprogesterone facilities estradiol induction of kisspeptin and its release via progesterone membrane-initiated, Src-dependent signaling. Estradiol-induced neuroprogesterone synthesis is both sexually differentiated and developmentally regulated: only postpubertal females exhibit this phenomenon – which maps onto the estradiol augmented cell addition in the female RP3V. A plurality of the newly born cells are astrocytes that are distinguished from existing astrocytes by having a greater number of estrogen receptor-α on the membrane, allowing them to stimulate neuroprogesterone synthesis. Thus, positive feedback, which develops in females after puberty, is dependent on EMS in astrocytes. Support: DA013185 & HD04246.

CS3R.004 CONTEXTUAL CONTROL OF SEXUALLY DIMORPHIC SOCIAL BEHAVIOUR

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No Abstract Submitted

CS3S.001 PVN CRH NEURONS CONTROL THE SOCIAL TRANSMISSION OF STRESS

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Hypothalamic paraventricular nucleus (PVN) corticotropin- releasing hormone (CRH) neurons are regulators of the canonical endocrine response to stress. Here, using an approach that combines in vivo optogenetics, behavior and in vitro electrophysiology, we show they also control the transmission of a putative alarm signal from a stressed mouse to others. The transmission of this alarm signal is sufficient to cause synaptic changes at glutamate synapses on CRH neurons in the recipient that resemble the synaptic changes observed following authentic stress. These synaptic changes could also be transmitted sequentially from the stressed subject to multiple partners. Our findings demonstrate that transmitted stress has the same lasting effects on glutamate synapses as authentic stress and reveal an unexpected role for PVN CRH neurons in transmitting distress signals among individuals.

CS3S.002 ROLES OF OXYTOCIN IN THE CONTROL OF STRESS AND SOCIAL BEHAVIOR

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Oxytocin has been shown to have anxiolytic and prosocial actions. We have previously reported that oxytocin facilitates serotonin release and reduces anxiety-related behavior in a way dependent on serotonin receptors. Activation of oxytocin neurons increases social behavior including social recognition memory. Oxytocin has been reported to have not only anxiolytic and prosocial actions but also anxiogenic actions dependent on situations. However, precise mechanisms of these oxytocin actions remain unknown. Oxytocin neurons are located mainly in the hypothalamus and project their axons to various brain regions. We examined anatomical distribution patterns of oxytocin fibers and the oxytocin receptor. Projecting fibers of oxytocin neurons were visualized by use of an anterograde viral tracer, which induces selective expression of membrane-targeted palmitoylated green fluorescent protein in oxytocin neurons. Projecting fibers from paraventricular hypothalamic oxytocin neurons were found in various

brain regions including the thalamus, the ventrolateral part of the ventromedial hypothalamus and the ventrolateral periaqueductal gray. Oxytocin neurons also extended their dendrites into the medial amygdala. These brain regions contained the oxytocin receptor. Oxytocin neurons in the hypothalamus and oxytocin receptor-expressing neurons in several brain regions were activated by stressful stimuli. Oxytocin receptor-deficient mice showed abnormalities in stress responses and affiliative behavior. Physiological functions of oxytocin will be discussed.

CS3S.003 USING ZEBRAFISH TO STUDY STRESS AND RESILIENCE

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The zebrafish, danio rerio, is a low-cost vertebrate model with conserved yet simpler nervous system compared to mammals that offer sophisticated and versatile toolkit for genetic, pharmacological, optogenetic and behavioral manipulations. Therefore it offers an excellent animal model to mechanistically dissect both the acute and chronic effects of stress on brain and behavior both during development and in adulthood. Here I discuss our recent work dissecting the effects of stress exposure on physiology and behavior in larval and adult zebrafish. Our approach combines two main methods: 1) genetic and optogenetically engineered transgenic zebrafish to manipulate and sense the activities of the cells of the Hypothalamo-pituitary-adrenal axis precisely in an intact animal and 2) detection of stress-induced alterations in behavior using a set of novel behavioral paradigms.

CS3S.004 CONTROL OF ANTERIOR PITUITARY CORTICOTROPH EXCITABILITY IN HEALTH & DISEASE

Mike Shipston
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The ability of an organism to respond appropriately to stress is essential for survival. A key determinant of the neuroendocrine response to stress are the electrically excitable anterior pituitary corticotroph cells of the hypothalamic-pituitary-adrenal (HPA) stress axis. Corticotrophs are stimulated by hypothalamic neuropeptides and inhibited by the negative feedback effects of circulating glucocorticoid hormones however, the ionic mechanisms that control excitability are poorly understood. By integrating electrophysiological, molecular, genetic and mathematical modelling approaches we are beginning to understand how corticotroph excitability is controlled and how this may be disrupted in disease. Importantly, the hypothalamic neuropeptides corticotrophin releasing hormone (CRH) and vasopressin (AVP) can promote distinct patterns of electrical excitability through the regulation of different ionic

conductances. Corticotrophs typically display spontaneous action potentials and CRH predominantly evokes a transition to a 'pseudo-plateau' bursting behaviour. This evoked bursting is, in part, dependent upon the properties of large conductance voltage- and calcium-activated potassium (BK) channels and predicted to support robust secretion of adrenocorticotrophin (ACTH). In contrast, AVP largely evokes an increase in action potential firing frequency through a BK-independent mechanism. Glucocorticoids, in the timescale of minutes to hours, attenuate CRH-evoked bursting through disrupting the BK dependent mechanism. In addition, glucocorticoids can also inhibit excitability through BK-independent mechanisms, controlling primarily spike frequency. Importantly, the ion channel expression landscape of corticotrophs can be significantly modified in chronic stress and we are beginning to dissect how the interplay between key ionic conductances control corticotroph function in both health and disease.

CS3TR.001 FROM BENCH TO PATIENTS: FROM MELATONIN TO AGOMELATIN®

Jean A. Boutin
Prospective, Insitut de Recherches Internationales SERVIER, Suresnes, France

Melatonin is an endogenous compound, synthesized at night by the pineal gland. Although some alerts were published from various agencies on its misused, it is considered as a very safe compound. We aimed at finding a substitute to melatonin: a compound with balanced agonist affinities at both melatonin receptors, MT_1 and MT_2, and some serotoninergic properties. The ways we undertook when we started our studies on melatonin receptor pathways and the search for alternative ligands will be shortly documented. Based on a few observations on animal models, the candidate drug was pushed to clinic and show surprisingly good results in treating depressive patients. Considered as being different from standard anti-depressive drugs (particularly because it is fast-acting in patients), Agomelatinin® was recently and independently considered one of the 4 best anti-depressive agents of the Pharmacopea out of 24 such agents.

CS3TR.002 CLINICAL STUDIES WITH KISSPEPTIN

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Kisspeptin has been identified as a key regulator of the reproductive system. We have recently determined the therapeutic potential of kisspeptin in patients with reproductive disorders: (i) Hypothalamic amenorrhea is defined as the cessation of menstruation due to abnormal signalling between the hypothalamus and the pituitary gland. It accounts for over 30 percent of

cases of amenorrhea in women of reproductive age. Kisspeptin administration stimulates reproductive hormone release and can restore LH pulsatility in with hypothalamic amenorrhea[1]. (ii) IVF treatment is now widely and successfully used to enable infertile couples to conceive. However, IVF treatment can result in the potentially life threatening condition, ovarian hyperstimulation syndrome (OHSS) due to the pharmacological use of human chorionic gonadotrophin (hCG) to stimulate oocyte maturation in current IVF protocols. Kisspeptin acts more physiologically to effectively and safely trigger oocyte maturation resulting in the birth of healthy children without OHSS[2,3,4]. (iii) Psychosexual disorders are common in men and women. Current medical treatments are limited yet the costs of medical and psychological therapy is estimated at $85 million/year in the USA alone. Kisspeptin administration enhances limbic brain activity specifically in response to sexual and couple-bonding stimuli in men[5] and hence has potential to treat patients with psychosexual disorders. Our future work will deliver novel kisspeptin based therapies into the clinic to improve the outcomes of patients with reproductive disorders. 1.JCEM 2014;99(6):E953-61 2.JCI 2014;124(8):3667-77 3.JCEM 2015;100(9):3322-31 4.Hum Reprod 2017;32(9):1915-1924 5.JCI 2017;127(2):709-719

CS3TR.003 THE ROLE OF GDF15 AND ITS RECEPTOR GFRAL IN THE REGULATION OF FOOD INTAKE AND BODY WEIGHT

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Growth / differentiation factor 15 (GDF15) or alternatively macrophage inhibitory cytokine 1 (MIC-1) has been shown to be increased in biological stress conditions including cancer, kidney failure, heart failure, myocardial infarction and atherosclerosis however its' role in these states remains unclear due to the lack of an understanding of its receptor. The observation that circulating GDF15 levels correlate with weight loss in prostate cancer-induced cachexia led to significant efforts to understand the receptor(s) involved in its' control of metabolism. Using a directed search of orphan receptors, we identified an orphan in the GDNF receptor family as the receptor for GDF15. GDNF-family receptor alpha-like (GFRAL) binds GDF15 and initiates signaling through interaction with its' coreceptor RET. Using GFRAL deficient mice and an anti-GFRAL antagonistic antibody, the metabolic actions of GDF15 were shown to be entirely dependent on interaction with GFRAL. In mice, GFRAL expression was found to be limited to the brainstem area postrema (AP). No other tissues expressed this receptor. Systemic administration of GDF15 activated GFRAL neurons in the AP and decreased meal size without an effect on meal number confirming that GDF15 induces satiety through engagement of brainstem centers. Importantly, a similar restricted distribution of GFRAL in the AP was found across species including rat, cynomolgus monkey and human suggesting these metabolic effects are conserved. This data demonstrates that the metabolic actions of GDF15 are mediated through the orphan receptor GFRAL and will lead to a further more comprehensive understanding of the metabolic effects of this protein.

CS3TR.004 NOVEL ORALLY ACTIVE SOMOTOSTATIN ANALOGS

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There are more than 80 known peptide hormones acting at more than 120 known different receptors. Small molecule, nonpeptide ligands that act at these receptors can provide important new therapeutic options that cannot be achieved with peptide based drugs. Using an iterative medicinal chemistry approach, we designed and optimized orally bioavailable, non-peptide small molecules with agonist activity at the somatostatin sst2 receptor. This led to the discovery of CRN00808, a low molecular weight (<500 daltons), potent agonist at hsst2 (EC_{50} = 0.25 nM) with high selectivity against other somatostatin receptor subtypes (>4000-fold). It is also a biased-agonist that is ~75 times more potent for cAMP inhibition than receptor internalization. We conducted a Phase 1, double blind, placebo controlled trial to assess the safety, tolerability, PK and PD of CRN00808 in 99 healthy human volunteers. To assess pharmacodynamics responses, suppression GHRH-stimulated GH secretion and basal IGF-1 levels was measured. The dose range evaluated was 1.25 mg to 60 mg administered as a single dose, and 5 mg to 30 mg administered QD for up to 10 days. CRN00808 was well tolerated when administered as a single or multiple doses. Single doses of CNR00808 suppressed mean GHRH-induced GH release in a dose-dependent fashion with a maximal effect (92% suppression) observed at the 10 mg dose. QD administration of 10 mg CRN00808 capsules for 10 days also maximally suppressed IGF-1. We are currently preparing for Phase 2 studies to evaluate the efficacy and safety of CRN00808 in patients with acromegaly.

CS3TR.005 REDUCING THE HEAT WITH NEUROKININ ANTAGONISM; A NEW NON-HORMONAL APPROACH FOR TREATING HOT FLASHES

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During the menopausal transition and for an extended period afterwards, ~75% of women will experience menopause related symptoms, most notably hot flashes (vasomotor symptoms). These are common and disruptive symptoms which arise due to changing circulating estrogen levels produced by the ovaries. HRT is an effective remedy for vasomotor symptoms, but its risk profile has restricted its use. Other hot flash treatments have limited efficacy and therefore there is a clear unmet need for an effective non-hormonal therapy. Recently, Neurokinin(NK)-B signalling has been identified as a key factor in hot-flash aetiology raising the prospect that pharmacological blockade of its cognate NK-3 receptor could represent a novel treatment paradigm. It is postulated that reducing the hyperactive state of estrogen deprived centrally located hypothalamic Kisspeptin-Neurokinin-B-Dynorphin (KNDy) neurones through NK-3 receptor antagonism in particular, returns to normal functioning the disrupted heat dissipation

effector systems driving the hot flash symptoms. Substance-P the cognate ligand for NK-1 receptors is also expressed on human KNDy neurons, though its role is much less well characterised. Non-clinical evidence suggests that the SP/NK-1 receptor system may act in unison with the NK-3/NK-B receptor system in regulating KNDy neuron outputs. This biological rationale is now supported by emerging evidence from clinical studies in post-menopausal women. Trials with three different NK antagonists have demonstrated that the NK-receptor antagonist class has the potential to be a game-changing therapy for debilitating vasomotor symptoms. The underlying science and recent clinical data which shows rapid and sustained reduction in hot flash frequency will be reviewed.

CS3TR.006 READJUSTING GUT-BRAIN SIGNALING WITH UNIMOLECULAR POLYAGONISTS: TRANSLATIONAL POTENTIAL

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Emerging insights from recent advances in metabolic diseases research suggest that one or several patterns of multiple neuro-endocrine factors are necessary for sustained modulation of body fat or metabolism set points. Gut hormones appear to reside at the core of these master–key-like signaling patterns, as indicated for example by bariatric surgery research. Over the last 7 years, we have tested a large series of combination therapies based on multiple gastrointestinal and adipocyte derived signals. Balanced single molecule peptide hormone based GLP1-glucagon and GIP-GLP1 co-agonists exhibited superior body weight loss and glucose metabolism benefits in mouse models of obesity and diabetes, as compared to any established mono-agonists. Since co-infusion of a soluble and stable glucagon mono-agonist in parallel with GIP-GLP1 co-agonist treatment provided additional benefits, a series of single molecule GIP-GLP1-glucagon tri-agonists were generated and validated. These novel tri-agonists again showed unprecedented metabolic and body weight benefits in mouse and rat models of obesity and diabetes. In a parallel approach single molecule conjugates combining a peptide (e.g. GLP1) with a steroid (e.g. estrogen) were generated to maximize metabolic benefits and minimize potential toxicity by specifically targeting a subset of estrogen receptors in GLP1-receptor carrying cells. Administration reversed hallmarks of the metabolic syndrome in diet induced obese and insulin resistant mice without causing any detectably side effects or toxicity. The above described novel single molecule approaches to polypharmaceutical therapeutics carry the potential to open new perspectives for the treatment of metabolic diseases such as diabetes and obesity.

CS4M.001 NUTRIENT SENSING BY GUT ENTEROENDOCRINE CELLS

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The gut endocrine system comprises a collection of enteroendocrine cells scattered through the intestinal epithelium. Enteroendocrine cells detect the local luminal composition and release hormones that regulate post-prandial physiology, including gut motility/secretion, and regulation of insulin secretion and food intake. We explored nutrient sensing pathways in primary and organoid-derived enteroendocrine cells using tissue from mouse models that exhibit cell specific fluorescence, enabling cell identification, purification and monitoring of intracellular signalling pathways. We identified a range of sensory pathways including G-protein coupled receptors and nutrient transporters that detect the digestion products of carbohydrates, fats and protein, triggering the release of gut hormones such as GLP-1 and PYY. Gut hormones have proven translational value in the therapeutics of diabetes, obesity and short bowel syndrome, and are dramatically altered after gastric bypass surgery. It is hoped that further exploitation of the enteroendocrine system will lead to the development of new therapies for diabetes and obesity.

CS4M.002 ROLE OF THE GUT MICROBIOTA IN CENTRAL ENERGY BALANCE REGULATION

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The gut microbiota affects the host's energy balance. It increases the energy harvest from the diet, and microbial products and metabolites can modulate appetite, gut motility, energy storage and energy expenditure. Several findings suggest that the gut microbiota can affect the development of obesity. Obese individuals have an altered gut microbiota composition compared to lean individuals, and transplantation of the gut microbiota can transfer the obese phenotype. Furthermore, germ-free (GF) mice are leaner than conventionally raised (CONV-R) mice and they are protected against diet-induced obesity when fed a Western diet. Feeding a Western diet has previously been shown to cause hypothalamic inflammation, which has been suggested to cause resistance to the anorexigenic hormone leptin. We found that feeding a Western diet led to hypothalamic inflammation in CONV-R mice, but not in GF mice. We observed an enhanced leptin sensitivity in mice lacking a microbiota i.e. GF and antibiotic-treated mice compared to CONV-R mice. Colonization of GF mice led to hypothalamic gliosis and diminished hypothalamic leptin signaling. Collectively, these findings suggest that the gut microbiota can modulate the host's energy balance and contribute to obesity.

CS4M.003 MECHANISMS UNDERLYING WEIGHT LOSS AFTER BARIATRIC SURGERY

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Bariatric surgery is currently our most effective strategy at sustained weight loss and improvements in metabolic co-morbidities of obesity. Weight loss is on average ≥30% over a 10 y period and importantly leads to a 40% remission of T2DM, where patients can be completely removed from diabetic medications. There is no other treatment that can claim this kind of remission in T2DM. Vertical sleeve gastrectomy (VSG), a surgery where 80% of the stomach along the greater curvature is removed with no intestinal rearrangement, is one type of bariatric surgery that is currently the most performed bariatric surgery in the state of Michigan and is a close second in frequency across the United States. At present, the underlying mechanisms for the success of bariatric surgery remain unclear but several hypotheses have revolved around changes in the gut-brain-axis. One hypothesis has surrounded the role of a glucoregulatory peptide called glucagon-like peptide-1 (GLP-1) which increases to a much greater extent after bariatric surgery. GLP-1 is made in the gut, brain, and to a lesser extent in the pancreas. Our work focuses on understanding the source of GLP-1 that increases with surgery, the mechanisms for that increase, and whether this increase is critical in the responses to bariatric surgery.

CS4M.004 METABOLIC MECHANISMS OF ROUX-EN-Y GASTRIC BYPASS: ROLE OF GLP-1, OXYNTOMODULIN AND PEPTIDE YY

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Bariatric surgery, for example Roux-en-Y gastric bypass (RYGB), is an effective treatment for obesity and type 2 diabetes. RYGB causes a major increase in postprandial secretion of the hormones GLP-1, oxyntomodulin (OXM) and peptide YY (PYY). The presentation will focus on the evidence for the important role of these hormones in mediating the metabolic effects of RYGB. Evidence from a study on the infusion of combined GLP-1, OXM and PYY (GOP) will be presented which shows that the elevation of gut hormones after surgery is likely to play an important role in the observed weight loss and improvement in glycaemia. By mimicking this rise by infusion, a safe and physiological treatment of obesity and diabetes may be achieved.

CS4R.001 MIRNA-BASED NETWORKS IN PUBERTY ONSET AND FERTILITY

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A sparse population of a few hundred primarily hypothalamic neurons forms the hub of a complex neuroglial network that controls reproduction in mammals by secreting the "master molecule", gonadotropin-releasing hormone (GnRH). Both the Kisspeptin input on GnRH neurons and timely changes in GnRH expression are necessary for the onset of puberty. However, the exact molecular mechanisms underlying this process remain elusive. Here, we report that a dramatic inversion in microRNA expression profile within postnatal GnRH neurons acts as an epigenetic switch that triggers the prepubertal increase in GnRH expression and controls the ability of GnRH neurons to sense Kisspeptin input, for a correct initiation of puberty. The disabling of this microRNA-mediated switch leads to hypogonadotropic hypogonadism and infertility in mice. The underlying mechanism involves a complex and multilayered network of transcriptional activators and repressors reciprocally controlled by several microRNA species, including miR-200 and miR-155. These microRNAs tune the balance between inductive and repressive signals to trigger a rise in hypothalamic GnRH expression and to control the expression of Kisspeptin receptor. Anomalies in this microRNA-embedded genetic network, which appears essential for the neuroendocrine control of reproduction by controlling both Kisspeptin sensitivity and GnRH production, could thus underlie dysfunctions of human puberty and fertility when a genetic cause is not apparent.

CS4R.002 CILIARY GPCR SIGNALING: LESSONS FROM KISSPEPTIN RECEPTOR 1

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Primary cilia are typically solitary appendages that extend from nearly all mammalian cell types, including most, if not all, neurons throughout the brain. Neuronal cilia are enriched for specific signaling proteins, including certain G protein-coupled receptors (GPCRs), such as serotonin receptor 6, and dopamine receptor 1. It is thought that neuronal cilia sense neuromodulators in the extracellular milieu and support specialized signaling of ciliary GPCRs. Yet, the precise roles of cilia on central neurons and their specific impact on neuronal GPCR function remain largely unknown. We discovered that the kisspeptin receptor 1 (Kiss1r) is enriched in cilia projecting from mouse gonadotropin-releasing hormone (GnRH) neurons. Interestingly, GnRH neurons in adult animals possess multiple Kiss1r-positive cilia and the proportion of multiple cilia increases in parallel with pubertal maturation. Disruption of cilia selectively on GnRH neurons does not affect neuron migration or sexual maturation. However, kisspeptin-mediated increases in GnRH neuron firing rate are reduced in the absence of cilia, indicating that cilia enhance Kiss1r

signaling on GnRH neurons. We are currently extending these findings to determine the roles of neuronal cilia in signaling of other ciliary GPCRs throughout the brain.

CS4R.003 THE CRITICAL ROLE OF KISSPEPTIN REGULATION OF REPRODUCTION AND METABOLISM

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The Critical Role of Kisspeptin Regulation of Reproduction and Metabolism The coordinated expression and release of Gonadotropin-releasing hormone (GnRH) from the hypothalamus is critical for mammalian reproduction. The goal of our studies is to characterize the signals that mediate hypothalamic GnRH gene expression. Kisspeptin, a protein controlling GnRH-mediated pubertal maturation and reproduction is expressed in specific neurons of the hypothalamus. We have shown that kisspeptin binds to a specific G-protein coupled receptor (GPR54 or Kiss1R) and stimulates GnRH release and increases GnRH mRNA levels. Further, we have localized a kisspeptin-response element (KsRE) within the previously identified GnRH enhancer element. To define the role of kisspeptin-Kiss1r signaling directly at the level of the GnRH neuron, a GnRH neuron-specific Kiss1r knockout (GKirKO) mouse model was generated. Female and male GKirKO mice exhibited a delay in pubertal onset, reduced gonadotropin and sex hormone levels and infertility. These data provide in vivo evidence that the Kiss1r in GnRH neurons is critical for proper and timely reproductive development and fertility. Kisspeptin has also been reported to be expressed in peripheral tissues including the liver, however, the role of hepatic kisspeptin and is regulation is not been clear. Mice lacking the KissR in b cells exhibited depressed glucose stimulated insulin secretion and improved glucose tolerance. Our observations identify the liver, regulation by glucagon, as the site of kisspeptin synthesis, define a liver-to-islet endocrine circuit in glucoregulation, and suggest a pathogenic mechanism in T2DM.

CS4R.004 IMMUNODETECTION OF TRKA AND ITS ASSOCIATION WITH GNRH AND KISSPEPTIN NEURONS IN THE HYPOTHALAMUS

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Nerve growth factor (*β*-NGF) from seminal plasma of llamas (*Lama glama*) is able to elicit a preovulatory LH surge and subsequent ovulation in llamas. However, it is unknown the mechanism by which seminal plasma *β*-NGF mediates this process. The goal of this study was to identify trkA receptor and *β*-NGF, and their association with distributions of GnRH, Kisspeptin neurons and the glial cells tanycytes at the medial basal hypothalamus, in pituitary gland and the circumventricular organs (CVOs): choroid plexus (CP); median eminence (ME); and the Organum Vasculosum Laminae Terminalis (OVLT), as potential seminal plasma *β*-NGF target. The encephalon and the pituitary gland of three mature female llamas were processed to

obtain sections for immunocytochemistry and immunofluorescence studies. It was found that trkA receptor and *β*-NGF immunolabeling are distributed in close spatial relationship with kisspeptin and GnRH neural bodies and fibers at the arcuate nucleus (AN), also abundant fibers of these two neuronal types were immunostaining in the ME. In this zone, fibers for GnRH and Kisspeptin neurons were intermixed with *β*-NGF reactive processes of tanycytes of the third ventricle. Expression of *β*-NGF and its receptor was also detected in the pituitary gland, specifically on pars tuberalis cells. Finally, trkA receptor was identified in PCs but not in ME or OVLT, although *β*-NGF immunostaining was established in all three CVOs included in this study. We conclude that seminal plasma *β*-NGF could trigger the ovulatory cascade by signaling or entering the environment of the third ventricle through CVOs. **Supported: Fondecyt 1160934**

CS4S.001 STRESS: LOCAL EVENTS WITH GLOBAL CONSEQUENCES

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No Abstract Submitted

CS4S.002 THE ROLE OF 11B-HSD2 IN GLUCOCORTICOID PROGRAMMING OF AFFECTIVE AND COGNITIVE BEHAVIOURS

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Prenatal exposure to excess glucocorticoids may be causal in programming psychiatric disorders in later life. In support of this hypothesis, maternal stress, treatment during pregnancy with dexamethasone (which crosses the placenta) or inhibitors of feto-placental 11ß-hydroxysteroid dehydrogenase type 2 (11β-HSD2), the physiological 'barrier' to maternal glucocorticoids, reduces birth weight and programmes offspring cardio-metabolic and affective behaviours. The enzyme 11β-HSD2 appears key to prenatal glucocorticoid programming, and is expressed in both the placenta and the developing fetal brain. Mice lacking 11β-HSD2 ($Hsd11b2^{-/-}$) have reduced angiogenesis in the placenta, which affects nutritional supply to the fetus, altered flow and resistance in the umbilical vessels and modification of fetal heart function and development. As adults the mice exhibit increased anxiety and depressive-like behaviours. However, deletion of 11β-HSD2 solely in the brain (HSD2BKO) programmes depressive-like behaviour in the absence of altered placental function. In this talk we will discuss potential mechanisms underpinning early-life programming of adult neuropsychiatric disorders and the novel therapeutic potential of statins. *Funding: European Commission, Seventh Framework*

Programme, project acronym: DORIAN, grant agreement n°278603 and The Wellcome Trust. We also acknowledge The University of Edinburgh Centre for Cognitive Ageing and Cognitive Epidemiology, part of the cross council Lifelong Health and Wellbeing Initiative (G0700704).

CS4S.003 PROGRAMMING OF COGNITIVE FUNCTIONS BY EARLY-LIFE STRESS, A ROLE FOR NUTRITION AND NEUROINFLAMMATION

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Early-life stress (ES) is associated with increased vulnerability to cognitive impairments as well as metabolic disorders like obesity later in life. We investigate the role of a synergistic effect of stress, metabolic factors, nutrition and the neuroimmune system in this early-life induced programming. We use an established model of chronic ES and expose mice to limited nesting and bedding material during first postnatal week and study the central and peripheral systems under basal and challenged conditions (i.e. LPS, amyloid accumulation, western style diet (WSD) and exercise) to gain further insight in the functionality of brain plasticity, microglia and adipose tissue. In addition, given the high nutritional demand during development, we propose that early nutrition is critical for programming of brain and body. We focus on essential micronutrients and fatty acids and propose that an early dietary intervention with a diet enriched with these nutrients might protect against ES-induced functional deficits. We show that ES leads to cognitive impairments associated with reduced hippocampal neurogenesis at basal conditions as well as in response to exercise, primed microglia with exaggerated response to LPS or amyloid accumulation. Metabolically, ES mice exhibit a leaner phenotype but they accumulate more fat in response to WSD. Finally, with an early dietary intervention with micronutrient or fatty acid we were able to (at least partly) prevent ES-induced cognitive decline, likely mediated by modulation of microglia, without however affecting the ES-induced metabolic profile. These studies give new insights for the development of targeted dietary interventions for vulnerable populations.

CS4S.004 SUPPRESSED CALBINDIN LEVELS IN HIPPOCAMPAL EXCITATORY NEURONS MEDIATE STRESS-INDUCED MEMORY LOSS

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As a Ca^{2+} buffer, sensor and transporter, calbindin critically modulates synaptic plasticity. Reduced hippocampal calbindin levels have been implicated in cognitive disorders, including those induced by early-life stress. However, it is unclear how early-life stress modulates calbindin expression in distinct hippocampal neurons and the contribution of each calbindin-

expressing neuronal population to memory. Here, we report that hippocampal excitatory and inhibitory calbindin neurons modulate the susceptibility and resilience to early-life stress-induced spatial memory deficits respectively. Stress exposure during early postnatal period lastingly reduced calbindin levels in all CA1 and DG neurons. Reduced calbindin levels in CA1 or DG excitatory neurons, but not CA1 interneurons, strongly correlated with spatial memory impairments. Accordingly, selective knockdown of calbindin in CA1 or DG excitatory neurons mimicked postnatal stress-induced memory deficits in adulthood. By contrast, calbindin knockdown in CA1 interneurons preserved memory both under basal conditions and after an acute stress challenge. Moreover, calbindin expression levels were suppressed by early-life stress through the cell adhesion molecule nectin3, and in turn reduced IMPase levels. Our findings highlight calbindin as a key molecule for the reprogramming effects of early-life stress on cognition, and exemplify how distinct neurons sharing a same molecule confer the susceptibility or resilience to stress.

CS4T.001 NEW MECHANISMS UNDERPINNING SEASONALITY IN SHEEP.

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Living organisms show seasonality in many functions such as reproduction, fattening and hibernation. It is accepted that light acts upon an endogenous clock, the “circannual clock”, to achieve environmental synchronization of seasonal rhythms. In mammals, the *pars tuberalis* (PT) of the pituitary has emerged as the key tissue responsible for photoperiod-decoding. Thyrotropin secretion by the PT is a major photoperiod-dependent upstream regulator of *Dio2*/*Dio3* gene expression, which drives seasonal control of thyroid hormone (TH) production in tanycytes of the medio-basal hypothalamus (MBH). TH plays a pivotal role in the control of seasonal rhythms and might be a component of the circannual clock. We used RNAseq to probe the impact of photoperiod and TH upon the ovine reproductive status. We identified >3000 genes with altered hypothalamic expression during the transition from non-breeding to breeding seasons. We further identified a much smaller core of ~130 genes, which are directly responsive to long days (LD). Finally, reproductive switch-off at the end of the winter breeding season was completely blocked by thyroidectomy (THX), despite a very modest effect on the hypothalamic transcriptome: only 49 genes displayed altered expression between intact and THX ewes, including less than 10% of the LD-induced gene set. Neuroanatomical mapping showed that many LD-induced genes are expressed in the PT, independently of the TH status. In contrast, TH-sensitive seasonal genes are principally expressed in the ependymal zone. Our data suggest a hierarchical model for the seasonal control of reproduction by the MBH.

CS4T.002 TANYCYTES AND SEASONAL TIMING

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Our studies in the Siberian hamster (*Phodopus sungorus*) maintained under changing photoperiods reveal profound seasonal changes in expression of many genes in hypothalamic tanycytes. These are glial cells whose cell soma are embedded in the ependymal lining of the third ventricle, but elaborate projections through the surrounding hypothalamus that appose neurons, capillaries in the median eminence, and probably cells in the surrounding *pars tuberalis* (pituitary stalk). This complex neuroanatomy underlies their multiple proposed functions as sensors of nutrients in the circulation, and as regulators of transport of hormones and metabolites into the hypothalamus. Many of the seasonal changes in tanycyte gene expression relate to the transport and bioactivity of thyroid hormone (eg deiodinase II and III), retinoic acid signalling, or metabolism. Manipulation of intrahypothalamic thyroid hormone concentrations in hamsters by surgical implantation of microimplants demonstrate a causal role for thyroid hormone in promoting appetite and reproductive activity in long day photoperiods. However, although we have inferred that changes in thyroid hormone availability in the hypothalamus can regulate annual cycles in energy intake, storage and other physiological adaptations, analysis of tanycyte gene expression changes over the course of one year in natural photoperiod paint a more complex picture for thyroid hormone involvement. As tanycytes are also a stem cell niche and function as hormone, nutrient and metabolite sensors that impact upon neuronal function in the surrounding hypothalamus, we hypothesise that seasonal cycles in metabolism and reproduction reflect long-term reversible plastic changes in the hypothalamus.

CS4T.003 A BINARY SWITCH MECHANISM OPERATING IN THE PITUITARY CONTROLS SEASONAL REPRODUCTION

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Life in seasonal environments is challenging, requiring accurate timing and anticipation of seasonal environmental changes in order to adapt and survive. Biological systems are adapted to respond quickly to changes in their environment. Signal processing often leads to all-or-none switch-like activation of downstream pathways, therefore, binary decisions are at the core of many cellular processes. Binary switches can be defined as a change in phenotype between two stable states. Endocrine cells have acute, medium, and long-term outputs and these must be regulated across different time scales and in response to diverse environmental signals, while achieving accurate control of hormone expression. We recently presented evidence for a binary switch operating in the pars tuberalis (PT) of the pituitary, timing seasonal reproduction, and

therefore yearly cycles. In this talk, I will assess the evidence for a binary switching mechanism timing seasonal reproduction in mammals and, hypothesise how a binary switch would allow biological processes to be timed over weeks to years. I draw parallels with mechanisms used in development, cell fate determination, and seasonal timing in plants to hypothesise how these process may occur in the PT. I propose that the adult PT is a plastic tissue, showing a seasonal cycle of differentiation, and that the PT may offer a unique tissue to explore cellular plasticity in an adult mammal. I conclude with evidence that the underlying processes may be epigenetically regulated, and that a framework to test how a long-term timer functions within the PT is needed.

CS4T.004 MECHANISMS UNDERPINING SEASONAL REPRODUCTION AMONG VERTEBRATES

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No Abstract Submitted

YA3.001 THE ROLE OF SEX HORMONES VERSUS SEX CHROMOSOMES IN THE SEXUAL DIFFERENTIATION OF THE HUMAN BRAIN

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Men and women clearly differ in cognitive abilities, brain morphology, emotion processing, and vulnerability for psychiatric disorders. Many of our ideas about the origins of these sex differences are derived from research on animal models which have convincingly shown that sex differences in the brain and behavior are induced by sex hormones during specific, hormone sensitive periods during development. Likewise, the sexual differentiation of the human brain seems to be primarily driven by gonadal hormones during fetal development. However, direct genetic factors might also contribute to the sexual differentiation of the human brain since several behavioral and neuroimaging studies of Turner (XO) or Klinefelter (XXY) syndrome point to a role for X-chromosomal dosage in human brain differentiation. Since in both disorders there is an aberrant number of sex chromosomes, along with reduced gonadal hormone levels it is difficult to determine whether the results from these syndromes reflect genes on the X or Y chromosome, chromosomal dosage or sex hormone levels. Therefore, we studied brain structure and function in women diagnosed with complete androgen insensitivity disorder (CAIS). CAIS are genetically male (46, XY) but phenotypically female since they are androgen resistant due to mutations in the androgen receptor gene. Overall we found female-

typical brain function and structure in women diagnosed with CAIS pointing to a predominant role for androgens in the sexual differentiation of the brain.

YA3.002 MATERNAL EXPOSURE TO LOW-LEVEL CHEMICAL MIXTURES: CONSEQUENCES ON OFFSPRING NEUROENDOCRINE SYSTEM

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It is well recognised that maternal factors during pregnancy can alter normal fetal development and programme later life disease susceptibility. Exposure to exogenous chemicals during pregnancy can alter fetal development and lead to reproductive dysfunction in male and female offspring. Environmental chemicals are classified as endocrine disrupting chemicals (EDCs) if they disrupt normal endogenous hormone release/action. Indeed, EDC exposure is a candidate contributory factor to observed changes in human reproductive health. Reproductive function is dependent upon the reproductive neuroendocrine system, controlled by the hypothalamic gonadotrophin-releasing hormone (GnRH) neurosecretory system which is sensitive to the effects of endogenous steroids and is a target through which EDCs could cause reproductive dysfunction. Understanding the consequences of in-utero chemical exposure on the neuroendocrine systems poses several challenges: 1) effects of the timing of exposure 2) sexually differentiated effects 3) effects of single chemicals may not reflect reality where we are typically exposed a mixture of chemicals at low individual levels. Here we present findings where we have addressed these challenges using an ovine model reflecting everyday chemical exposure, by grazing sheep on pasture treated with biosolids (sewage sludge) containing EDCs. We have demonstrated that maternal exposure to biosolids is associated with altered offspring neuroendocrine systems, including altered mRNA expression of GnRH and kisspeptin in the the hypothalamus, and pituitary expression of receptors for GnRH, kisspeptin and estrogen. Effects are dependent on the timing of exposure and the sex of the offspring. These effects could have long term consequences for reproductive function later in life.

YA3.003 IMPACT OF GENDER ON THE BEHAVIOURAL PROCESSES AND NEUROBIOLOGY OF MOTIVATION FOR VOLUNTARY ACTIVITY

Joan I. Morrell[1], Julia C. Basso[2]

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All mammals engage in voluntary activity including sexual and parental activity, foraging, drug seeking, and other activities such as wheel running. Voluntary wheel running (VWR) is often used in the laboratory to assess the effect of activity on various endpoints of brain function. VWR can also be considered a preclinical model for physical activity in human making such data relevant for important clinical problems that are generated by inactivity. As a sequel to our studies on motivation of parental and drug seeking behaviors in rats we have examined the detailed behavior and neurobiology of VWR in rats. We confirmed or newly discovered both marked and subtle gender differences in VWR behaviors (Basso and Morrell, 2017 https://doi.org/10.1016/j.jneumeth.2017.07.009) and its incentive salience (Basso and Morrell, 2015 http://dx.doi.org/10.1037/bne0000070), under conditions that carefully controlled variables such as housing, running experience, age, gender, gonadal hormonal status, and wheel apparatus. Female rats exhibit a more rapid onset of maximal VWR, run greater distances at a faster rate compared to males. Circulating gonadal hormones are a significant basis for gender differences in running, but they are not the sole basis. While both genders are motivated to wheel run, rate and amount of running are not correlated to its incentive salience. The after-effects of running have incentive salience for males but not females. Both prefrontal cortex and nucleus accumbens are necessary for motivation to engage in VWR, the first proof that these components of the motivational neural circuit are required for the motivation for VWR.

YA3.004 HYPOTHALAMIC CHANGES IN HUNTINGTON DISEASE

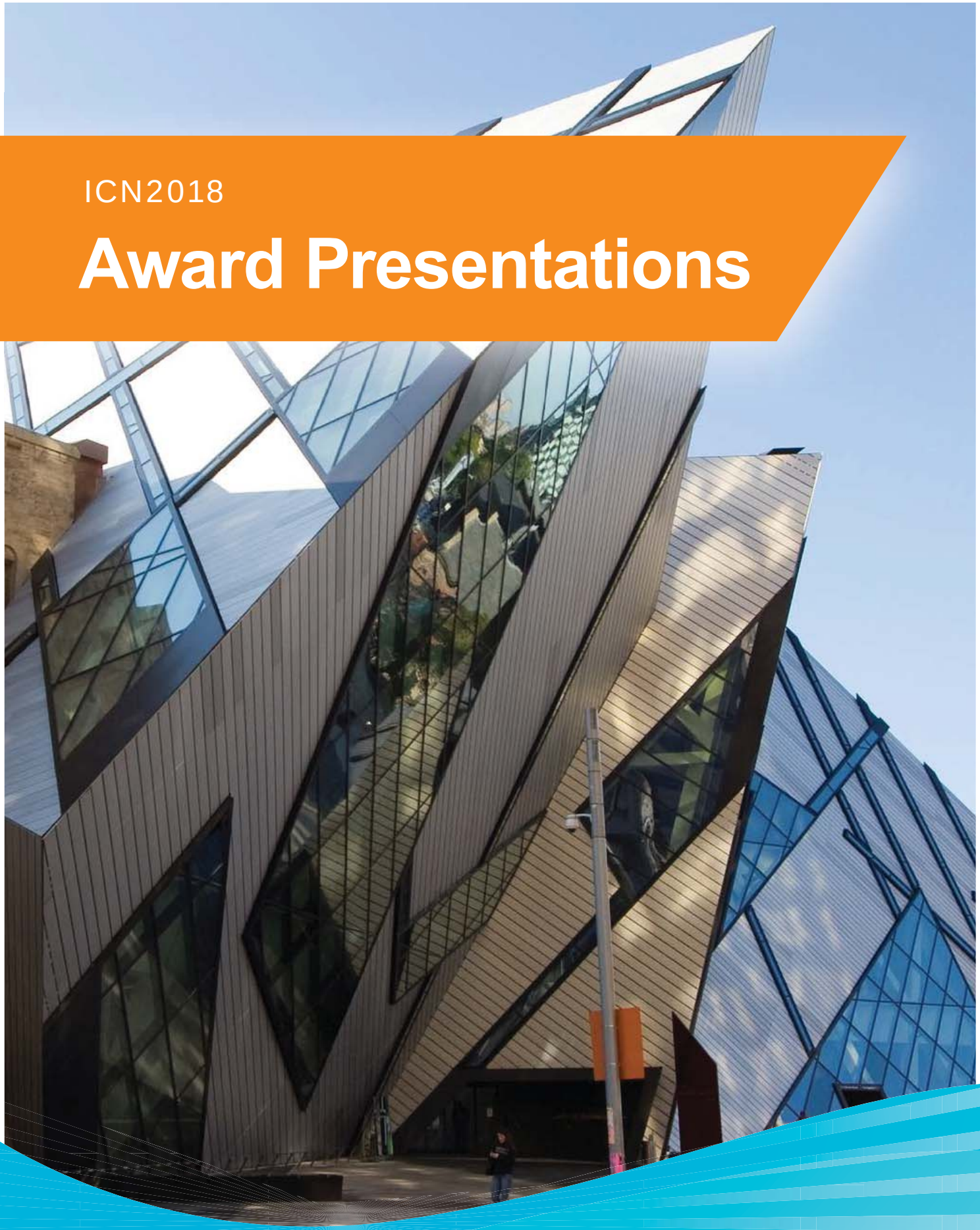
Asa Petersen
Experimental Medical Sciences, Lund University, Lund, Sweden

Huntington's disease (HD) is a fatal neurodegenerative disorder caused by an expanded CAG repeat in the HD gene. There is no disease-modifying treatment available. Typical motor symptoms such as chorea form the clinical diagnosis together with a positive gene test. The motor symptoms are associated with neuropathology in the striatum of the basal ganglia. Importantly, early psychiatric symptoms, metabolic dysfunction and sleep problems are key non-motor features in Huntington's disease (HD). The underlying neurobiological mechanisms for these aspects of the disease are not well known. Changes in the hypothalamus and its neuroendocrine network may constitute the core of the underlying pathology for the non-motor features of HD. In fact, our research has identified a number of hypothalamic changes in HD. In MRI from pre-motor HD participants, we have detected changes in the hypothalamic region at least a decade before predicted time of motor diagnosis. In post-mortem hypothalamic tissue from manifest HD, we have found loss of the hypothalamic neuropeptides orexin, oxytocin and vasopressin, involved in the regulation of sleep, emotion and metabolism. Using gene-expression analyses, we have demonstrated that the lateral hypothalamus is selectively affected and share a similar disease signature with the striatum. These findings have been replicated in mouse models of the disease. Importantly, we have shown that mutant

huntingtin expression in the hypothalamus is causally linked to the development of depressive-like behavior and metabolic dysfunction in mice. Finally, our recent data indicate that expression of mutant huntingtin selectively in the lateral hypothalamus leads to neuropathology in the ventral striatum. These results open up for the possibility that hypothalamic dysfunction may not only be important for the non-motor aspects of HD but may also be involved in the pathogenesis of the disease.

ICN2018

Award Presentations



Award Presentations – Sunday, July 15, 2018

AW1.001 SEXUAL DIFFERENTIATION OF BRAIN AND BEHAVIOR: SEX HORMONES, SITE OF ACTION AND SEXUAL PREFERENCES

Ashlyn Swift-Gallant
Neuroscience, Michigan State University, East Lansing, MI, United States of America

Since Young and colleagues (1959) posited that the organizing effects of androgens on behavior are likely due to androgens acting via neural tissue, the field of behavioral neuroendocrinology has largely focused on the effects of hormone action directly on the brain. For my doctoral work, I revisited the idea championed by Frank Beach that hormone action in *non*-neural tissues can affect the brain and behavior. To do so, we created two novel transgenic mouse models: one with neural-specific overexpression of androgen receptors (AR), and the second with global overexpression of AR. Our work comparing these two models supports a role for androgen action via both neural and *non*-neural tissues in sexual differentiation of brain and behavior. We also found evidence for a nonlinear relationship between androgens and male-typical behaviors: for example, male mice with global AR overexpression displayed an increased preference for same-sex sexual stimuli, akin to the effects of global loss of AR function in male mice. This observation in male mice spurred an independent line of questioning into the biological basis of human sexual orientation, in which I asked 1) whether high levels of androgens affect sexual preferences in men, and 2) whether there are subgroups of gay men who owe their sexual orientation to multiple distinct biological pathways (e.g., hormones [high and low], genetics, immunology). In this talk, I will present my findings comparing the socio-sexual behaviors of mice with global versus neural-specific AR overexpression, and I will share evidence suggesting that findings from my rodent work hold translational value for human research.

AW2.001 NOVEL REGULATORS OF THE CENTRAL CONTROL OF FEEDING AND SYSTEMIC INSULIN SENSITIVITY

Sophie M. Steculorum
Group of Neurocircuit Wiring and Function, Max Planck Institute for Metabolism Research, Cologne, Germany

Over the last decades, our understanding of the fundamental processes governing energy balance and glucose homeostasis has largely evolved and pinpointed a pivotal role of the central nervous system and more particularly of the arcuate nucleus of the hypothalamus (ARH). Notably, activation of orexigenic AgRP-expressing neurons located in the ARH potently promotes feeding. We demonstrate that in addition to its orexigenic effects, chronically altering AgRP-neurons activity also affects peripheral glucose homeostasis. Acute activation of AgRP-neurons causes insulin resistance through impairment of insulin-stimulated glucose uptake into brown adipose tissue (BAT) and decreased sympathetic nerve activity. Optogenetic circuitry

mapping reveals that feeding and insulin sensitivity are controlled by both distinct and overlapping AgRP-projections. We notably find that activation of AgRP→aBNST$_{vl}$ (ventro lateral part of the anterior bed nucleus of the stria terminalis) projections mediates the effect of AgRP-neuron activation on insulin sensitivity and BAT. Our results thus reveal a mechanism by which these neurons rapidly coordinate hunger states with glucose homeostasis. Along with this line, we discovered a novel AgRP-neurons' stimulatory pathway able to modulate both feeding and insulin sensitivity. We show that AgRP-neurons express the purinergic receptor 6 (P2Y6) and that activation of P2Y6 by its endogenous ligand uridine-diphosphate increases AgRP-neuron's action potential firing and promotes feeding. Further, selectively abrogating P2Y6-signaling in AgRP-neurons alleviates obesity-associated adiposity, hyperphagia, and insulin resistance. Our work, therefore, reveals that modulating AgRP-neurons by targeting P2Y6-signaling improves obesity-associated metabolic outcomes.

AW3.001 BSN ALISON DOUGLAS AWARD LECTURE

Peter J. Morgan
School of Medicine Medical Sciences and Nutrition, University of Aberdeen, Aberdeen, United Kingdom

No Abstract Received

AW4.001 INTRINSIC AND EXTRINSIC FACTORS REGULATING THE ACTIVITY OF VASOPRESSIN NEURONS

Masha Prager-Khoutorsky
Physiology, McGill University, Montreal, QC, Canada

Vasopressin-secreting neurons are an integral part of the magnocellular neuroendocrine system, playing a key role in water and salt homeostasis. Increases in plasma sodium and osmolality activate magnocellular vasopressin neurons, resulting in enhanced vasopressin release from their nerve terminals located in the posterior pituitary into the peripheral circulation. Magnocellular vasopressin neurons are intrinsically osmosensitive, and are activated by cell shrinking in response to increased extracellular sodium levels and osmolality. Our recent studies have demonstrated that magnocellular vasopressin neurons harbor a unique cytoskeletal scaffold attached to ion channels on the cell surface, which translates cell shrinking into mechanical activation of the channels, leading to the increase in the firing rate of the neurons, and enhanced vasopressin release. In addition to the intrinsic mechanisms mediating the activation of magnocellular neurons, extrinsic factors contribute to the stimulation of magnocellular neurons and vasopressin release via synaptically-mediated inputs arising from sodium and osmolality sensing neurons located in several interconnected hypothalamic nuclei.

Additionally, local glia cells play an important role in controlling the activity of magnocellular neurosecretory cells. During the lecture, Dr. Prager-Khoutorsky will present recent advances in the understanding of the intrinsic and extrinsic mechanisms regulating the activity of magnocellular vasopressin neurons in healthy organism and will describe how their malfunction can be associated with pathological conditions such as salt-sensitive hypertension.

Award Presentations – Wednesday, July 18, 2018

AW5.001 PROLACTIN SIGNALING IN THE MEDIAL PREOPTIC AREA IS CRITICAL FOR MATERNAL BEHAVIOR

Rosie S.E. Brown
Centre For Neuroendocrinology and Department of Anatomy, University of Otago, Dunedin, New Zealand

Parental care is critical for the survival of dependent offspring, with maternal care being the predominant form of care in mammals. The brain's medial preoptic area (MPOA) is central to integrating sensory and hormonal cues to generate appropriate maternal responses. The specific role of prolactin in contributing to the onset of maternal behavior has previously been unclear. By deleting prolactin receptors specifically from the MPOA using an adeno-associated virus expressing Cre-recombinase injected into prolactin receptor flox mice, we were able to show that prolactin signaling in the MPOA is critical for the normal onset of maternal behavior. Although fertility, gestation length and parturition were unaffected in these mice, no litters survived beyond day 2 of lactation, due to a complete failure of maternal care. Using a conditional transgenic strategy to remove prolactin receptors from specific neuronal populations, we probed the role of GABAergic and glutamatergic neurons in maternal behavior within the prolactin responsive neural network. Although GABAergic and glutamaterigc neurons represent the majority of prolactin responsive cells in the brain, there were only subtle deficits in maternal care in these mice. These data are the first to demonstrate that prolactin action is essential for normal maternal care, and highlight the MPOA as being key in hormonal regulation of maternal behavior.

AW6.001 NEURO- AND GLIOGENESIS IN THE PUBERTAL RAT BRAIN: IMPLICATIONS FOR FEMALE REPRODUCTION

Margaret A. Mohr
University of California, Los Angeles, Los Angeles, CA, United States of America

Puberty is an intricate process that results in reproductive maturation. In females, reproductive circuits are mature when estrogen positive feedback is able to elicit a luteinizing hormone (LH) surge. The LH surge triggers ovulation, a critical event in reproduction. The canonical view of reproductive maturation has been that the HPG axis is awakened at puberty through some combination of removal of inhibition and application of an accelerator, suggesting that the cellular machinery involved in the regulation of the HPG axis is already in place. My research has shed light on the possibility that the cells responsible for reproductive maturation are *not* in place before puberty, given that during puberty a new population of neurons and glia are added to the anteroventral periventricular nucleus (AVPV), the brain region that controls estrogen positive feedback. This addition of neurons and glia to the AVPV (and suprachiasmastic nucleus) continues into adulthood in the female rat. ~50% of these newborn cells expressed Fos during the LH surge, indicating responsiveness to hormonal signals. Inhibiting cell proliferation during puberty or adulthood reduced and delayed the estradiol and progesterone-induced LH surge. Ongoing postnatal addition of new cells to the preoptic area/hypothalamus may maintain the capacity to regulate the gonadotropin surge and ovulation. Currently, in my postdoctoral lab, I am investigating if pubertally born astrocytes are estrogen-responsive, and whether these cells increase synthesis of progesterone in response to estrogen, a critical step in estrogen positive feedback and the generation of the LH surge.

AW6.002 THE ROLE OF PROLACTIN IN AVIAN PARENTAL CARE

Kristina O. Smiley
Centre for Neuroendocrinology, Department of Anatomy, University of Otago, Dunedin, New Zealand

Parental care is a widespread phenomenon observed in many diverse taxa. Neuroendocrine systems have long been thought to play an important role in stimulating the onset of parental behavior. In particular, the hormone prolactin (PRL), which is significantly elevated during late pregnancy, has a well-established role in mediating mammalian maternal behavior through its actions on central prolactin receptors. Similarly, in most birds with altricial young, circulating PRL levels are low during non-breeding times and significantly increase during late incubation and early post-hatch chick care. Because of this pattern, PRL has been suggested to be involved in the initiation of parental care in birds, but rarely has this hypothesis been causally tested. My dissertation work is the first to establish that PRL plays a causal role in zebra finch parental behavior and to show that the central PRL receptor distribution and neural PRL signaling patterns are altered during times of parental care. This work will allow for opportunities to begin integrating this important group into comparative analyses to test whether parental brain networks are conserved across species and whether hormones such as PRL have conserved roles in parental care across taxa.

AW6.003 SBN YOUNG INVESTIGATOR AWARD LECTURE

Joanna Spencer-Segal
University of Michigan, Ann Arbor, United States of America

No Abstract Submitted

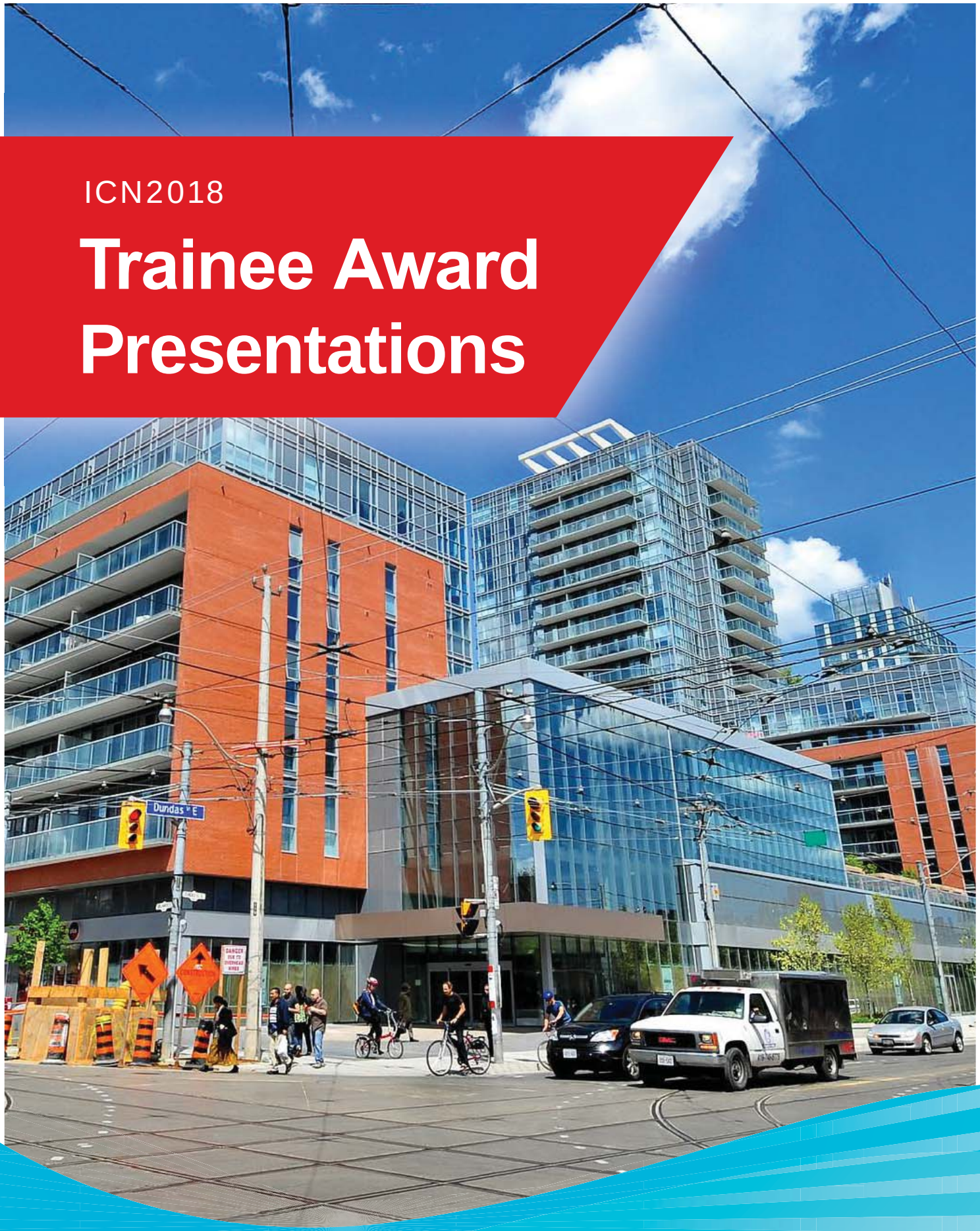
AW7.001 SBN LEHRMAN AWARD

Jeffrey D. Blaustein
University of Massachusetts, Boston, United States of America

No Abstract Submitted

ICN2018

Trainee Award Presentations



Trainee Award Presentations

Oral Presentations – Sunday, July 15, 2018

BOP1M.002
BOP1R.001
BOP1R.004

Poster Session 1 – Sunday, July 15 to Monday, July 16, 2018

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Oral Presentations – Tuesday, July 17, 2018

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BOP2S.001

Poster Session 2 – Tuesday, July 17 to Wednesday, July 18, 2018

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ICN2018

Oral Presentations

BOP1B.001 A PROLACTIN-MEDIATED HYPOTHALAMO-PITUITARY LOOP THAT DETERMINES SPECIES-SPECIFIC PATERNAL BEHAVIOUR.

Christian Broberger[1], Paul Williams[2], Sarah Kakadellis[2], Stefanos Stagkourakis[2]

[1]Dept Of Neuroscience, Karolinska Institutet, Stockholm, Sweden, [2]Dept Of Neuroscience, Karolinska Institutet, Stockholm, Sweden

Parental care is necessary for species survival. While maternal investment is near-universal, the relative contribution of fathers varies substantially between species, and its mechanisms are poorly understood. Using Sprague-Dawley rats and C57 mice, we identify how a difference in the electrical activity of tuberoinfundibular dopamine (TIDA) neurons, could account for the respective uni- and biparental strategies of the two species. While maternal behaviour was similar between species, paternal behaviour was remarkably different. C57Bl6/J mouse sires consistently performed pup retrieval, constructed nests and spent time in proximity to the pups. Sprague-Dawley rat sires did not exhibit any such paternal behaviour. Searching for a mechanism to explain this striking behavioural discrepancy, we identified a species difference in TIDA neuron activity, whose inhibitory influence control pituitary prolactin release. We found that fast mouse and slow rat TIDA oscillations translate to anticorrelated levels of dopamine release and serum prolactin, and identified strong activation of the mouse, but not rat, medial preoptic area, previously implicated in maternal behaviour. To test the hypothesis that different firing patterns in TIDA neurons account for the different parenting strategy through a neuroendocrine loop, we applied *in vivo* optogenetic inhibition to impose a range of oscillation frequencies, encompassing the natural rat and mouse rhythms, upon TIDA neurons in mouse sires. Distinct frequencies of optogenetic TIDA neuron stimulation resulted in defined prolactin serum concentrations and correlated levels of paternal investment. This work identifies a neuroendocrine circuit, tuned by membrane potential oscillations, that drives paternal care for offspring.

BOP1B.002 EVIDENCE FOR DOPAMINE AS A MODULATOR OF SEASONAL AUDITORY PLASTICITY IN THE PLAINFIN MIDSHIPMAN FISH

Jonathan Perelmuter[1], Joseph A. Sisneros[2], Paul M. Forlano[3]

[1]Behavioral & Cognitive Neuroscience, City University of New York, The Graduate Center, New York, NY, United States of America, [2]Psychology, University of Washington, Seattle, WA, United States of America, [3]Biology, City University of New York, Brooklyn College, Brooklyn, NY, United States of America

The dopaminergic system is considered integral to attentional and social incentive processes, but studies are largely restricted to the central nervous system, particularly the mesolimbic pathway. In rodents, central dopamine is known to project to the inner ear, and therefore may modulate the detection and encoding of acoustic signals at the earliest stages of reception,

including transduction. Accumulating evidence from the plainfin midshipman fish, *Porichthys notatus*, suggests peripheral dopaminergic modulation is important for perception of acoustic signals in the context of social communication and reproduction. During the summer breeding season, nesting males produce long duration vocalizations at night, which females use to locate males for spawning. Females undergo steroid-dependent enhancement of peripheral auditory sensitivity that facilitates mate localization. Crucially, during the breeding season, dopamine puncta are reduced in the saccule, the main organ of hearing in the midshipman ear. In contrast, dopaminergic innervation is increased in the hindbrain cholinergic nucleus that sends efferent projections to the saccule. Electron microscopic analysis of the saccule revealed that terminals do not form traditional synapses but contain vesicles in proximity to hair cells, suggesting paracrine release and the potential for dopamine to modulate hair cells directly. Application of exogenous dopamine increases saccule hair cell receptor thresholds, as evaluated by saccular potential recordings, in both summer and winter fish. Quinpirole, a D2 agonist, also increases saccular thresholds. We suggest that the seasonal decrease of dopamine in the inner ear serves as a release of inhibition, adaptively improving saccular auditory sensitivity for mate detection and localization.

BOP1B.003 ADAPTIVE EVOLUTION OF A NOVEL AVPR1A ENHANCER ASSOCIATED WITH THE ONSET OF PRAIRIE VOLE MONOGAMY

Alejandro Berrio[1], Steven M. Phelps[2]

[1]Biology, Duke University, Durham, NC, United States of America, [2]Integrative Biology, University of Texas, Austin, TX, United States of America

Changes in regulatory DNA play a critical role in the evolutionary diversification of form. Nevertheless, we know little about how such changes influence behavior. In monogamous voles, pairbonding is influenced by increased transcription of the neuropeptide receptor Avpr1a within the ventral pallidum, a brain region critical to reward. Furthermore, monogamous species like prairie and pine voles exhibit remarkably similar patterns of Avpr1a expression. We began with a phylogenetic analysis of 8 related vole species with known mating-system, which suggests that monogamy evolved once in this group, at the common ancestor of prairie and pine voles. Next we identified active enhancers within the prairie vole pallidum by performing ChIP-seq targeting the histone modification H3K27ac. This revealed two regulatory regions neighboring the Avpr1a locus. One of these putative enhancer sequences showed extremely rapid evolution coinciding with the origins of monogamy and high pallidal Avpr1a. Lastly, we generated lines of transgenic mice in which a reporter (LacZ) was expressed under the control a minimal Hsp68 promoter and flanked by the putative enhancer element. Our results show that the enhancer is able to drive LacZ expression in diverse regions of the mouse forebrain, including the pallidum. Together our data suggest that evolutionary changes in pallidal Avpr1a expression have been shaped by a novel enhancer that emerged in the common ancestor of prairie and pine voles. More generally, these results illustrate how functional genomics,

evolutionary genetics and behavioral neuroscience can be combined to understand the evolution and mechanisms of complex behaviors.

BOP1B.004 SF1 NEURONS ARE TIMING AND FIRING FREQUENCY-DEPENDENT REGULATORS OF METABOLISM AND BEHAVIOUR

Paulius Viskaitis[1], Elaine E. Irvine[1], Mark A. Smith[1], Agharul I. Choudhury[1], Mahesh M. Karnani[2], Elisa Alvarez-Curto[3], Justyna A. Glegola[1], Darran G. Hardy[1], Silvia M..A. Pedroni[1], Maria R. Paiva Pessoa[1], Anushka B..P. Fernando[1], Loukia Katsouri[1], Alessandro Sardini[1], Mark A. Ungless[1], Graeme Milligan[3], Denis Burdakov[4], Dominic J. Withers[1]

[1]Institute Of Clinical Sciences, Imperial College London,, London, United Kingdom, [2]Francis Crick Institute, London, United Kingdom, [3]Centre For Translational Pharmacology, University of Glasgow, Glasgow, United Kingdom, [4]ETH Zürich, Schwerzenbach, Switzerland

The ventromedial hypothalamus (VMH) was historically considered a satiety centre implicated in the regulation of affective states and stress response. More recently, with advent of cell-type specific techniques, steroidogenic factor 1 (SF1) neurons, which constitute the major neuronal population in the VMH, have been shown to regulate feeding, glucose homeostasis, adiposity and stress-responses. However, the full extent of SF1 neuronal functions as well as how a single neuronal population is responsible for such a plethora of roles remain unclear. In a series of pharmacogenetic, optogenetic and *in vivo* calcium imaging studies, we demonstrate that SF1 neuronal function depends on the kinetics as well as the type and intensity of modulation, while endogenous activity responses differ significantly depending on the external stimuli. Specifically, we show that pharmacogenetic alterations in SF1 neuronal activity acutely regulate hunger and affective states, but these effects are absent with prolonged pharmacogenetic modulation instead altering the fat mass set-point. Furthermore, using optogenetics we reveal a frequency-dependent switch between the regulation of feeding and stress responses: tonic low-frequency stimulation decreased feeding without affecting stress response, while higher-frequency activation lead to overpowering stress-related outcomes. Finally, calcium imaging in freely behaving animals revealed that feeding required sustained suppression of SF1 neuronal activity, while stressful stimuli induced activity significantly above the levels observed during feeding. Overall, we comprehensively describe various roles SF1 neurons play in facilitating survivability and present a mechanism where different functions can be partially segregated based on timing, type and intensity of changes in SF1 neuronal excitability.

BOP1M.001 THE ROLE OF MICROGLIAL ESTROGEN RECEPTORS IN OBESITY PATHOGENESIS

Mauricio D. Dorfman, Rachael Fasnacht, Jineta Banerjee, Alice Wyse-Jackson, John D. Douglass, Joshua P. Thaler

University of Washington, Seattle, WA, United States of America

Obesity is associated with a hypothalamic injury response that includes increased microglial activation and inflammatory signaling. Rodent studies have demonstrated that reducing hypothalamic inflammation and microglial activation limits weight gain and the metabolic complications of diet-induced obesity (DIO). Similarly, estrogen reduces food intake, increases energy expenditure, and can potently suppress brain inflammation. In addition, female mice are more resistant to diet-associated microglial activation and weight gain, suggesting a potential link between estrogen and microglial signaling. Therefore, we hypothesize that estrogen reduces DIO susceptibility through central anti-inflammatory action on microglial cells. To test this possibility, we generated mouse with microglia-specific deletion of either estrogen receptor alpha (ERa-KO) or estrogen receptor beta (ERb-KO). We found that male and female ERb-KO mice exposed to high-fat diet (HFD) are more susceptible to weight gain than their wild-type (WT) littermate controls. The predisposition to obesity in ERb-KO mice is not due to altered food intake, suggesting a reduction of energy expenditure. Body composition analysis revealed that ERb-KO mice have higher fat mass, but not lean mass than WT controls. Surprisingly, despite the increase of adiposity, ERb-KO mice were not glucose intolerant. In contrast with ERb-KO, ERa-KO mice displayed glucose intolerance after 12 weeks on HFD, but did not exhibit a body weight phenotype. Together, these preliminary observations suggest that estrogen signaling through microglial ERb protects from diet-induced weight gain and through microglial ERa improves glucose tolerance.

BOP1M.002 ASTROCYTE ACTIVITY STATE IN THE HYPOTHALAMIC ARCUATE NUCLEUS REGULATES SYSTEMIC GLUCOSE METABOLISM

Ophelia Le Thuc[1], Beata Legutko[1], Tim Gruber[1], Claire Martin[2], Daniela Herrera Moro Chao[2], Dongdong Li[3], Serge Luquet[4], Matthias Tschöp[1], Cristina García-Cáceres[1]

[1]Helmholtz Center Munich, Helmholtz Diabetes Center & German Diabetes Research, Neuherberg, Germany, [2]Unit Of Functional and Adaptive Biology, Université Paris Diderot-Paris 7, Paris, France, [3]Mix Research Unit 8118, National Center for Scientific Research, Paris, France, [4]Unité Biologie Fonctionnelle et Adaptative, Cnrs Umr 8251, University Paris Diderot, Sorbonne Paris Cité, Paris CEDEX, France

Hypothalamic arcuate nucleus (ARC) is a primary sensor for nutrients and hormones regulating energy homeostasis *via* its unique location next to the median eminence, where blood-brain-barrier protection is reduced. Most studies aiming at deciphering central control of systemic metabolism focused exclusively on neurons. However, we recently revealed that astrocytes respond to metabolic cues, controlling energy balance together with neurons. Furthermore, high-fat high-sugar diet has been found to induce astrogliosis specifically in the ARC before any significant body weight gain, suggesting a role in promoting obesity. We now hypothesize that ARC astrocytes actively participate in governing systemic energy homeostasis. To test this hypothesis, we used Designer Receptors Exclusively Activated by Designer Drugs (DREADD) to remotely increase *in vivo* the $[Ca^{2+}]_{int}$ in distinct ARC astrocyte subpopulations targeted via glia-enriched proteins (glial fibrillary acidic protein (GFAP) and aldehyde dehydrogenase 1 family member L1 (Aldh1L1)). We therefore induced expression of hM3Dq (Gq-coupled DREADD)

spcifically in $GFAP^+$ or $Aldh1L1^+$ ARC astrocytes to evoke astrocyte intracellular Ca^{2+}release *via* clozapine-n-oxide (CNO). We found that elevation of $[Ca^{2+}]_{int}$ in $GFAP^+$ and $Aldh1L1^+$ ARC astrocytes induced a fast increase in systemic blood glucose levels (peak 15 min following CNO injection). Interestingly, increasing $[Ca^{2+}]_{int}$ in $Aldh1L1^+$ ARC astrocytes triggered a decrease in food intake, an effect not found when $GFAP^+$ cells were targeted. Therefore, these results suggest that ARC astrocytes might participate in the central control of key metabolic processes such as hepatic glucose production and/or pancreatic insulin secretion, but also influence feeding centers, leading changes in feeding behavior.

BOP1M.003 BRAINSTEM-HYPOTHALAMUS LONG FEEDBACK LOOP MEDIATING NORMAL SATIETY

Simon M. Luckman
University of Manchester, Manchester, United Kingdom

We have shown previously the importance of neurons in the brainstem nucleus of the tractus solitarius, which contain prolactin-releasing peptide (PrRP), for mediating the satiating effects of the gut-produced factor, cholecystokinin (CCK).[1,2] Here we map ascending projections of $PrRP^{NTS}$ neurons, including towards oxytocin neurons in the paraventricular nucleus of the hypothalamus. Selective, chemogenetic activation of either $PrRP^{NTS}$ or Oxt^{PVH} neurons, using the stimulatory designer receptor (hM3Dq) produced a reduction in normal food intake. In contrast, site specific deletion of PrRP in the NTS or the selective inhibition of Oxt^{PVH} neurons, using the inhibitory designer receptor (hM4Di), blocked the satiating effect of a low dose of CCK. Using complementary optogenetic and tracing techniques, we have mapped key targets of Oxt^{PVH} neurons and, in particular, confirmed the descending projection back down to the brainstem. These data demonstrate the $PrRP^{NTS}$-Oxt^{PVH} pathway modulates food intake, in the context of normal satiety signalling. [1]Bechtold and Luckman, 2006, Endocrinology 147: 4723-4729. [2]Dodd *et al.*, 2014, Cell Metab. 20: 639-649.

BOP1M.004 DEPLETION OF EMBRYONIC MICROGLIA ALTERS HYPOTHALAMIC CIRCUITS AND CAUSES ACCELERATED WEIGHT GAIN

Jessica M. Rosin, Deborah M. Kurrasch
Medical Genetics, University of Calgary, Calgary, AB, Canada

Microglia are the resident immune cells in the CNS. Originally thought to be primarily responsible for disposing of cellular debris and responding to neural insults, emerging research now shows that microglia are highly dynamic cells involved in a variety of developmental processes. The hypothalamus is a brain region critical for maintaining homeostatic processes such as energy balance and food intake. Given that microglia colonize the embryonic brain

alongside key steps of hypothalamic development, we tested whether microglia are required for the proper establishment of this brain region. To eliminate microglia from the fetal brain, we treated pregnant dams with the Csf1r inhibitor PLX5622. We showed that approximately 99% of microglia are eliminated by E15.5 when pregnant dams are placed on a PLX5622 diet starting at E3.5. Following microglia depletion, we observed an increase in cell death throughout the developing hypothalamus. Embryonic microglia depletion also resulted in a decreased litter size, as well as an increase in the number of pups that died within the first two postnatal days of life. In pups that survived, the elimination of microglia in the brain resulted in a decrease in POMC neurons and a concomitant accelerated weight gain starting at P5, suggesting that microglia are important for the development of hypothalamic satiety centers. Finally, the depletion of microglia during embryogenesis had long-term sex-specific effects on behaviour, including the appearance of hyperactivity and anxiolytic-like behaviour in female mice. Together, these data demonstrate that microglia likely play an important role during the development of the hypothalamus.

BOP1R.001 RABIES-MEDIATED MONOSYNAPTIC TRACT-TRACING OF THE AFFERENT KNDY NETWORK IN THE MOUSE

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A variety of neurochemical systems are implicated to regulate fertility by relaying endogenous and peripheral signals to arcuate nucleus (ARC) Kisspeptin/Neurokinin B/Dynorphin (KNDy) neurons, which in turn regulate GnRH secretion; however, the location of these upstream cells is largely unknown. We provide a preliminary report of monosynaptic inputs to KNDy neurons using rabies-mediated tract-tracing. Adult female Kiss1-Cre mice (n=4) were stereotaxically injected in the unilateral ARC with Cre-dependent adenoassociated viruses (AAVs) to express avian receptor protein with GFP (TVA-GFP) and rabies glycoprotein (G) in ARC kisspeptin (KNDy) neurons. Three weeks later, the glycoprotein-deleted rabies virus containing mCherry (RVdG-mCherry) was injected into the same location. RVdG exclusively infects KNDy cells expressing TVA, and, as G is required for rabies to cross transynaptically, RVdG-mCherry will cross into primary afferents of KNDy neurons. After one week, mice were perfused and coronal sections immunolabelled for GFP to identify Cre-positive KNDy cells and mCherry to visualize KNDy afferents. Sections were imaged using epifluorescent microscopy and slide scanning software (MBFbioscience). Afferents were mapped, and the average number of cells and percentage of total afferents per nuclei determined, by overlaying sections with Paxinos and Franklin mouse brain atlas. KNDy afferents were detected within 23 different nuclei in the hypothalamus, amygdala, subiculum and brainstem of all animals. The majority of afferent cells were located within the ARC (33.5±7.3%, excluding reciprocally-connected KNDy cells), followed by the

paraventricular (11.5±6.2%) and dorsomedial hypothalamic nuclei (10.9±1.5%). Future studies will identify the phenotype of KNDy afferent populations to define inputs critical for fertility.

BOP1R.002 DISCLOSING NEUROENDOCRINE MECHANISMS OF SEASONALITY, A STEP TOWARDS GENETICALLY MODIFIED MODELS.

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Previous studies on seasonal mammals have highlighted the key role of melatonin for synchronizing reproductive activity with the seasons. Melatonin controls thyroid stimulating hormone (TSH) production from the pituitary *pars tuberalis* so that its secretion is higher under long summer days. TSH in turn acts on tanycytes, to regulate the balance in deiodinase2/3 activities leading to increased hypothalamic 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 inhibited by melatonin in short days adapted sexually inactive Syrian hamsters, but whether this seasonal regulation depends on a direct effect of T3 is unknown. Because studies on hamsters are limited by the lack of genetically modified models, we explored whether mice, although showing no overt seasonal functions, could help disclosing the link between hypothalamic T3 and kisspeptin/RFRP. First, we observed that melatonin-deficient C57 mice supplemented with melatonin at night display the same melatonin-dependent regulation of TSH, Dio2/Dio3 and RFRP than hamsters. Next, by comparing the effect of melatonin supplementation in wildtype C57 or C57 mutated for the T3 receptor TRalpha, we found that in mice lacking TRalpha, melatonin no longer inhibits RFRP expression. Altogether our data indicate that mice, like seasonal mammals, are able to integrate the melatonin signal up to the hypothalamic RFRP and that the melatonin-driven inhibition of RFRP neurons appears to depend on the effect of T3 on TRα.

BOP1R.003 FEMALE-BIASED DEPENDENCE OF PUBERTY AND FERTILITY ON MICRORNA SYNTHESIS IN KISS1 NEURONS

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Kisspeptins, produced by Kiss1 neurons, are an essential component of the GnRH pulse generator, with indispensable roles in the control of puberty and fertility. We report here that mice with congenital ablation of the miRNA-synthesizing enzyme, Dicer, in *Kiss1*-expressing cells (named KiDKO) display postnatal-onset hypogonadotropic hypogonadism (HH) in both sexes, but failure to complete puberty and early-onset infertility occurs selectively in females. This HH phenotype resembled that induced by conditional elimination of *Dicer* in GnRH neurons, albeit with a milder juvenile progression of gonadal failure. In fact, male and female KiDKO mice showed phenotypic markers of initiation of puberty, and preserved Kiss1 neuronal populations, both in the arcuate nucleus (ARC) and antero-ventral periventricular (AVPV) hypothalamus, at the infantile period. Yet, while females never reached an ovulatory stage, KiDKO males completed spermatogenesis and were fertile at young adult age. Disparate changes of Kiss1 cell numbers and *Kiss1*/kisspeptin expression were detected between ARC and AVPV after congenital ablation of Dicer. Likewise, divergent alterations of kisspeptin *vs.* neurokinin B (co-transmitter of ARC Kiss1 neurons) profiles were detected in infantile KiDKO mice. Our data unveil that miRNA biosynthesis in Kiss1 neurons is indispensable for pubertal completion and fertility selectively in females, but dispensable for early stages of postnatal sexual maturation in both sexes. The disparate impact of *Dicer* ablation on reproductive maturation and function between sexes and ARC *vs.* AVPV Kiss1 neurons surfaces intrinsic differences in the roles of Kiss1 miRNA regulatory machinery in the fine control of the male and female reproductive brain.

BOP1R.004 ESTROGEN RECEPTOR ALPHA DELETION IN AVPV KISSPEPTIN NEURONS IN ADULTHOOD DECREASES CELL EXCITABILITY

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The brain regulates fertility through gonadotropin-releasing hormone (GnRH) neurons. Estradiol acts via estrogen receptor-alpha (ER-alpha)-expressing afferents of these cells, including arcuate and anteroventral-periventricular (AVPV) kisspeptin neurons. Estradiol induces negative feedback on pulsatile GnRH release and positive feedback generating GnRH/luteinizing hormone (LH) surges. AVPV kisspeptin neurons exhibit increased spontaneous activity and burst firing during positive feedback. This increased excitability was lost in kisspeptin-specific ER-alpha knock-out (KERKO) mice, which also fail to exhibit estradiol-induced LH surges. If these phenotypes are due to organizational and/or activational changes is unknown, as ER-alpha is

deleted before puberty in KERKO mice and is important for development of AVPV kisspeptin cells. We utilized CRISPR-Cas9 AAV to deliver a single-guide RNA to target disruption of *Esr1*, or *Lacz* as a control. AAVs were injected bilaterally into the AVPV of adult females expressing Cas9-GFP in kisspeptin cells. In mice with *Esr1* sgRNA, only 26±1% of AVPV kisspeptin cells expressed ER-alpha vs the typical 71±1% in *Lacz* controls ($p<0.01$). We recorded firing signatures of infected and uninfected cells ($n>8$ cells each) in ovariectomized, estradiol-replaced mice using the whole-cell configuration, and confirmed ER-alpha status *post hoc*. ER-alpha deletion caused loss of depolarization-induced bursts (*Esr1* 0% of cells, *Lacz* 45%, uninfected 37%, $p<0.01$) and reduced rebound bursts (*Esr1* 26% of cells, *Lacz* 90%, uninfected 75%, $p<0.01$) compared to neurons infected by *Lacz* or uninfected controls. Firing signatures of AVPV kisspeptin neurons after adult *Esr1* knockdown were comparable to those in KERKO mice, suggesting activational effects of estradiol can regulate firing activity. NIH-HD41469

BOP1S.001 MATERNAL STRESS EFFECTS IN THE WILD: FROM MECHANISM TO FUNCTION IN NORTH AMERICAN RED SQUIRRELS

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The intergenerational consequences of maternal stress are appreciated in biomedicine and increasingly so in evolutionary biology and ecology. Theoretical models suggest that maternal stress effects could enable organismal resilience across environmental fluctuations by generating adaptive changes in offspring characteristics that prepare them for stressful environments. Testing the consequences of maternal stress effects on offspring survival and reproduction in the wild has remained challenging because of a lack of experimental studies in addition to the difficulties associated with tracking offspring over their lifetime. For the past 10 years, we have been examining the ecological factors (predators, competitors, and food availability) that induce maternal stress in wild North American red squirrels (*Tamiasciurus hudsonicus*) in the Yukon, Canada and its effects on maternal behaviour and a range of offspring characteristics. In a 3-year field experiment with wild female red squirrels, we experimentally elevated maternal glucocorticoid levels during pregnancy or lactation and to test the hypothesis that maternal stress effects increase offspring survival early in life but reduce their longevity. We will present an overview of this work showing how elevated gestational stress can alter offspring physiology, postnatal growth, and behavior that increases their early life survival but may carry costs for their overall lifespan. Overall, our work highlights the causes for the evolution of maternal stress effects and their widespread occurrence in mammals.

BOP1S.002 MINERALOCORTICOID RECEPTORS ARE NECESSARY FOR THE PLASTICITY PHENOTYPE OF HIPPOCAMPAL CA2 NEURONS

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Exposure to stress is a risk factor in the development, onset, and exacerbation of several neuropsychiatric disorders, including depression, schizophrenia, and PTSD. In the brain, glucocorticoid (GR) and mineralocorticoid (MR) receptors mediate behavioral and physiological responses to stress. Although much is known about the roles of MRs and GRs in neuronal response to stress, very little is known about their role in the development of neuronal phenotype. In adult mice, we found MR transcripts far outnumber GR in hippocampal area CA2, where we previously found the neurons to have a peculiar 'lack of LTP' phenotype. We also noticed that MRs are highly expressed in the embryonic hippocampus, thus we sought to test whether MRs regulate CA2 neuronal development. Indeed, we found that neuronal MR deletion disrupted the normal expression of several of the molecules that make CA2 molecularly distinct, including RGS14, PCP4, and NECAB2. We observed these effects regardless of whether MR deletion occurred embryonically (Nestin-cre), postnatally (Amigo2-cre), or in adulthood (virally expressed cre), and in both mRNA and protein. Interestingly, we also found several CA1 markers increased in (what might have been) CA2 after MR deletion, as compared with cre-negative control mice. In addition, several stress-related genes were also differentially expressed between cre+ and cre- animals. Furthermore, this loss of MR, and/or the resulting disruption of CA2 gene expression, enabled synaptic potentiation of CA2 excitatory currents. These results suggest a unique role for MRs in regulating synaptic plasticity phenotype in CA2 during development and, perhaps, in response to stress.

BOP1S.003 EFFECTS OF INTRANASAL OXYTOCIN ON STRESS-EVOKED BY CESAREAN SECTION IN RATS

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Milk shortage in women with cesarean section (CS) along with aberrant maternal behaviors has become an increasing concern for the health of mothers and the babies, and intranasal application of oxytocin (IAO) has been considered a potentially effective therapy. However, the effect of this treatment and the underlying mechanism remains unclear. Here, we reported our findings in rats that the influence of CS and IAO on lactation performance is largely determined by the timing of postoperative dam-pup reunion. That is, early dam-pup contact has relative less adverse effect on lactation while delayed maternal contacts resulted in severe hypogalactia. There is a trend of spontaneous recovery of the lactation performance with the

extension of postoperative days and IAO is effective only at the first several days but lacks long lasting effect. Moreover, CS can activate both vasopressin neurons and the hypothalamic pituitary adrenal axis. The effect of IAO is associated with the activation of oxytocin neurons and hypothalamic pituitary thyroid axis; however, it further increases the activity of the hypothalamic pituitary adrenal axis. These results indicate that CS can lead to hypogalactia in association with the timing of postpartum mother-baby contact, which is largely because of activation of the hypothalamic pituitary adrenal axis; IAO transiently improves lactation performance by activation of oxytocin neurons and increased secretion of thyroid hormone; however, that is compromised by the further activation of hypothalamic pituitary adrenal axis.

BOP1S.004 DEVELOPING A MULTISCALE MATHEMATICAL UNDERSTANDING OF THE HPA AXIS

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The stress response depends on the coordination of the hypothalamus, the pituitary and the adrenal glands for the rapid synthesis and secretion of glucocorticoid hormones. To mount an efficient response to stress, circulating levels of these steroid hormones are dynamically regulated by a multitude of factors, spanning different levels of organisation (molecular, cellular, systemic). Existing experimental protocols often struggle to combine data at these different levels and, as a result, the mutual interactions between factors underlying endocrine regulation and the different time scales at which they occur are often ignored. Data-driven mathematical models can provide novel insight by simultaneously considering the contribution of factors across differing scales of space and time, and by realising the system as a network, rather than as a composition of isolated components. Here, we present mathematical models of the interactions between the pituitary and adrenal glands, with special attention to the adrenal Steroidogenic Regulatory Network (SRN). We postulate how steroidogenic factors respond collectively to ACTH stimuli from the pituitary, and used our model to predict the time evolution of steroidogenesis in response to a range of physiological ACTH perturbations, including basal and acute stress scenarios (e.g. inflammation). We discuss the implications of the model predictions in the light of *in vivo* experiments in the rat.

BOP2B.001 NON OBESE TYPE 2 DIABETES LEADS TO EMOTIONAL, ENDOCRINE AND MEDIAL PREFRONTAL CORTEX ALTERATIONS

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A large literature documents an increased co-occurrence of type 2 diabetes (T2D) and psychiatric disorders such as anxiety and depression; however the nature of the underlying co-morbidity remains unclear. In animals, emotional disturbances have been recurrently reported in obese models of T2D. These models do not adequately reflect the full clinical context of T2D which is not always associated with obesity. Our study aims to explore emotional behaviors in Goto Kakizaki (GK) rats, a non-obese genetic model of T2D. 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. GK rats displayed hyper-anxiety in the light-dark and open-field tests, reduced social interaction and hyper-response of the HPA axis to stress compared with Wistar rats. Neurobiological measures reveal that the mPFC was the most affected brain area showing increased expression of 5HT-R1a (serotonin receptor 1a), 5HTT (serotonin transporter) and a marked increase of Interleukin-6 (IL-6). GK rats also exhibited decreased astrocytes number and increased microglia process length in the prelimbic subregion of the mPFC. In conclusion, our study reveals that T2D, independently of the 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 test the role of inflammatory processes in the reported alterations in GK rats.

BOP2B.002 ANABOLIC-ANDROGENIC STEROIDS AND COGNITIVE EFFORT DISCOUNTING IN MALE RATS

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Anabolic-androgenic steroids (AAS) are drugs of abuse taken to increase muscle mass and athletic performance, but which also impair behavior and cognition. In particular, a rodent model of AAS abuse demonstrates that testosterone-treated male rats will expend more effort in an operant discounting task where rats press a lever repeatedly for a large reward. However,

since modern society prioritizes cognitive over physical effort, it is important to evaluate the extent to which AAS limit cognitive effort. Here we test the effects of AAS on a novel cognitive-effort discounting task. Each operant chamber has 3 nose-pokes on 1 wall, opposite 2 retractable levers and a pellet dispenser. Rats press a lever to illuminate 1 nose-poke for 1s, and they must respond in the illuminated nose-poke to receive sugar pellets. The small reward lever activates a 1s illumination, and a correct response earns 1 pellet. The large reward lever decreases illumination from 1s to 0.2s, but a correct response earns 4 pellets. During training, rats completed 78.8±9.3 trials with 95.6±2.6% accuracy and 16.5±3.3% omissions in each 40m session. There was no effect of testosterone on task acquisition. In previous discounting tasks, testosterone-treated rats preferred the large reward even when it was discounted by delay, effort, and punishment. Thus, we hypothesize that testosterone increases willingness to expend cognitive effort to obtain a large reward. Recent studies show that AAS impair visuospatial memory in humans, and set-shifting and reversal learning in rats. Therefore, we hypothesize that testosterone reduces accuracy to earn the large reward.

BOP2B.003 TRANSGENERATIONAL EFFECTS OF EXPOSURE TO AN EDC MIXTURE ON MATERNAL BEHAVIOR AND SEXUAL DEVELOPMENT

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Environmental factors such as endocrine disrupting chemicals (EDCs) have been proven to produce transgenerational inherited modifications. A rising public health challenge is to determine the effect of complex mixtures of EDCs on the developing body throughout generations. In this study we aim to determine the transgenerational effects of a mixture of EDCs on female sexual development and behavior. Female rats were orally exposed from 2 weeks before gestation until weaning to corn oil or a mixture of 14 anti-androgenic and estrogenic EDCs at low doses. Sexual development (sex ratio, vaginal opening (VO), GnRH interpulse interval and estrous cyclicity) as well as maternal behavior were measured from F0 to F3 generation. *In utero* exposed females (F1) when raising pups, showed an increased time resting alone and decreased time licking and grooming pups. F2 (animals whose germlines were exposed) and F3 exposed animals showed an altered sex ratio in favor of males and F2 and F3 females showed delayed VO. F2 and F3 females followed for estrous cyclicity showed significant alterations of estrous cyclicity characterized by a significant increase in the time spent in estrus and decreased time spent in diestrus. F3 females presented an increased GnRH interpulse interval compared to control. Overall, data shows that gestational exposure to an EDCs mixture can affect maternal behavior and sexual development during several generations. The effects observed in the F3 generation suggest the presence of transgenerational epigenetic mechanisms.

BOP2B.004 LEARNING INFANT CUES: ESTROGEN AND SOCIAL EXPERIENCE'S EFFECTS ON MECHANISMS FOR SENSORY PLASTICITY

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While infant cues are often assumed to innately motivate maternal response, recent research highlights how cue processing is enhanced through sensory cortical plasticity. Evidence from mice suggests experience caring for pups induces plasticity in the auditory cortex (AC), which improves pup vocalization detection and discrimination. However, little is known about the molecular mediators for such AC plasticity during the initial pup experience. Here, we used ovariectomized and estrogen (E2) or blank implanted virgin female mice to explore the behavior and AC molecular changes induced by the very first pup-caring and vocalization experience. As expected, E2 increases the overall time spent performing maternal behavior during one-hour of pup experience. Surprisingly, while E2 animals display more licking/grooming and crouching behaviors, blank implanted animals display significantly more nest building. We used qPCR to assay how the memory associated gene, brain derived neurotrophic factor *(bdnf),* is altered by the playback of pup-calls depending on the availability of pups to retrieve. AC *bdnf* mRNA increases significantly in pup and pup-call stimulated females relative to those who only heard calls, suggesting that hearing these stimuli in the social context induces immediate molecular changes at the site of auditory cortical processing. To our knowledge, this is the first time *bdnf* is associated with processing novel social stimuli in the AC. Examining behavior and *bdnf* expression enables us to investigate the molecular underpinnings of early plasticity at the AC and other sites associated with maternal response, which are responsible for enhancing future recognition of infant cues.

BOP2B.005 EARLY LIFE FAMILY ENVIRONMENT IMPACTS BEHAVIOR, NONAPEPTIDES, AND THE EPIGENOME OF OFFSPRING

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The nonapeptides (vasopressin and oxytocin) are important modulators of aggression, anxiety, and social behavior. Early life experiences alter behavior and gene expression by genomic and epigenomic means. However, our knowledge of gene x environment influences on brain and behavior are grossly limited to the influence of mothers in uni-parental species. Yet, a father's behavior should have enormous impact on offspring development and the extent to which paternal care influences the development and epigenetic modification of nonapeptide systems has been comparatively underdeveloped. To this end, we raised prairie voles (a bi-parental rodent) (1) in the presence or absence of a father, and (2) manipulated parental feeding effort forcing mothers and fathers to care for themselves or care for their pups (i.e., "working" or "not-working" for food). We found single mothers increased their parental effort compared to

bi-parental mothers. The 'working' condition impacted parental care by only reducing care from 'working' fathers. Variation in parental care resulted in several behavioral differences in adult offspring. Both sexes raised in single mother families were less exploratory and exhibited impaired performance in a spatial learning and memory task. Male, but not female, pairbonding and non-reproductive social preferences were sensitive to the presence/absence of the father. Social approach was particularly sensitive to the reduced paternal care from 'working' family units. Astonishingly, methylation of the nonapeptide receptor genes in the lateral septum (crucial for parental care) directly accounted for the differences in social approach. Thus, complex interactions of postnatal social experience influenced the epigenetic regulation of behavior.

BOP2B.006 LONGITUDINAL CHANGE IN COGNITIVE PERFORMANCE OF WOMEN POST-OOPHORECTOMY

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Women who have oophorectomy prior to spontaneous menopause are at increased risk for dementia and cognitive decline in later years. Furthermore, estrogen interacts with the e4 variant of the apolipoprotein E gene (APOE4), a risk factor associated with Alzheimer's disease, thereby increasing cognitive decline in an estrogen-depleted state. While the immediate and long-term effects of surgical menopause are better understood, less is known about the rate of cognitive decline over time in these women. Therefore, this study investigated longitudinal changes in verbal memory in women with a bilateral salpingo-oophorectomy prior to spontaneous menopause through a multilevel modeling approach. Our analyses revealed significant decline with time post-oophorectomy on key measures of verbal memory in the Rey Auditory Verbal Learning, Logical Memory, and Verbal Fluency tasks with a deleterious interaction effect of APOE4 on performance while controlling for effects of aging. Overall, this research supports a longitudinal decrement in verbal memory performance following oophorectomy, and furthers implications for the role of estrogen depletion in the development of cognitive decline and dementia.

BOP2M.001 TRIGLYCERIDES SENSING IN THE MESOLIMBIC SYSTEM AND THE REGULATION OF FOOD REWARD

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Circulating triglycerides (TGs) normally increase after a meal but are altered in pathophysiological conditions, such as obesity in both human and rodent. TG-hydrolyzing enzymes are expressed in the mesolimbic dopaminergic system suggesting that central TG-sensing might regulate dopamine neurons activity and ultimately reward-driven behaviour. Using brain-specific TG delivery (BTGD), we show nutritional TG access mesocorticolimbic (MCL) structures where they modulate dopamine (DA) neurons activity and signalling. Central TG sensing is mediated, at least in part, by the lipoprotein lipase (LPL) whose transcript is specifically found onto DA and medium Spiny Neuron (MSN) neurons. TG detected centrally create positive reinforcement in a conditioned place preference paradigm (liking) but lead to reduced motivation to work for reward (wanting) as assessed by both operant conditioning and self-administration. Finally, we find that TG action on reward seeking behaviour primarily rely on the indirect Dopamine receptor DRD2 pathway. Using functional magnetic resonance (fMRI) we found that in human, post-prandial TG excursions modulate brain response to food versus non-food cues. The response of the ventromedial prefrontal cortex (vmPFC) was specific to TG and independent of other energy-related signals. Finally, the action of TG onto brain response was driven by the genetic polymorphism TaqAI, a mutation known to affect D2DR signaling and susceptibility to addiction. Collectively, these findings reveal new mechanisms by which dietary TG alter mesolimbic circuit function and reward seeking behaviour, and provide a novel hypothesis by which energy-rich diet might lead to dopamine circuitry adaptation and ultimately addictive behaviour.

BOP2M.002 HYPOTHALAMIC NEURODEGENERATION AND INFLAMMATION IN PRADER-WILLI SYNDROME

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Prader-Willi syndrome (PWS) is a severe neurodevelopmental condition characterised by hypotonia and feeding difficulties at birth, followed by development of disordered eating behaviour, obesity, hypogonadism, growth retardation, learning difficulties and behavioural abnormalities in early childhood, largely due to hypothalamic dysfunction. Genetically, the condition is a result from loss of expression of paternally expressed genetic material from chromosome 15q11 region. Over the past decades many rodent models have been successfully created to study disease mechanisms; yet, none of these fully recapitulate the PWS-associated phenotypes, possibly due to inherent metabolic, as well as genetic differences between human and rodents. In order to understand the molecular events in the PWS, we have now analysed the gene expression and post-transcriptional events directly in the human hypothalamus. Using high-throughput RNA-sequencing of donated post mortem samples from patients and matched control samples, we uncovered a distinct genome-wide molecular PWS signature. Two striking features of the PWS transcriptomic profile, neurodegeneration and neuroinflammation, are of particular interest, as they offer a novel insight into the molecular and neurodevelopmental events affected in PWS brain. Specifically, we have observed early neuronal loss, with reduced expression of BDNF, as well as gliosis with particular increase of the expression microglia-specific genes in the patients. We have also observed abnormalities in the alternative splicing events as a further molecular mechanism underlying neuronal dysfunction in PWS.

BOP2M.003 NEUROENDOCRINE AND METABOLIC ADAPTATIONS TO CALORIE RESTRICTION AND EXERCISE

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Caloric restriction causes a homeostatic reduction in thermogenesis to impede weight loss. We aimed to determine whether exercise could overcome the inhibitory effect of food restriction on thermogenesis in ewes. We further sought to characterise the neuroendocrine and tissue-specific mechanisms that drive altered thermogenesis. Ewes (54.8 ± 0.96kg) were divided into 4 groups: sedentary fed *ad lib*, exercise fed *ad lib* (30 min/day, 5 days/week), sedentary food-restricted (30%) or combined exercise and food-restricted. Dataloggers continuously recorded temperature in adipose tissue (sternal and retroperitoneal) and skeletal muscle. After 4 weeks, combined diet and exercise reduced ($P<0.05$) adiposity. Calorie restriction decreased ($P<0.05$) overnight thermogenesis in adipose tissue only and this effect was counteracted ($P<0.05$) by regular exercise. There was no effect of exercise on markers of 'browning' or thermogenesis in either fat depot. At 4 weeks, animals were euthanised and hypothalami collected for *in situ* hybridisation. Expression of neuropeptide Y (NPY) and agouti-related protein (AgRP) were increased ($P<0.05$) in the combined diet/ exercise group, consistent with decreased adiposity. Expression of pro-opiomelanocortin mRNA was similar in all groups, but calorie restriction increased ($P<0.05$) the expression of melanocortin 4 receptor in the paraventricular nucleus. Exercise alone increased ($P<0.05$) orexin expression in the lateral hypothalamus, which was obviated by food restriction. In summary, we demonstrate a novel effect of exercise to

counteract the decrease in thermogenesis caused by food restriction. Despite this, altered thermogenesis did not coincide with the diet and exercise-induced changes in the expression of appetite-regulating peptides in the hypothalamus.

BOP2M.004 ASTROCYTIC UCP2 IS REQUIRED FOR HYPOTHALAMIC RESPONSE TO METABOLIC CHALLENGES

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Years of intense research dissecting how hypothalamus controls systemic metabolism in health and disease exclusively focused on neuronal networks. We recently discovered that astrocytes, and not only neurons, respond to hormones and nutrients and, in turn, cooperate with neurons to control energy homeostasis[1,2,3]. Mitochondrial uncoupling protein 2 (UCP2) was previously described as a key determinant of cellular energy metabolism in hypothalamic neurons and we sought to investigate if astrocytic UCP2 affects the control exerted by the brain over systemic energy balance. To that end, we generated an inducible astrocyte-specific UCP2 knock-out by crossing hGFAP (glial fibrillary acidic protein)-Cre^{ERT2} mice with $UCP2^{fl/fl}$ mice (GFAP-UCP2 KO mice). Mice lacking UCP2 in astrocytes exhibited hyperphagia on chow diet, which was associated with a dramatically reduced feeding response to orexigenic signals such as triiodothyronine (T3) or ghrelin. Interestingly, the lack of UCP2 in astrocytes worsens glucose intolerance, excessive calorie intake and body weight gain upon high-fat high-sugar diet. Overall these findings suggest that UCP2-dependent astrocytic metabolic processes play an important role for engaging appropriate hypothalamic responses to whole-body nutritional and endocrine status, presumably by modifying the activity of hypothalamic neurons involved in the regulation of feeding behavior. 1. Garcia-Caceres C. *et al.,* Cell 2016. 2. Kim JG. *et al.,* Nat Neurosci. 2014. 3. Horvath TL. *et al.,* PNAS. 2010.

BOP2M.005 THE 26RFA/GPR103 SYSTEM IS A KEY RELAY FOR INSULIN SIGNALING IN THE CENTRAL REGULATION OF GLYCAEMIA

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The neuropeptide 26RFa and its receptor, GPR103, form a hypothalamic system known to strongly stimulates food intake. We recently showed that this system regulates glucose homeostasis at the periphery and that 26RFa acts as an incretin. As it is now well accepted that the hypothalamus is also involved in the control of glucose homeostasis, we investigated whether 26RFa may play a role in the hypothalamic regulation of glycaemia. For this, we performed a glucose challenge concurrently with a central administration of 26RFa and we found that hypothalamic 26RFa exerts an anti-hyperglycemic effect similar to that observed peripherally. This is associated with an insulinotropic activity of the neuropeptide. This central anti-hyperglycemic effect is partially abolished by a central administration of a GPR103 antagonist. To understand how the 26RFa/GPR103 peptidergic system is involved in the central glycemic regulation, we examined whether the expression and the secretion of 26RFa by hypothalamic neurons may be regulated by factors known to control glucose homeostasis. Using mouse hypothalamic explants, we showed that insulin strongly stimulates 26RFa secretion by hypothalamic neurons. We also found that hypothalamic 26RFa-neurons express the insulin receptor, that insulin induced c-fos expression in these neurons and that the central anti-hyperglycemic effect of insulin is partially abolished by the GPR103 antagonist. Together, these data reveal, for the first time, that the hypothalamic 26RFa/GPR103 system plays a pivotal role in the hypothalamic regulation of glucose homeostasis and act as a relay of insulin signalization in the brain.

BOP2M.006 BPA, PALMITATE AND NITRIC OXIDE INDUCE SPEXIN, GALR2 AND GALR3 EXPRESSION IN HYPOTHALAMIC NEURONS

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Spexin (SPX) is a recently discovered peptide that binds to galanin receptors 2 (GalR2) and 3 (GalR3). Emerging evidence implicates SPX as a modulator of satiety and energy balance as circulating SPX levels appear to be dysregulated in obesity. It is currently unknown how SPX and its receptors are regulated in the hypothalamus, an area critical for energy homeostasis. We therefore examined hypothalamic SPX, GalR2 and GalR3 gene expression in response to several compounds in a clonal adult-derived hypothalamic cell model, mHypoA-59, using quantitative reverse transcription PCR (qRT-PCR). We hypothesized that SPX and its receptors would be regulated by bisphenol A (BPA), an endocrine disrupting chemical and obesogen; palmitate, a dietary saturated fatty acid; and PKA, PKC or PKG. SPX and GalR3 mRNA was maximally upregulated at 8 hours when treated with: 50 uM of palmitate, 100 uM of bisphenol A and 100 uM of sodium nitroprusside (SNP), a nitric oxide donor that can activate the PKG pathway. GalR2 mRNA was maximally upregulated at 8 hours when treated with 50 uM of palmitate and

100 uM of SNP. Conversely, 100 uM of BPA modestly downregulated GalR2 mRNA at 16 and 24 hours. These results suggest regulatory roles for BPA, palmitate, and nitric oxide in SPX, GalR2 and GalR3 gene expression. Elucidating the pathways that regulate gene expression of SPX and its receptors in the hypothalamus will contribute to current models of energy balance and provide insight into how components of this regulatory circuit may be dysregulated in obesity.

BOP2R.001 OPTOGENETIC STIMULATION OF KISSPEPTIN IN THE AMYGDALA INCREASES LH PULSE FREQUENCY IN FEMALE MICE

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Kisspeptin within the arcuate nucleus of the hypothalamus is a critical neuropeptide in the regulation of reproduction. Together with neurokinin B and dynorphin, hypothalamic kisspeptin provides the oscillatory activity that drives the pulsatile secretion of GnRH, and therefore LH, and is believed to be a central component of the GnRH pulse generator. It is well established that the amygdala also exerts an influence over gonadotropin secretion and reproductive physiology. The discovery of kisspeptin and its receptor within the posterodorsal medial amygdala (MePD), and our recent finding showing administration of kisspeptin or a kisspeptin receptor antagonist results in increased LH secretion and decreased LH pulse frequency, respectively, suggests an important role for amygdala kisspeptin in the regulation of the GnRH pulse generator. To further investigate the function of amygdala kisspeptin, the present study has used an optogenetic approach of stimulating MePD kisspeptin neurones followed by analysis of blood hormone levels. Kisspeptin neurones in conscious Kiss1-CRE mice, virally infected with a channelrhodopsin gene, received light of wavelength 473nm via an implanted fibre optic cannula for highly-selective stimulation. Frequent blood sampling showed that acute stimulation lasting 10 or 20 minutes with light at pulse frequency 10Hz and 20Hz was able to elicit an LH pulse. Moreover, continuous stimulation using 5Hz resulted in an increased LH pulse frequency. In wild-type animals, neither acute nor continuous stimulation influenced LH pulse secretion. These results demonstrate that selective stimulation of MePD kisspeptin can trigger the secretion of LH pulses and alter their frequency.

BOP2R.002 KISSPEPTIN ACTS ON BONE CELLS TO PREVENT OBESITY-INDUCED OSTEOPENIA

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Kisspeptin (Kp) plays a major role in luteinizing hormone (LH) control. Obesity is associated with reduced LH secretion and hypogonadism. We examined the effects of Kp on metabolic, hormonal, and bone phenotypes in high-fat diet (HFD)-induced obese rats. Weaning male rats were treated with HFD or standard diet (CT) *ad libitum* for 18 weeks. From the eighth week on, HFD rats received daily s.c. Kp-10 (HFD+Kp, 1 or 3 µg/rat/day; n = 12) or vehicle (HFD, n = 7). CT group received only vehicle (CT, n = 8). Blood samples were collected for hormonal and metabolic analyses. Femur and tibia were evaluated by micro-computed tomography. HFD rats displayed increased caloric intake, body weight, visceral adiposity, and glucose intolerance. All these responses were attenuated in HFD+Kp-10 rats. However, Kp-10 treatment was unable to prevent HFD-induced inhibition of LH pulses and did not change plasma testosterone or estradiol levels. In addition, HFD induced bone loss in the femur and tibia, which was fully prevented in HFD+Kp rats. Bone marrow cells (BMC) from CT and HFD rats were further investigated for *in vitro* differentiation into osteoclasts (OCL) and osteoblasts (OBL). OCL and OBL counts were increased in HFD rats compared to CT. Moreover, incubation with Kp-10 decreased OCL and further increased OBL differentiation in the BMC of HFD rats. The antagonist Kp-234 blocked these effects of Kp-10. Our findings provide evidence of a yet unrevealed direct osteoprotective role of Kp on bone cells, which can protect against obesity-induced bone loss. Support: CNPq, FAPEMIG.

BOP2R.003 NKB ANALOGS AND THEIR EFFECT ON REPRODUCTION IN TILAPIA.

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Neurokinin B (NKB) and its cognate receptor (NK3R) are emerging as important components of the neuroendocrine regulation of reproduction. Two putative tachykinin3 (tac3) homologs and two tac3 receptor genes were identified in the zebrafish genome. Unlike mammalian tac3, which encodes only one mature peptide (namely NKB), two mature peptides are predicted for each tac3 gene in zebrafish. All the piscine and frog tac3 sequences contained the second predicted peptide; therefore, it was designated as Neurokinin F (NKF). Hormone analogs with high and long-lasting biological activity are important tools for physiological and biological research, however, the availability of piscine-specific analogs is very limited. Such piscine-specific neuropeptide analogs that can enhance gonadotropin secretion will potentially serve as "next-generation" piscine spawning inducers. Therefore, we tested specific NKB and NKF analogs based on the structure of the mammalian NKB analog - Senktide. These analogs, specifically designed for longer half-lives by methylation of proteolysis sites, exhibited activity equal to those of the native NKB and NKF in signal-transduction assays of NKB receptors. The

analogs were found to be able to induce the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) in tilapia as fast as 1 hour after IP injection. The impact of the analogs on LH and FSH secretion lasted longer compared to the effect of native peptides and salmon GnRH analog (sGnRHa). These results suggest that novel synthetic NKB analogs are biologically active and may serve as a tool for both research and agricultural purposes.

BOP2R.004 RESCUE OF THE ACTIVITY OF MUTANT LH RECEPTORS IMPLICATED IN REPRODUCTIVE DYSFUNCTION

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G protein-coupled receptors (GPCRs) play a crucial role in neuroendocrine signalling and GPCR mutations have been implicated in many diseases. The majority of inactivating mutations cause receptor mis-folding, preventing translocation to their site of action at the cell membrane. Some cell-permeant ligands (pharmacoperones) have been shown to 'rescue' cell surface expression of such mutant GPCRs, presumably by stabilising correct folding of the nascent protein. Here we describe a pharmacoperone that can restore function of mutant luteinising hormone receptors (LHRs). Twenty mutant LHRs, reported as causing reproductive dysfunction in humans, were examined. Receptor expression/localisation (measured by ELISA), hormone binding affinity (measured by radioligand binding assay) and signalling competence (measured by $G\alpha_{16}$-linked inositol phosphate accumulation) was determined in order to establish the cause of mutant receptor non-functionality as loss of ligand binding, loss of signal transduction and/or loss of cell surface receptor expression. The ability of a small molecule LHR agonist (LHR-CHAP) to rescue the localisation and/or function of these mutant receptors was then examined. The majority (70%) of the mutant receptors were found to be poorly expressed at the cell surface, and LHR-CHAP was able to 'rescue' cell surface expression and hormone responsiveness of a subset of these. Furthermore, mutant receptors with normal cell surface expression, but no response to hormone stimulation (due to impaired hormone-binding or hormone-induced signalling ability) also elicited a robust response to allosteric stimulation by LHR-CHAP. In summary, we have identified a small-molecule that functionally rescues a number of intracellularly retained, hormone binding-deficient or signalling-deficient mutant LHRs.

BOP2R.005 SUPRACHIASMATIC NUCLEUS MONOSYNAPTIC INPUT TO THE ROSTRAL PERIVENTRICULAR AREA OF THE 3RD VENTRICLE

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In female rodents, the preovulatory surge in gonadotropin-releasing and luteinizing hormone secretion is controlled by the suprachiasmatic nucleus (SCN) – where the central circadian clock resides. Neurons within the rostral periventricular area of the third ventricle (RP3V), which are activated at the time of the surge, are estrogen-sensitive and receive projections from the SCN, are thought to play a prominent role in generating the surge by integrating humoral and circadian cues. To examine the functional impact of SCN projections to the RP3V, we targeted channelrhodopsin (ChR2) to the SCN of mice expressing Cre recombinase (Cre) in GABAergic neurons (vGAT-Cre mice), and interrogated this input in brain slices using patch-clamp electrophysiology. Unilateral stereotaxic injections of viral vectors carrying a Cre-dependent ChR2-mCherry construct successfully transfected SCN neurons in most animals. Blue-light stimulation reliably evoked action potential firing in mCherry-expressing SCN neurons (n=9 in 5 mice), confirming the functional expression of ChR2. In the RP3V, blue-light stimulation evoked inhibitory post-synaptic currents (IPSCs) in a majority of neurons (>60%). Blue-light evoked IPSCs were abolished by the GABA-A receptor antagonist gabazine (5 microM; n=6 in 5 mice), indicating that they resulted from the release of GABA. Evoked IPSCs were also inhibited by the sodium channel blocker TTX (0.5 microM) and subsequently restored by the addition of the potassium channel inhibitor 4-AP (100 microM; n=4 in 2 mice), suggesting these events could be accounted for by activation of a monosynaptic input. Together, these data indicate that GABAergic SCN neurons extend a monosynaptic input to RP3V neurons.

BOP2R.006 FERTILITY DEPENDS ON THE ACTIVIN TYPE II RECEPTORS, ACVR2A AND ACVR2B, IN PITUITARY GONADOTROPES

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Activins are selective regulators of follicle-stimulating hormone (FSH) production by pituitary gonadotrope cells. In an immortalized gonadotrope cell line, activins stimulate FSH via the type II receptors ACVR2A and/or BMPR2. *In vivo*, global *Acvr2a* knockout mice have ~50% lower serum FSH levels than controls. In contrast, gonadotrope-specific *Bmpr2* knockouts exhibit normal FSH. Another type II receptor, ACVR2B, can bind activins but appears dispensable for activin-stimulated FSH production *in vitro*. *Acvr2b* global knockout mice die soon after birth, precluding their use to assess ACVR2B's role in FSH production. In light of these previous results, we hypothesized that both ACVR2A and ACVR2B are required for normal FSH production *in vivo*. To investigate this idea, we crossed floxed *Acvr2a* or *Acvr2b* mice to GRIC mice, which express Cre recombinase specifically in gonadotropes. The resulting conditional knockout (cKO) animals were compared to their littermate controls.
Both *Acvr2a* and *Acvr2b* cKO females exhibited normal puberty onset (assessed by vaginal opening) and regular estrous cyclicity. However, when paired to wild-type males, *Acvr2a* and *Acvr2b* cKO females displayed ~70% and ~35% reductions in litter sizes.

Similarly, *Acvr2a* and *Acvr2b* cKO males exhibited ~50% and ~20% reductions in testicular weights. Both of these phenotypes are consistent with FSH deficiency. We are currently assessing production or secretion of the hormone in these animals and in a new model in which both receptors are deleted in combination. These data suggest that activins (or related TGFβ ligands) signal through both ACVR2A and ACVR2B in gonadotropes.

BOP2S.001 SALT LOADING INCREASES BRAIN DERIVED NEUROTROPHIC FACTOR IN SUPRAOPTIC VASOPRESSIN NEURONS

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Salt loading (SL) upregulates Brain Derived Neurotrophic Factor (BDNF). BDNF diminishes the $GABA_A$ inhibition in vasopressin neurons in the supraoptic nucleus (SON) by increasing intracellular chloride ([Cl]i) through tyrosine receptor kinase B (TrKB) phosphorylation. This produces sustained increase in arginine vasopressin (AVP) release. However, the source of BDNF is not known. We hypothesize that SON is the source of BDNF contributing to increased AVP release in SL rats. Adult male Sprague Dawley rats were bilaterally injected in the SON with AAV2- shRNA-BDNF-mCherry or AAV2- shRNA-SCR-mCherry and given either water or 2%NaCl to drink for 7 days. At the end of the protocol, SONs were collected by Laser Capture Microdissection for qRT-PCR and by punches for Western blot. Plasma osmolality, hematocrit and AVP concentration were measured. Other rats were injected in the SONs with rAAV2-0VP1-ClopHensorN for [Cl]i imaging. Their SONs were dissociated and tested with $GABA_A$agonist(Muscimol;100uM) to measure changes in [Cl]i and its flux. Data were analyzed by one-way ANOVA with Bonferroni comparisons. BDNF knockdown significantly blocked the increases in BDNF mRNA, AVP hnRNA and TrkB phosphorylation (all $P<0.05$;5-9) in SON of SL rats and prevented the increases in plasma AVP associated with SL ($P<0.05$;5-6). Muscimol application to SL SONs caused either significant increase in chloride efflux ($P<0.05$;6/10) or no change in chloride flux (4/10). TrKB antagonist (AnA,50uM) significantly blocked the Cl effluxes. Cells from control rats showed muscimol induced Cl influx ($P<0.05$;5/9). BDNF produced in the SON contributes to increased AVP and altered [Cl]i during SL. Supported by R01 HL11945

BOP2S.002 INHIBITION OF FKBP51 ALTERS ULTRADIAN AND STRESS-INDUCED CORTICOSTERONE SECRETION IN THE RAT

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The hypothalamic-pituitary-adrenal (HPA) axis regulates the release of glucocorticoids (CORT). CORT secretion is characterised by both circadian and ultradian rhythms which are strongly affected by age, gender, and disease states in the rat. The FK506 binding protein 51 (FKBP51) regulates the effects of CORT by inhibiting nuclear translocation of the glucocorticoid receptor (GR), thus affects the negative feedback of CORT release. Indeed, clinical studies have shown that FKBP5 gene polymorphism (i.e. overexpression) is associated with elevated levels of CORT in patients affected by psychiatric disorders including major depression. To further investigate the role of GR and FKBP51 in regulating ultradian rhythm of CORT, we used the FKBP51-specific antagonists SAFit2 (which has central and peripheral effects) and SAFit1 (peripheral effects only). Adult male Sprague-Dawley were given SAFit2 (20mg/kg, 09.00h and 17.00h, SC) or SAFit1 (20mg/kg, 09.00h, 14.00h and 19.00h, SC) for five consecutive days. The ultradian rhythmicity of CORT was assessed using an automated blood-sampling system, collecting blood every 10 minutes for 24 hours. A noise stress was used to investigate the effects of SAFit2 and SAFit1 on stress-induced CORT secretion. Both treatment with SAFit2 or SAFit1 decreased basal and stress-induced CORT concentrations. Furthermore, RTqPCR experiments showed enhanced GR-regulated gene expression in the hypothalamus of rats treated with SAFit2. Our data provide insights into the role of FKBP51 and GR in regulating CORT release, and suggest that inhibition of FKBP51 may represent a novel therapeutic approach for disorders associated with increased HPA axis activity.

BOP2S.003 EFFECTS OF ADOLESCENT SOCIAL STRESS ON SYNAPTIC PLASTICITY MARKERS AND NEURON MORPHOLOGY IN RATS

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Adolescent male rats exposed to social instability stress (SS; 1h isolation and return to an unfamiliar cagemate daily from postnatal day 30-45) have deficits in many social behaviours (reduced social interaction, impaired social recognition, reduced sexual performance in adulthood, increased aggression in adulthood) compared with non-stressed controls (CTL). In 2 experiments we investigated whether SS affects several markers of synaptic plasticity using western blotting (Expt. 1) and stellate neuron morphology using the Golgi-Cox method (Expt. 2) in the medial amygdala (MeA) and lateral septum (LS) (two brain regions with roles in social behaviour) on postnatal day 46. In Expt. 1, lower synaptophysin in the MeA ($p=0.03$) and greater CamkII in the LS ($p=0.027$) were found in SS rats compared with CTL rats. In Expt. 2, SS rats were found to have reduced dendritic arborisation ($p=0.023$) and reduced total length of dendrite matter ($p=0.026$) in the anteroventral subregion of the MeA, a greater number of dendrite terminals ($p=0.047$) and the highest dendrite branch order ($p=0.045$) in the anterodorsal MeA, and a reduced number of dendrite terminals ($p=0.03$) in the posterodorsal MeA compared with CTL rats. SS rats had reduced total dendritic spines in the lateral septum compared with CTL rats, but the effect did not meet statistical significance ($p=0.057$). Differences in markers of synaptic plasticity and in neural structure in the MeA and LS of CTL

and SS rats may underlie their differences in social behaviour. Our findings highlight the susceptibility of the MeA and LS to social stressors in adolescence.

BOP2S.004 THE ROLE OF RFRP NEURONS IN PUBERTY ONSET AND DEPRESSION

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RF-amide related-peptide (RFRP) neurons are thought to modulate reproductive function and stress responses Using transgenic mice which have stimulatory and inhibitory designer-receptors exclusively activated by designer-drugs (DREADDs) selectively expressed in RFRP neurons via a Cre-loxP system, we explored the reproductive and behavioural effects of RFRP neurons non-invasively in vivo. The role of RFRP neurons in puberty onset was investigated by stimulating and inhibiting RFRP neurons through administration of the DREADD ligand clozapine-n-oxide (CNO) from post-natal days 26-31 (~5mg/day p.o). Stimulation of RFRP neurons in male mice led to delayed preputial separation (stimulated mice: 31.7±0.8 vs controls: 29.3±0.3 days old; $P<0.05$) and inhibition of RFRP neurons led to a delay in age at first successful mating (inhibited mice: 50.6±2.8 vs controls: 47.6±1.4 days old; $P<0.05$). In females, there was no difference in puberty onset. The role of RFRP neurons in anxiety-like and depression-like behaviours was investigated in 8-week-old male mice following acute CNO administration (1 mg/kg s.c.). There were no changes in anxiety-like behaviours. There was, however, an increase in depression-like behaviour following stimulation of RFRP neurons. Stimulated mice spent more time immobilised (66.5±4.1%) than control mice (38.4±6.9%; $P<0.05$) in the last 2 minutes of the forced-swim test. This finding indicates a novel role for RFRP neurons in the control of depression-like behaviour in mice, and more behavioural testing will be conducted to elucidate this role. Characterizing the functions of RFRP neurons is an important step towards understanding their role and therapeutic potential in human infertility and mental illness.

BOP2S.005 TRIIODOTHYRONINE INTEGRATES NA+/K+ -ATPASE-DRIVEN NA+ SIGNALING DURING STRESS IN MICE BRAIN

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Thyroid hormones (TH) are critical for the differentiation and maintenance of brain functions. It is likely that triiodothyronine (T_3) could interact with cortisol, a prominent stress hormone that would modify the neuronal functions. Further, it is not certain how Na^+/K^+-ATPase, a ubiquitous

Na^+ transporter (NKA), responds to these hormones during stress or recovery response. We, thus, tested the action of T_3on (NKA) transporter functions and examined whether T_3 integrates Na^+ signaling during restraint-induced stress response in mice brain. Transcript analyses of neuronal-specific Atp1a1, Atp1a3 and Atp1b1 isoforms in cortex, hippocampus and cerebellum of eight weeks old mice brain showed differential regulation after T_3 challenge and that indicates a direct role of T_3 in Na^+ and Ca^{2+}signaling. These molecular markers that showed spatial and temporal distributions, produced a modified but integrated pattern in restraint-stressed mice brain, providing clues on the vital role of these transporters in neuronal functions during stress response. Molecular analysis of NKAα1 protein abundance, immunoprecipitation expression pattern, phosphorylation status and transporter kinetics in tested brain segments of restraint-stressed mice following T_3, supported an integrative role of T_3 in NKA transporter function. Taken together, these data provided evidence for a critical stress modifier role for T_3 in Na^+signaling in restraint-stressed mice brain (supported by grants by Higher Education Department of GoK for iCEIB project).

BOP2S.006 ADOLESCENT STRESS REPROGRAMS THE MEDIAL AMYGDALA TRANSCRIPTIOME AND SEX DIFFERENCES IN REWARD

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Adolescence, a time of heightened sensitivity to rewarding stimuli, is associated with vulnerability to psychiatric disorders. Male rodents that experience adolescent social isolation (SI) form stronger preferences for drugs of abuse but little is known about how females respond to SI. The medial amygdala (meAMY) is sexually dimorphic, develops during adolescence and is sensitive to SI. Our preliminary data suggest that SI reverses sex differences in reward behaviors and reduces baseline sex differences (M>F) in projections from meAMY to ventral tegmental area (VTA). Across adolescent development (postnatal day (P)22, P32, P42 & P72), SI females show a male-typical developmental pattern in corticosterone and progesterone is reduced in SI adults (M & F). Given these peripheral and behavioral alterations, we tested the hypothesis that SI alters the meAMY transcriptome in a persistent and sex-specific manner. Mice were isolated or group housed (GH) from P22 - P42, then GH until ~P90. Transcriptome-wide changes in meAMY were investigated by RNA-seq after cocaine (acute/chronic) or saline. Sexually dimorphic genes were disproportionately affected by SI (Sex X SI: 869 genes). Gene co-expression analysis revealed that SI results in the loss of sexually dimorphic gene co-expression and identified key drivers of sexually dimorphic expression. Together, these data suggest that

the meAMY plays an important role in sex differences in cocaine reward and SI disrupts sex-specific adolescent development of brain connectivity, transcription and endocrinology.

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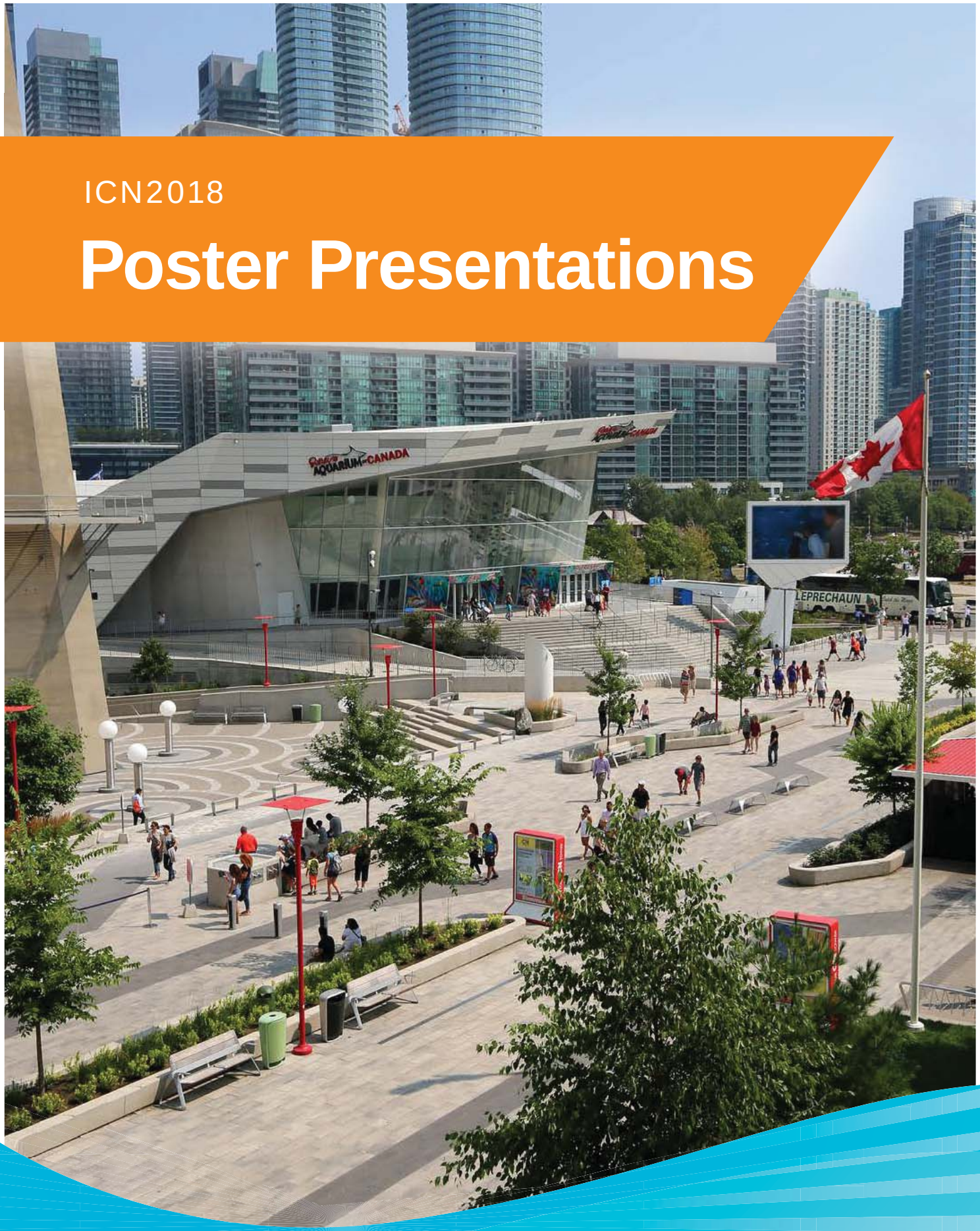
Poster Presentations



PS1.001 RECONSTRUCTING THE HYPOTHALAMO-NEUROHYPOPHYSIS CONNECTION BY VIRAL TRACING

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Virus-based tracing has become an important tool in dissecting neural circuits in the brain. It is well known that neurohypohysis (NH) receives projections from arginine vasopressin (AVP) and oxytocin (OXT)-containing magnocellular neurons within the paraventricular hypothalamic nucleus (PVN), supraoptic nucleus (SON) and accessory magnocellular nucleus (AN). However, a clear and comprehensive overview of the hypothalamus-NH connection remains unclear. We used both retrograde and anterograde virus to systematically map the connection. Injection of the retrograde-transported recombinant adeno-associated virus (retro-rAAV) into NH not only traced prominent projections from known PVN, SON and AN, but also revealed multiple previously unexpected areas in the hypothalamus. Anterograde viral tracing into these newly found areas verified their direct projection to NH. Systematically-administered fluorogold revealed the neuroendocrine features of these newly-traced neurons by projecting beyond the blood-brain barrier. Reconstructing the connections between the hypothalamus and neurohypophysis provides new insights into further understanding the regulation of the neuroendocrine system.

PS1.0010 ENHANCEMENT OF SEXUAL BEHAVIOR IN V1A AND V1B DOUBLE KO MALE MICE

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Many studies have investigated the regulatory roles of two types of vasopressin receptors, V1a and V1b, in various behaviors, such as anxiety-like behavior, social interaction, social recognition, pregnancy block (Bruce effect), and so on. However, it has been not known whether they have interactive (or synergistic) effects on behavior. In this study, we report the effect of v1a and v1b double knockout (dKO) on sexual and anxiety-like behaviors in male mice. C57BL/6 (wild-type, WT) and dKO mice were firstly subjected to weekly tests for olfactory preference (male odor vs. estrous female odor) and sexual behavior with receptive females in a large cage with enriched environment. Although no difference between them was found in olfactory preference for estrous odor, dKO males showed significantly greater number of mounts and pursuits to receptive females in the sexual behavior sessions. Secondly, they were tested for anxiety-like behavior in an elevated plus maze and an open field, suggesting the

lower level of anxiety-like behavior in dKO males than that of WT males. Finally, we examined approach behavior to various objects placed in the open field apparatus. While WT and dKO males spent equivalent time in exploration of non-biological objects, dKO males spent significantly longer time in the vicinity of estrous females than WT males. In summary, the combination of v1a and v1b deficiencies enhances sexual behavior under enriched environment, at least partly being contributed by the lowered emontionality.

PS1.00100 HYPOTHALAMIC BMAL1-KNOCKOUT CELL LINES FOR THE STUDY OF CIRCADIAN FEEDING REGULATION.

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Circadian rhythms drive the timing of daily physiological functions, including food intake. These rhythms depend on core clock genes, including BMAL1. Disruptions of the molecular clock in rodents have been shown to induce obesity and metabolic syndrome. We hypothesize that clock genes, specifically BMAL1, contributes to central energy homeostasis by altering the expression of feeding-related neuropeptides. We have previously demonstrated the rhythmic expression of neuropeptides in clonal neuropeptide Y (NPY)/agouti-related peptide (AgRP)-expressing cells. BMAL1 is hypothesized to play a role in neuropeptide gene expression; thus, hypothalamic cell lines were derived from adult BMAL1-wildtype or knockout (KO) C57BL/6J mice. Hypothalamic primary cultures were immortalized with SV40 T-antigen. Characterization of the mixed neuronal cell line demonstrated a robust expression of several neuropeptides, and the mixed line is currently being subcloned to obtain clonal neuronal cell lines. Our recent results show that bisphenol A (BPA), an endocrine disrupting chemical, alters proopiomelanocortin (POMC) and clock mRNA levels in POMC-expressing neurons. However, preliminary results indicate that BPA-mediated increases in POMC mRNA are not changed in BMAL1-KO cells, suggesting that BMAL1 may not be involved in the control of POMC by BPA. However, endogenous expression of NPY, AgRP, and POMC in the BMAL1-KO line appears to differ significantly from those in the wild-type cell line, suggesting a role for BMAL1 in regulating the basal expression of these neuropeptides. The newly-generated cell lines will serve as models of hypothalamic circadian disruption and will be valuable to define the roles of BMAL1 in specific hypothalamic neurons.

PS1.00101 HYPOTHALAMIC AND VAGAL SIGNALING IN OBESITY DEVELOPMENT IN BARDET-BIEDL SYNDROME

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Obesity results from dysregulated food intake. The brain, specifically the arcuate nucleus of hypothalamus receives and processes input from a number of peripheral orexigenic and anorexigenic signals to regulate food intake and energy expenditure. These signals include leptin, insulin, ghrelin, galanin, cholecystokinin (CCK) and PY that are projected to the second order neuron populations of the lateral and paraventricular nucleus of the hypothalamus. In addition to blood circulation, compelling evidence suggests, for gut hormones, there is also a vagal afferents route that modulates food intake. Complex genetic and/or environmental factors makes it difficult to unravel the aetiology of obesity. Therefore, models of monogenic obesity such as Bardet-Biedl syndrome(BBS) may help us identify the pathways associated with obesity and dis-regulated food intake and may have an impact on the understanding of the mechanisms of more common eating disorders. BBS is a ciliopathy where majority of the patients develop morbid obesity (BMI>35) from very early age and moreover, this phenotype is recapitulated in mice models. We performed RNASeq and bioinformatic analyses of *Bbs5* pre-obese (5 wks) and obese (12 wks) core hypothalamic tissues. Our results highlighted a number of dysregulated pathways associated with neurodevelopment and vagal afferent neuron signaling. For the first time, we have shown the presence of primary cilia on vagal nodose neurons and dis-regulation of cilia and other obesity related genes in *Bbs5*$^{-/-}$ nodose ganglia neurons. Our findings support the role of vagal afferent neuron signaling in developing obesity in Bbs using *Bbs*5 conditional and constitutive knockout models.

PS1.00102 TBX3 CONTROLS THE IDENTITY OF HYPOTHALAMIC NEURONS TO MAINTAIN ENERGY HOMEOSTASIS

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Neurons of the hypothalamus are crucial for the regulation of energy homeostasis. Within the arcuate nucleus, a balanced number of POMC and AgRP neurons allows fine tuning of energy intake and expenditure. However, the main molecular signals underlying the specification of hypothalamic cell identities and functions remain unknown. The transcription factor Tbx3 is implicated in the control of cellular differentiation and expressed in the hypothalamus.

Although haploinsufficiency of Tbx3 is associated with human obesity, the role of this factor in the context of systemic metabolism is unexplored. We investigated whether Tbx3 action in hypothalamic neurons may affect neuronal identity and energy homeostasis. Tbx3 hypothalamic expression was restricted to the arcuate nucleus as observed in our reporter mouse. Via the generation of several loss-of-function models, we highlighted a crucial role for Tbx3 in POMC-expressing neurons to maintain body weight and glucose homeostasis, in both constitutive and adult-onset models of Tbx3 deletion. Using lineage tracing, IP-mass-spectrometry, murine and human hypothalamic neuronal cultures, and RNA-sequencing, we demonstrated that these metabolic effects are achieved via the control of neuronal identity by Tbx3. Thus, Tbx3 signaling in the brain plays a key role in the maintenance of the identity of both immature and terminally differentiated hypothalamic POMC neurons, thereby promoting changes in energy balance and systemic glucose control. Our data represent a first step towards the identification of the molecular machinery controlling the maintenance of functional identities in hypothalamic neurons, which may have future therapeutic relevance for the pharmacological treatment of obesity and type-2 diabetes.

PS1.00103 KISSPEPTIN MODULATES B-CELL FUNCTION AND THE METABOLITE PROFILE IN HUMANS

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Background Neuroanatomical and hormonal mediators of the cross-talk between reproductive and metabolic systems are emerging. Kisspeptin is essential for reproductive function but animal data suggests it has additional roles in metabolism. Delineating the effects of kisspeptin on human metabolism may provide information about novel kisspeptin-mediated links between reproduction and metabolism. **Experimental Design/Methodology** Intravenous glucose tolerance tests (IVGTTs) and mixed meal tolerance tests (MMTTs) were performed in 15 healthy men (age 25±1y, BMI 22.3±0.5kg.m^{-2}); once during 1nmol.kg^{-1}.hr^{-1} kisspeptin infusion and once during rate-matched vehicle infusion. Blood samples were collected at baseline and during infusions (pre-glucose load/pre-meal) to determine if kisspeptin alters metabolite profiles.

Static incubation experiments were performed using human donor islet cells (n=4) to assess *in vitro* effects of kisspeptin on glucose stimulated insulin secretion (GSIS). **Results** During IVGTTs, kisspeptin infusion stimulated greater insulin secretion (mean serum insulin concentration kisspeptin minus vehicle: 4.1μU.mL^{-1} [95%CI: 0.9 to 7.3], p=0.01; disposition index: kisspeptin 2768±484 vs vehicle 2061±255, p<0.05). Kisspeptin produced dose-dependent increases in insulin secretion *in vitro* in human islet cells. Kisspeptin infusion resulted in changes in serum metabolites including lysophosphatidylinositol and sphingomyelins, which are associated with insulin dynamics. During MMTTs, glucose concentrations, insulin concentrations, disposition indices, appetite scores and food intake were similar during kisspeptin and vehicle infusions. **Conclusions** This is the first study in humans to examine the effects of kisspeptin on metabolism. We demonstrate that kisspeptin increases GSIS with metabolite changes suggesting novel kisspeptin-mediated connections between reproduction and metabolism, which have importance for the ongoing development of kisspeptin-based treatments.

PS1.00104 OLFACTORY RECEPTOR OR51E1 MEDIATES GLP-1 SECRETION IN HUMAN AND RODENT ENTEROENDOCRINE L CELLS

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Glucagon-like peptide-1 (GLP-1), produced in intestinal enteroendocrine L cells, is an important gut hormone in coordinating gastrointestinal physiology, metabolism and appetite. The present study aimed to investigate the role of the olfactory receptor (OR) OR51E1 in GLP-1 secretion. We verified the expression of olfactory marker protein (OMP), an indicator of OR-mediated events in non-olfactory systems, in human intestinal L cells. Furthermore, we analyzed OMP and OR51E1 expression in the human L cell line NCI-H716. To investigate whether odorant-activated OR signaling stimulates GLP-1 secretion, we employed nonanoic acid, a known OR51E1 ligand. Treatment with 100 μM nonanoic acid increased GLP-1 secretion by 2.09 ± 0.39 folds; however, this effect was attenuated on *OR51E1* knockdown. Oral administration of nonanoic acid to rats resulted in a 2.89 ± 0.53-fold increase in circulating GLP-1 levels and reductions in blood glucose levels compared to those in the control group. Our findings suggest that nonanoic acid stimulates GLP-1 secretion via OR51E1 signaling in intestinal L cells, thereby indicating a potential role of OR-mediated events and the corresponding odorants in GLP-1 secretion, which could be translated into a novel therapeutic approach in treating diabetes.

PS1.00105 EARLY LIFE EXPOSURE TO HIGH FAT DIET INDUCES WIDESPREAD CHANGES IN THE ADULT BRAIN

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About 2/3 of US women of reproductive age are overweight and 1/3 are obese. Human and animal studies show that maternal exposures to high-fat diets, and obesity, can induce long-term programming in offspring and are associated with neurological disorders, such as autism spectrum disorder. However, the effects of maternal *in utero* and perinatal exposures on adult brain structures have not been fully assessed. We acclimated C57BL/6 mice for 6 weeks with diets differing in fat percentage [control diet, 10 kcal % fat (CD); high-fat diet, 45 kcal % fat (HF45); and high-fat diet, 60 kcal % fat (HF60)] prior to breeding and maintained the diets throughout gestation and lactation. At weaning, all pups were switched to the CD until whole-brain magnetic resonance imaging (MRI) was performed in adulthood, day (D) D65. Offspring born from HF45 and HF60 diet weighed more at weaning than CD pups. Body weights converged among the male offspring by D35 and female offspring by D65; thus, at time of MRI, there were no differences in weights among all diets. Voxel-wise analysis of the effects of fat percentage on brain structure showed extensive changes in offspring from HF45 and HF60 diets compared to CD. Both relative increases (e.g, amygdala, hippocampus, and midbrain) and decreases (e.g. cerebellum, pons and medial preoptic area) were observed. Our findings demonstrate that early life exposure to high-fat diet can induce widespread changes in the adult brain. The potential link between these changes and the observed association with neurological disorders warrants further investigation.

PS1.00106 GLUCOKINASE WITHIN THE ARCUATE NUCLEUS REGULATES GLUCOSE HOMEOSTASIS

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Glucokinase is a key component of glucose sensing and is expressed in the arcuate nucleus. Evidence supports an important role for the brain in glucose homeostasis. Our novel data identifies a critical role for arcuate glucokinase within this. Using recombinant adeno-associated vector (rAAV) expressing either glucokinase or antisense glucokinase injected into the arcuate nucleus we are able to increase (iARC-sGK) and decrease glucokinase activity (iARC-asGK) specifically in the arcuate nucleus of male Wistar rats. During an oral glucose tolerance test increased glucokinase activity significantly improved glucose tolerance at 15minutes (7.43±0.23 mmol/L iARC-GFP vs 6.4±0.27 mmol/L iARC-sGK, $p<0.05$) and 30minutes (7.75±0.24 mmol/L

iARC-GFP vs 6.93±0.27mmol/L iARC-sGK, p<0.05). Insulin secretion was significantly increased at 15 minutes (2.68±0.38ng/ml iARC-GFP vs 3.94±0.33 ng/ml iARC-sGK, p<0.001) and 30 minutes (0.82±0.12ng/ml iARC-GFP vs 1.74±0.25 ng/ml iARC-sGK, p<0.05). Conversely, decreased glucokinase activity significantly worsened glucose tolerance at 15minutes (7.27±0.34mmol/L iARC-GFP vs 8.5±0.34mmol/L iARC-asGK, p<0.05). Insulin secretion was also significantly lower at 15 minutes (3.63±0.12ng/ml iARC-GFP vs 2.89±0.20 ng/ml iARC-asGK, p<0.05). Insulin sensitivity remained unchanged throughout. These results suggest an important role for arcuate nucleus glucokinase in glucose homeostasis and support the emerging evidence surrounding the brain's critical role in glucose processing. Type 2 diabetes is a growing epidemic yet its central pathophysiology is poorly understood. Understanding and targeting arcuate glucokinase and its downstream pathways may pave the way for developing centrally acting treatments for Type 2 Diabetes to improve patient morbidity and mortality.

PS1.00107 STRESS RESPONSE PATHWAYS REGULATE ISLET HOMEOSTASIS

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The islets of Langerhans contain several types of endocrine cells, and the functions of different islet cell types are precisely regulated by hormone and neurotransmitter. Somatostatin from pancreatic δ cells mediates important paracrine interactions in the islets, controlling the reciprocal insulin and glucagon release. Disruption of this islet circuit plays important roles in the development of diabetes. However, the mechanisms that control somatostatin synthesis and secretion from the islets remain elusive. Here, we found that the stress hormone adrenaline and the specific adrenergic agonists increased somatostatin content and transcription through b1-/b2- adrenergic receptors. We also found that the corticotropin-releasing hormone (CRH) family member Ucn3 epigentically regulates somatostatin secretion through Crhr2 and CRL4B/PRC2 complex. These regulations of pancreatic δ cells further affect insulin secretion and glucose metabolism. The paracrine interactions between pancreatic δ cells and β cells are critical to islet homeostasis and islet function in response to different physiological changes. Our results also reveal genetic and epigenetic mechanisms that modulate paracrine interactions between pancreatic δ and β cells.

PS1.00108 MEMBRANE ESTROGEN RECEPTORS ACTIVATE THE JAK-STAT PATHWAY IN THE HYPOTHALAMUS AND POMC NEURONS

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A growing literature supports involvement of membrane-bound estrogen receptors (mERs) in mediating estradiol's anorexigenic effect, and our group has begun to investigate the underlying intracellular signaling mechanisms. Here, we examined involvement of the JAK-STAT pathway, which is involved in mediating leptin's anorexigenic effect. In study 1, ovariectomized rats (n=8/group) received subcutaneous injections of G protein-coupled estrogen receptor 1 (GPER-1) agonist, G-1 (0.5µg), ERalpha agonist, PPT (50µg), or vehicle. Thirty min later, rats were euthanized and brain tissue was processed for expression of phosphorylated STAT3 (pSTAT3) protein via immunohistochemistry and Western blot analysis. ER agonist treatment increased the number of pSTAT3 immunolabeled cells in the arcuate nucleus of the hypothalamus (Arc) and the ratio of pSTAT3/STAT3 in Arc tissue, relative to vehicle treatment ($p<0.05$). In Study 2, we utilized a hypothalamic proopiomelanocortin (POMC) cell line to investigate the cell-specificity of estradiol-induced STAT3 phosphorylation. POMC neurons were treated with 10nM estradiol, 10nM PPT, 100nM G-1, or vehicle for 30 min. Following treatment, cells were either lysed for protein purification to enable pSTAT3 detection via western blot, or fixed with paraformaldehyde for immunofluorescent labeling of pSTAT3. ER agonist treatment increased pSTAT3 expression and nuclear translocation of pSTAT3 in POMC neurons. Our findings indicate that estradiol acts via GPER-1 and ERalpha to activate the JAK-STAT pathway in Arc tissue and POMC neurons over a time course in which mER agonists suppress feeding. Future studies will investigate the downstream consequences of activating the JAK-STAT pathway, including changes in Arc expression of anorexigenic and orexigenic genes.

PS1.00109 PHYSIOLOGICAL CONSEQUENCES OF SELECTIVE ABLATION OF BETA2 TANYCYTES IN ADULT MICE.

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Tanycytes, a specialized radial glial cell lining the third ventricular wall in the mediobasal hypothalamus, has been suspected as an important mediator between central and peripheral control of energy homeostasis. In particular, the beta subtype of tanycytes adjacent to the arcuate nucleus and median eminence (ArcN-ME) may directly sense or transfer metabolic signaling molecules through the CSF or fenestrated capillaries to regulate hypothalamic physiology. Despite steadily accumulating evidences of tanycytic capacities in this regard, still doubts remain over the real necessity and its extent in the adult mouse brain. Here, we report that tamoxifen-dependent expression of diphtheria toxin fragment A (DTA) under the control of *enolase2* (*Eno2*) mediated by tanycyte-specific Cre (Rax-CreER^{T2}) rapidly and effectively ablated beta2 tanycytes in adult animals. Without beta2 tanycytes, mediobasal hypothalamus was maintained intact, and distribution of the major neuronal populations in the ArcN was unaffected. A moderate decrease in the volume of ME was observed, along with the loss of ME neurons. Although tanycytes have been proposed to regulate energy metabolism, consistent

body weight and food intake differences were not observed in either male or female mutant mice. To confirm our current observations, insulin and leptin response, glucose homeostasis, body fat distribution will be presented. In addition, a more detailed characterization of physiological changes in the mutant mice will be discussed to further clarify function of beta2 tanycytes in the adult brain.

PS1.0011 SEX DIFFERENCES AND THE IMPACT OF ESTRADIOL IN THE FUNCTIONAL ROLE OF THE HIPPOCAMPUS IN THE RAT

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Studies in male animals demonstrate that the dorsal hippocampus (dHPC) is critical for spatial learning and memory, but not for object recognition memory (ORM). However, studies in female rodents suggest that 17β-estradiol (E2) modulates ORM via effects on the dHPC. One possible interpretation is that the dHPC plays a greater role in ORM in females than males. Sex differences are observed in the functional role of the entorhinal cortex in spatial memory and so perhaps similar differences exist in the role of the dHPC. Since E2 modulates spatial and object memory, a related question is whether elevated E2 levels increase the involvement of the dHPC in these abilities. The main goal of the present study is to investigate whether sex differences exist in the functional role of the dHPC in both spatial and object memory, and if so, whether E2 modulates this difference. Male (*n* = 16) and female (*n* = 32) Long Evans rats received either bi-lateral n-methyl d-aspartic acid (NMDA) lesions targeting the dHPC or sham lesions. All female rats also received bilateral ovariectomy (OVX), with half (*n* = 16) receiving E2 replacement (10 µg/kg, s.c.) within 24 h of testing. Rats were tested on two spatial memory tests (Morris watermaze and novel object-in-place preference test) and the novel-object preference (NOP) test. We observed no effect of sex, but some evidence of an effect of E2 on spatial and object memory.

PS1.00110 THE EFFECT AND MECHANISM OF SUBSTANCE P IN RAT PANCREATIC ISLETS

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Substance P (SP) is an important neuropeptide regulating pancreatic functions. Deficiency of SP release is closely linked to type I diabetes. However, the effect and mechanism of SP in pancreatic islets is still not clear. The aim of this study was to study the effect of SP in

pancreatic islets and investigate its mechanism. Islets were separated/collected from adult SD rat pancreases and then cultured for 3 days. SP at 10^{-9} – 10^{-6} M significantly enhanced insulin secretion at different levels of glucose. With SP (10^{-9} M), the insulin release was significantly increased (3.6 ± 0.2 μIU/IEQ , $p<0.05$) compared to the control (2.8 ± 0.3 μIU/IEQ) with 16.7 mM glucose. In islets treated with H_2O_2 (150 μM) to induce oxidative stress damage, SP also significantly enhanced insulin release ($P<0.05$) and significantly reduced the TUNEL positive cells ($P<0.05$). The increase of insulin by SP was totally abolished with NK-1 receptor blocker L703,606 (1 μM). Immunostaining for NK-1 receptor, glucagon and insulin indicated that NK-1 receptor was colocalized with α cells but not β cells in islets. SP failed to alter glucagon release from α cells but significantly increased GLP-1 release ($p<0.05$) compared to the control. L703,606 totally blocked the SP-induced additional release of GLP-1. Our results suggested that SP increased insulin release and protect β cells from oxidative stress damage through its action to NK-1 receptors on α cells stimulating GLP-1 release in rat islets. (supported by the National Natural Science Foundation of China , Grant No. 81270900, 81670492).

PS1.00111 BLOOD-BORNE LIRAGLUTIDE TRANSPORT INTO THE HYPOTHALAMUS: A TANYCYTIC ROUTE?

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Liraglutide is a GLP-1 analogue that mediates several energy balance regulation through the arcuate nucleus of the hypothalamus (ARH). In order to reach their neuronal targets, hormones like GLP-1 have to first pass the blood brain barrier (BBB) either at the level of BBB vessels or of circumventricular organs (CVO) such as the median eminence laying just adjacent to the ARH. The median eminence, contains specialized ependymoglial cells, called tanycytes. Tanycytes play an active role in shuttling circulating metabolic signals into the cerebrospinal fluid, which may then freely diffuse into the hypothalamus. It has been shown that liraglutide reaches different CVO in a GLP-1 receptor dependent manner. However, the cellular mechanism underlying its transport and the nature of the neuronal populations on which it exerts its action, remain unclear. We show that tanycytes are the first cells of the hypothalamus to sense liraglutide upon its intravenous (IV) injection. Liraglutide is indeed seen to induce CREB phosphorylation in tanycytes as early as 30-seconds after its injection in mice. Given the widespread expression of GLP-1Rs in tanycytes, it is conceivable that phosphorylation was mediated via its activation. CREB-phosphorylation was accompanied by rapid uptake in tanycytes as visualized using fluorescently-labeled liraglutide. Sixty seconds after injection, fluorescent-liraglutide was visualized in the ARH where it was found bound to POMC neurons.

At later time points, c-FOS activation in different areas including arcuate, ventromedial and dorsomedial hypothalamus was observed. Further studies are currently in progress to decipher the molecular mechanisms underlying transcytosis of liraglutide in tanycytes.

PS1.00112 LXR AGONISM AND PHOSPHOETHANOLAMINE: NEUROENDOCRINE IMPLICATIONS FOR MAJOR DEPRESSIVE DISORDER

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Plasma phosphoethanolamine (PEA) levels are significantly decreased in Japanese MDD patients compared to healthy controls. However, the neuroendocrine mechanism by which PEA changes *in vivo*; and its role in the pathobiology of MDD is not clearly understood. Previous *in vitro* studies reported that the endogenous LXR agonist 25-hydroxycholesterol increased PEA levels in cell lines. To find the role of PEA in the neuroendocrine circuitry of MDD, the *in vivo* effects of 25-hydroxylcholesterol on changes in plasma PEA, and in brain nuclei implicated in MDD were studied. Both targeted and untargeted metabolomics analysis using capillary electrophoresis-mass spectrometry were applied to measure plasma and brain tissue PEA and various metabolites in C57BL/6J mice. 25-hydroxycholesterol significantly decreased plasma and brain PEA levels in both male and female mice. Compared to vehicle control females, vehicle treated male mice had about 50% higher basal plasma PEA levels than females. Plasma PEA levels in both male and female mice decreased by about 25% from baseline levels after 25-OH-chol treatment. Both baseline and 25-OH-cholesterol treated levels of plasma PEA were gender dimorphic. Moreover, brain PEA levels were also significantly decreased in the 25-OH-chol treated male and female mice. 25-OH-chol caused significant changes in several metabolites in key brain areas of MDD. LXR agonism impacts PEA levels differently in cells and in the whole animal: Changes in plasma PEA levels are also reflected in brain nuclei implicated in the pathobiology of MDD. Further studies are underway to investigate and clarify the neuroendocrine mechanism of PEA in MDD.

PS1.00113 FINASTERIDE IMPAIRS MEMORY AND INCREASES HIPPOCAMPAL PATHOLOGY IN MALE 3XTG-AD MICE

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Women are at greater risk for developing Alzheimer's disease (AD) compared to men. This may be attributable to loss of neuroprotection when circulating ovarian steroids decline at menopause, while the age-related decline in testosterone levels in men is more gradual. Recent work has suggested that 5alpha-reduced metabolites of testosterone may contribute to the neuroprotection conferred by their parent androgen, as well as to sex differences in the incidence of AD. In this study, we investigated the effects of inhibiting synthesis of testosterone-derived neurosteroids on object recognition memory (ORM), hippocampal dendritic morphology, and pathological markers of AD in male triple transgenic AD mice (3xTg-AD). Male 6-month old wild-type (WT) or 3xTg-AD mice received daily injections of finasteride (5alpha-reductase inhibitor; 50mg/kg i.p) or vehicle (18% beta-cyclodextrin, 1% v/b.w.) for 20 days. Female WT and 3xTg-AD mice received vehicle injections only. Finasteride treatment in males differentially impaired ORM after short-term (3xTg-AD only) or long-term (3xTg-AD and WT) retention delays. Dendritic spine density (DSD) and dendritic branching of pyramidal neurons in the CA3 hippocampal subfield were lower in 3xTg-AD females than in males, while finasteride decreased DSD in both WT and 3xTg-AD males. Dendritic branching was significantly reduced by finasteride in 3xTg-AD males, abolishing the observed sex difference. Hippocampal amyloid beta deposition was substantially higher in 3xTg-AD females, but was unaffected by finasteride treatment of 3xTg-AD males. However, finasteride significantly increased tau hyperphosphorylation in 3xTg-AD males. These results suggest that 5alpha-reduced neurosteroids may contribute to sex differences in the development and severity of AD.

PS1.00114 LOSS OF THE IMPRINTED GENE NEURONATIN AFFECTS BOTH FOOD INTAKE AND ENERGY EXPENDITURE

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Neuronatin (*Nnat*) is an imprinted gene, expressed only from the paternal allele, and found within the brain, the pituitary gland, and a number of peripheral tissues such as adipose tissue and pancreas. A full understanding of its specific function remains elusive. We have previously shown that *Nnat* expression within the hypothalamic paraventricular nucleus is leptin-regulated and that mice carrying a paternally inherited null *Nnat* allele ($Nnat^{+/-p}$) display an unusual body weight distribution , with some phenotypically identical to wild type (WT) mice while others develop obesity. We have undertaken detailed metabolic phenotyping of $Nnat^{+/-p}$ mice to further understand the role of *Nnat* in energy homeostasis. In keeping with previous findings, on normal chow 15-20% of $Nnat^{+/-p}$ mice develop obesity by 12 weeks. $Nnat^{+/-p}$ mice are hyperphagic compared to WT, but intriguingly a proportion of *Nnat*-deficient mice that are weigh-identical to WT also show an increase in energy expenditure. Unsupervised multivariate analysis of energy phenotype data excluding body weight and genotype reveals 3 distinct clusters; WT, "lean $Nnat^{+/-p}$" and "obese $Nnat^{+/-p}$". When fed a 45% HFD, the between-genotype difference in body weight distribution persists with a proportion of $Nnat^{+/-p}$ also

remaining leaner than WT. These data indicate *Nnat* may play a crucial role in neuronal populations that control food intake and energy expenditure. Ongoing transcriptomic analysis of laser captured material from *Nnat* $^{+/-p}$ mice and functional analysis of hypothalamic *Nnat* +ve cells aims to further characterise the site of these actions.

PS1.00115 IS IRISIN A REGULATORY (F)ACTOR ON FOOD INTAKE?

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The hypothalamus is known as the most important center for control of feeding. Several hormonal factors play role in the regulation of this control mechanism. Irisin is a recently discovered myokine hormone, which secreted from skeletal muscle tissue during exercise. It has been reported that centrally irisin infusion can causes alterations in food consumption and body weight in rats. This study intends to elucidate possible effects of the irisin on feeding behavior. In this study, male Wistar-albino rats weighing 150-200 g were used. Primarily, the rats were divided into two main groups (n=63); first group was fed with standard-rat-chow (SRC) while second group was fed with high-fat-diet-chow (HFDC) for 12 weeks. At the end of this duration, development of obesity was corrected by measurement of the body weights and then each group was divided into 7 sub-groups (n=9). Two different doses of irisin were infused to all animals (except control and sham groups) as centrally and peripherally for 14 days. The mRNA gene expression/protein levels of UCP2, NPY and POMC in the hypothalamus tissue were determined. The central and peripheral administration of the irisin in both groups of animal fed with HFDC and SRC did not cause any change in food intake while irisin caused significant decreases in hypothalamic NPY, POMC and UCP2 gene expression/protein levels ($p<0.05$). Our results demonstrate that irisin plays a regulatory role on appetite by suppressing groups of neurons that secrete both orexigenic and anorexigenic neuropeptides. This study was supported by TUBITAK (Project no: 214S205)

PS1.00116 MATERNAL ANDROGENS AND OBESITY INDUCE SEXUALLY DIMORPHIC ANXIETY-LIKE BEHAVIOR IN THE OFFSPRING

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Maternal polycystic ovary syndrome (PCOS), a condition associated with androgen excess, is suggested to increase anxiety-like behavior in the offspring. Because PCOS is tightly linked to obesity, here we investigated the impact of an adverse hormonal and/or metabolic maternal environment and offspring obesity on anxiety in the offspring. The obese PCOS phenotype was induced by chronic high-fat/high-sucrose (HFHS) consumption in mice dams together with prenatal dihydrotestosterone exposure. Anxiety-like behavior was assessed in adult offspring with the elevated-plus maze and open field tests. The influence of maternal androgens, maternal and offspring diet on genes known to be implicated in anxiety in the amygdala and hypothalamus was analyzed with real-time PCR (n=47). Females exposed to maternal androgens were more anxious, independently of diet, and this was associated with upregulation of adrenoceptor alpha 1b in the amygdala and corticotropin-releasing hormone (*Crh*) in the hypothalamus. Moreover, the corticotropin-releasing hormone receptor 1 was downregulated due to DHT exposure and maternal HFHS-diet in the hypothalamus of the females. In contrast, males exposed to mothers fed with HFHS-diet had increased anxiety-like behavior and displayed upregulation in the expression of epigenetic markers in the amygdala along with upregulation of the hypothalamic *Crh*. Overall, there was a substantial sex difference in gene expression. In conclusion, maternal androgens and obesity exert sex-specific effects on behavior and gene expression in the amygdala and the hypothalamus. These findings provide novel insight into the mechanisms that lead to anxiety in the offspring of a PCOS mouse model.

PS1.00117 ROLE OF ESTRADIOL IN PROLACTIN-INDUCED SUPPRESSION OF LUTEINIZING HORMONE PULSATILE SECRETION

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Hyperprolactinemia causes infertility suppressing gonadotrophin-releasing hormone (GnRH) and luteinizing hormone (LH). Because prolactin (PRL) effects on the brain require estradiol (E_2), we investigated the role of E_2 in PRL-induced inhibition of LH pulses. Ovariectomized (OVX) rats treated with oil or E_2 (OVX+E_2) received a subcutaneous injection of ovine PRL (oPRL) 30 min before tail-tip blood collection for LH measurement by ultrasensitive enzyme-linked immunosorbent assay. E_2 reduced the frequency of LH pulses and mean LH levels in OVX+E_2 rats. The moderate dose of 0.5-mg/rat oPRL further reduced the frequency of LH pulses in OVX+E_2 but had no effect in OVX rats. The high dose of 2-mg/rat oPRL decreased pulse frequency equally in OVX+E_2 and OVX rats, whereas lowered pulse amplitude and mean LH levels only in OVX+E_2 rats. The effects of 2-mg/rat oPRL on kisspeptin neurons of the anteroventral periventricular (AVPV) and arcuate (ARC) nuclei were also investigated.

Kisspeptin immunoreactivity and *Kiss1* mRNA levels in the ARC were lower in OVX+E_2 compared with OVX rats. oPRL decreased both kisspeptin peptide and gene expression in the ARC of OVX rats whereas did not further reduce them in OVX+E_2 rats. In the AVPV, oPRL had no effect on kisspeptin immunoreactivity, although increased the stimulatory effect of E_2 on *Kiss1* mRNA levels. Additionally, *Gnrh* mRNA levels were suppressed by oPRL regardless of E_2. The present findings reveal that E_2 modulates the responsiveness of gonadal axis to PRL but is not essential to hyperprolactinemia-induced suppression of ARC kisspeptin and LH pulsatile secretion. Support: CNPq, FAPEMIG.

PS1.00118 ESTRADIOL FEEDBACK SHAPES POSTSYNAPTIC POTENTIALS IN ARCUATE KNDY NEURONS VIA K+ AND CA2+ CHANNELS

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Arcuate kisspeptin neurons help mediate estradiol feedback to GnRH neurons. We tested the hypothesis that estradiol feedback modifies postsynaptic potentials generated by synaptic conductances. We compared ovariectomized (OVX) and estradiol-treated (OVX+E) adult mice and performed recordings in the morning to focus on negative feedback. To examine postsynaptic potentials between experimental groups, we used dynamic clamp to deliver synaptic conductances modeled from either GABAA or AMPA postsynaptic currents to generate dynamic-clamp postsynaptic potentials (dcPSPs). Both GABA and glutamate are depolarizing from baseline potentials with reversal potentials of -55 and 0mV, respectively. Action potentials and receptors for fast synaptic transmission were blocked, and the same series of modeled conductances applied to each cell. The dcPSPs generated in response to the largest conductances were greater in the OVX+E group for both GABAA and AMPA models. Whole-cell potassium currents are smaller in cells from OVX+E mice; we thus tested the hypothesis that potassium currents modify the response to modeled synaptic conductances. The A-type potassium channel antagonist 4AP eliminated estradiol-dependent differences between groups. In half of cells from OVX+E mice, AMPA dcPSPs exhibited complex responses suggesting involvement of additional voltage-gated conductances. In these cells, blockade of T-type calcium channels with Ni^{2+} eliminated the complex component of dcPSPs, and reduced dcPSP amplitude and duration, with minimal effects on AMPA dcPSPs in cells from OVX mice or GABAA dcPSPs in either group. These results suggest estradiol feedback modulates voltage-gated potassium and calcium currents, increasing the response to synaptic input in arcuate kisspeptin neurons during negative feedback. NICHD41469

PS1.00119 CIRCADIAN RHYTHM SYNCHRONY IS REQUIRED FOR ESTROUS CYCLING AND FEMALE FERTILITY

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The suprachiasmatic nucleus (SCN), the master circadian pacemaker of the brain, transmits time-of-day information to the body, coordinating hormone release and maintaining fertility. However, technical challenges have limited scientific understanding of the neuronal network underlying SCN control of fertility. We here use synapsin-cre to delete the homeoproteins Ventral Anterior Homeobox 1 (VAX1) and SIX homeobox 3 (SIX3) in mature SCN neurons. VAX1 and SIX3 are two transcription factors required for SCN development, which maintain high expression in the adult SCN. Based on combined transcriptional analysis and immunohistochemistry, we found that VAX1 and SIX3 regulate expression of the SCN neuropeptides vasoactive intestinal polypeptide (VIP) and arginine vasopressin (AVP), both of which are important for SCN output. Indeed, both male and female Vax1-flox:synapsin-cre and Six3-flox:synapsin-cre mice had impaired circadian rhythms of wheel running activity in constant darkness, indicating weak SCN output. In females, but not males, deletion of Six3 or Vax1 with synapsin-cre resulted in prolonged estrous cycles, abnormal hormone release, and various degrees of subfertility or complete infertility. To determine whether the impaired fertility was due to misalignment of rhythms in the reproductive axis, we recorded Per2::luciferase rhythms in SCN and peripheral tissue explants as a direct measure of molecular circadian clock function. In Vax1-flox:synapsin-cre:Per2::luciferase mice, we found that circadian period was shorter than controls in the SCN, and also abnormal in peripheral reproductive tissues. These results suggest that in mice lacking functional Vax1, impaired SCN function disrupts circadian synchrony in reproductive tissues, impairing female but not male fertility.

PS1.0012 DEVELOPMENTAL ORIGINS OF VARIATION IN SOCIAL BEHAVIOR AND NEUROENDOCRINE FUNCTION

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Early-life social experiences shape adult behavior critical for evolutionary fitness and health, but fundamental questions remain about the behavioral and neuromolecular mechanisms underlying these effects. Social experiences vary across individuals, and accrued experiences can profoundly affect future behaviors, resulting in dramatically different developmental

trajectories. Such plasticity is especially likely in brain regions, neuromodulators, and gene networks that regulate adult social behavior. We use the highly-social African cichlid, *Astatotilapia burtoni*, to investigate juvenile social behavior, how it is shaped by early-life experience, and the underlying neuroendocrine mechanisms. First, juvenile behaviors appear largely similar to adults, but key differences reveal processes of behavioral development. The most striking difference relates to social status. Status forms when juveniles differ in size, but not when size-matched. Relative size not only affects the interacting pair, but also extends to other group members. Second, we show that juveniles are sensitive to multiple kinds of early-life experiences, including social and maternal effects. Juveniles reared in social groups are more active in open field and social cue investigation assays, and more interactive in a dominance assay, than juveniles reared in pairs. Rearing environment also significantly shifted neural gene expression networks of key neuroendocrine regulators of social behavior. Glucocorticoid and androgen receptor expression drives these differences. The effects of maternal brooding duration on juvenile behavior may be caused by similar neuroendocrine stress axis mechanisms. Together, this research demonstrates the important developmental origins of adult phenotypes and identifies factors contributing to social behavioral variation, which has consequences for fitness and health.

PS1.00120 SEXUAL DIFFERENTIATION OF A NOVEL FEMALE-BIASED SEXUALLY DIMORPHIC CELL GROUP IN MURINE BRAIN

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The principal nucleus of the bed nucleus of the stria terminalis (BNSTp) is a male-biased sexually dimorphic nucleus in the murine brain. The BNSTp contains numerous calbindin neurons, and the number of calbindin neurons is greater in males than in females, although details of BNSTp tissue structures are largely unknown. To evaluate BNSTp tissue structures of mice, we performed *in situ* hybridization for *p21 protein (Cdc42/Rac)-activated kinase 3* (*Pak3*) and immunohistochemistry for calbindin. Regions expressing *Pak3* and calbindin in the BNSTp were almost overlapped, but the ventral part of the BNSTp (BNSTpv) expressing *Pak3* had few calbindin neurons. Although the BNSTp showed male-biased sex differences in volume and neuron number, the BNSTpv exhibited female-biased sex differences in volume and neuron number. Next, we examined the effects of sex steroids during the neonatal and pubertal periods on sex differentiation of the BNSTpv. The volume and neuron number of the BNSTpv were increased in males by neonatal orchidectomy and decreased in females by neonatal treatment with testosterone, dihydrotestosterone or estradiol. Additionally, the volume and neuron number of the BNSTpv were decreased in females by prepubertal ovariectomy, and the effects of prepubertal ovariectomy were rescued by an estradiol treatment during the peripubertal period. These findings suggest that testicular testosterone during the postnatal period acts to defeminize the BNSTpv via binding directly to the androgen receptor and

indirectly to the estrogen receptor after aromatization. Ovarian estradiol during puberty may act to feminize the BNSTpv.

PS1.00121 AMH REGULATES DEVELOPMENT AND FUNCTION OF GNRH NEURONS AND IT IS MUTATED IN PATIENTS WITH CHH

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Gonadotropin releasing hormone (GnRH) neurons, critical for reproduction, originate in the olfactory placode and enter the brain along vomeronasal and terminal axons during embryonic development. Alterations either in the development of this system or in the secretion of GnRH are associated with congenital hypogonadotropic hypogonadism (CHH) in humans, a condition characterized by failure of sexual competence. Kallmann syndrome (KS) associates congenital hypogonadism due to GnRH deficiency and anosmia. Here, we report that Anti-Müllerian Hormone (AMH), a gonadal factor essential for male sexual differentiation, is expressed along the GnRH migratory pathway in mouse embryos and that it regulates GnRH cell motility through Amhr2/Bmpr1b signalling. Pathohistological analysis of *Amhr2*$^{-/-}$ mice revealed defective embryonic migration of GnRH cells, which results in a significant reduction of the GnRH population size in adulthood. *In utero* administration of an Amhr2-blocking-function antibody into the olfactory placodes recapitulates the GnRH migratory defects observed in the *Amhr2* deficient mice. Whole-exome sequencing of a cohort of 180 CHH probands, including 105 KS, identified several heterozygous missense mutations in *AMH* and *AMHR2*. Finally, *in vitro* functional validations of these variants indicate that they might have a pathogenic effect. These results uncovered a novel role for AMH in the correct development and function of GnRH neurons and suggest that AMH signaling insufficiency might be associated to CHH in humans.

PS1.00122 KISSPEPTIN AND RFRP3 MODULATE BODY MASS IN THE PHODOPUS SUNGORUS VIA DIFFERENT HYPOTHALAMIC PATHWAYS

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Seasonal species adapt their reproduction and metabolism so that offspring is born in the time of the year when resources are highly available in nature. *Phodopus sungorus* is known to inhibit its reproduction and to reduce its body fat mass when the photoperiod shortens in autumn. Hypothalamic peptides involved in the central control of reproduction, kisspeptin and RFRP3, and metabolism, POMC and somatostatin, are strongly regulated according to short winter-like(SD) or long summer-like (LD) day lengths. To investigate how these metabolic and reproductive hypothalamic networks interact with each other in the seasonal physiology, we performed a chronic central administration of either kisspeptin or RFRP3 in male and female hamsters adapted to the photoinhibitory-SD conditions. We found that kisspeptin restores reproductive activity in both males and females and increases bodyweight only in males. Notably, the metabolic effect of kisspeptin is abolished in castrated animals. RFRP3 does not restore male and female reproduction and only in males it increases bodyweight as well as circulating levels of leptin and insulin. In the hypothalamus of treated males, kisspeptin upregulated both NPY and POMC mRNA whereas RFRP3 targets are still unknown since we found no effect on NPY, POMC, somatostatin and orexin mRNA. We conclude that kisspeptin and RFRP3 are involved in the seasonal control of not only reproduction, but also bodyweight in a sex-dependent manner. Further, our data indicate that both peptides use different metabolic pathways with kisspeptin effects being testosterone-dependent and RFRP3 acting through a pathway probably involving the control of adiposity.

PS1.00123 GHRELIN REGULATES REPRODUCTIVE DEVELOPMENT

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A hunger-stimulating hormone ghrelin exists in circulation in two main forms, as des-acyl ghrelin (DAG) and acyl ghrelin (AG). AG is formed via posttranslational octanoylation by the enzyme ghrelin-O-acyltransferase (GOAT). Through its interaction with the growth hormone secretagogue receptor (GHSR), AG plays a role in the regulation of metabolism, cardiovascular function, stress, reproduction and other biological functions. The functional role of DAG, however, has been largely unexplored. While DAG does not act at the GHSR, it is known to inhibit the effects of AG and we have recently shown its independent role in regulating stress responsivity. Here, we used GOAT KO mice that have no AG and chronically high levels of DAG, to begin and elucidate the involvement of DAG in regulating reproduction, as well as the necessity of AG for optimal reproductive function. Our findings show that GOAT KO mice have persistent depletion of ovarian follicles and long-term changes in the ovarian transcriptome. These changes are not reflected in their reproductive capacity, and these mice breed normally under optimal animal husbandry. These novel data suggest that while AG is critical for normal ovarian development it is not essential for reproduction, at least under non-stressed conditions. Circulating AG and DAG levels are modulated by metabolic conditions, and some of

these, including obesity, are known to underlie reproductive dysfunction and infertility. Our findings may thus have important and encouraging implications for future research targeting obesity-related reproductive dysfunction.

PS1.00124 IN NON-BREEDING EWES THE KISSPEPTIN ANALOG C6 TRIGGERS OVULATION WITHOUT PROGESTOGEN PRIMING

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The kisspeptin (Kp) system governs the reproductive timeline of mammals. In the ewe Kp infusion triggers ovulation in the absence of progestogen priming. Infusion, however, is not suitable for field applications. Hence, we created a Kp analog (C6) with improved pharmacological features. In the ewe (Ile de France breed) during the non-breeding season and in the absence of progesterone priming, a single injection of C6 (1, 5, 15, 45 or 100 nmol/ewe, N=6 per group) increased LH but did not induce ovulation. Next, we investigated the effect of multiple injections. Ewes (N=6 per group) were injected intramuscularly thrice with C6 (15 nmol/ewe) at either 36 (group 1) or 48 (group 2) hour-intervals. After the first injection LH plasma concentration increased above 10 ng mL^{-1} in both groups. Subsequent injections, regardless to the groups, had minor if any effect on LH plasma concentration. However, an increase of progesterone above the 1 ng mL^{-1} threshold, a biomarker of ovulation and *corpora lutea*formation, was observed in 3 out of 6 (group 1) and in 4 out of 6 (group 2) ewes. Ovulation occurred 2 to 4 days after the LH surge induced by the first injection, suggesting a possible additional effect of C6 on ovaries. Experiments are ongoing to characterize further the neuroendocrine responses triggered by this dosing regimen. Nonetheless, these results suggest that kisspeptin analogs could substitute hormonal treatments to induce ovulation and to manage livestock reproduction during the non-breeding season. Supported by Région Centre-Val de Loire (Capriss) and ANR-15-CE20-0015-01 grants.

PS1.00125 THE RESPONSE TO SENKTIDE ADMINISTRATION IN THE RETROCHIASMATIC AREA IS SEXUALLY DIMORPHIC IN LAMBS

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Neurokinin B (NKB) is critical for fertility in humans and stimulates GnRH/LH secretion in humans, primates, and sheep. In sheep, NKB actions in the retrochiasmatic area (RCh)

contribute to the induction of the preovulatory LH surge. Because the LH surge only occurs in female sheep, we tested if the response to NKB receptor agonist, senktide, in the RCh is sexually dimorphic. To normalize steroid milieu, age-matched, yearling wethers (rams castrated at a young age) and ovariectomized ewes (10 – 11 months) with chronic guide tubes in the RCh were treated with estradiol and progesterone (CIDR) implants that mimicked luteal phase concentrations of these steroids. After two artificial luteal phases, CIDRs were removed and the next day, blood samples were collected from 36 min before to 8 hrs after insertion of bilateral empty (control) or senktide-containing microimplants. Microimplants were then removed, CIDRs reinserted, and the protocol was repeated using a cross-over design two weeks later. As seen previously in adults, senktide microimplants in the RCh of female lambs significantly ($P < 0.02$) increased mean LH concentrations from 2.97 ± 0.99 ng/mL before to 8.15 ± 1.81 ng/mL during treatment. In contrast, senktide microimplants had no effect in male lambs (pre-treatment: 20.2 ± 3.0 ng/mL; during treatment: 28.0 ± 5.0 ng/mL, $P = 0.20$). These data demonstrate that senktide acts in the RCh to stimulates LH secretion only in females, and raise the possibility of sex differences in the RCh that contribute to the sexual dimorphism of the estrogen induced-LH surge in sheep.

PS1.00126 ESTROGEN SIGNALING IN GHRH CELLS IS REQUIRED FOR NORMAL GROWTH AND PUBERTY IN FEMALE MICE

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Growth hormone (GH) secretion, regulated by GH-releasing hormone (GHRH), is also modulated by sex steroids, with higher GH circulating levels at birth and puberty. It has been described that GHRH-containing cells in the hypothalamus express the estrogen receptor alpha (ERα) mRNA, suggesting a direct effect of estrogens on GH secretion through GHRH neurons. Recently, a mouse model expressing Cre-recombinase driven by GHRH promoter has been generated. Exploration of the distribution of GHRH-GFP immunoreactivity and GHRH mRNA expression in the hypothalamus of young adult female mice showed cells distributed in the preoptic area (POA), paraventricular, periventricular, dorsomedial (DMH) and arcuate (Arc) nuclei. To evaluate the role of GHRH in the female reproductive physiology and its modulation by estrogens, a mouse model with specific deletion of ERα in GHRH-Cre expressing cells (GHRH-ERKO) was generated. High co-expression of GHRH-GFP and ERα immunoreactivity was located in Arc, with more than 80% of GHRH-GFP positive cells expressing the ERα, while a low co-localization was identified in POA and DMH. Phenotypically, GHRH-ERKO females displayed a reduced body weight at 5 weeks-old and they were shorter at adult age compared to controls. Timing of onset of puberty (day of vaginal opening) was similar in both groups, but pubertal completion was delayed in GHRH-ERKO females. Collectively, our data show the requirement of estrogen signaling directly in GHRH cells for normal growth and sexual maturation, and highlights the close relationship between growth and reproductive axes during the establishment of reproductive function.

PS1.00127 THE NEUROANATOMICAL RELATIONSHIP OF NNOS TO KISSPEPTIN AND GNRH IN ADULT FEMALE SHEEP AND PRIMATES

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Neuronal intermediates that communicate steroid feedback to gonadotropin-releasing hormone (GnRH) neurons are essential for reproductive cyclicity. Individually, kisspeptin and nitric oxide (NO) influence GnRH secretion, but these two neuronal intermediates may also interact to affect reproduction. Thus, we investigated the neuroanatomical relationship of neuronal nitric oxide synthase (nNOS) to kisspeptin or GnRH in adult female rhesus monkeys and sheep. Additionally, we evaluated if the phase of the reproductive cycle would affect these relationships. However, no effect of stage of cycle was observed for any variable in this study. In the arcuate nucleus (ARC) of sheep, 98.8±3.5% of kisspeptin neurons colocalized with nNOS, and kisspeptin close-contacts were observed onto nNOS neurons. In contrast to ewes, no colocalization was observed between kisspeptin and nNOS in the ARC of primates, but kisspeptin close-contacts were apposed to nNOS neurons. In the preoptic area (POA) of ewes, 15.0±4.2% of GnRH neurons colocalized with nNOS. In primates, 38.8±10.1% of GnRH neurons in the MBH colocalized with nNOS, and GnRH close-contacts were observed onto nNOS neurons in both sheep and primates. Thus, although species differences were observed, a neuroanatomical relationship exists between nNOS and kisspeptin and nNOS and GnRH in primates and sheep, and NO may act either directly or indirectly to affect GnRH secretion in these species.

PS1.00128 ACTIVATION OF CRH RECEPTOR TYPE-1, BUT NOT TYPE-2, INCREASES GABAERGIC TRANSMISSION TO GNRH NEURONS

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GnRH neurons integrate multiple inputs to regulate reproduction. CRH, released during stress, alters GnRH neuron activity in the presence of circulating estradiol and in a CRHR-receptor-dependent manner; activation of CRHR-1 stimulates, whereas activation of CRHR-2 suppresses GnRH neuron firing activity. The neurobiological mechanisms of how CRH alters GnRH neuron activity are not fully understood. GABAergic fast synaptic transmission is a major input to GnRH neurons. We hypothesized that activation of CRHR-1 and CRHR-2 differentially regulates GABAergic transmission to GnRH neurons. Mice were ovariectomized and implanted with a capsule producing a physiological level of estradiol. Whole-cell voltage-clamp recordings of GABAergic postsynaptic currents (PSCs) were made during a control period, followed by bath application of either the CRHR-1-specific agonist stressin I or the CRHR-2-specific agonist urocortin III (10nM each), and wash out periods. Stressin I increased PSC frequency in GnRH neurons (control 0.4±0.2 Hz, stressin 0.6±0.2 Hz, wash 0.5±0.2 Hz, n=7, $p<0.05$). GABA can be

excitatory to GnRH neurons; therefore, this result supports the finding that activation of CRHR-1 leads to stimulation of GnRH neuron activity. In contrast, urocortin III did not affect PSC frequency in GnRH neurons (control 0.5±0.1 Hz, urocortin III 0.5±0.1 Hz, wash 0.4±0.1 Hz, n=7, p>0.5). This suggests that urocortin III acts via other mechanisms such as direct action on GnRH neurons or via other upstream neuromodulators. Neither stressin I nor urocortin III altered any other PSC property. These results imply that CRH acts via different neurobiological mechanisms to exert stimulatory and inhibitory effects on GnRH neurons. NIH-R01HD41469

PS1.00129 ELEVATED FIRING RATE IN GNRH NEURONS IN PRENATALLY ANDROGENIZED MICE IS OVARY-DEPENDENT

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GnRH secretion drives the downstream reproductive axis, including gonadotropin release and ovarian steroidogenesis. In the common fertility disorder polycystic-ovary syndrome (PCOS), both ovarian and neuroendocrine defects are present. Prenatally androgenized (PNA) mice exhibit elevated LH pulse frequency and testosterone, similar to women with PCOS, and increased GnRH neuron firing and excitatory GABAergic transmission to GnRH neurons. Changes in these latter parameters occur before puberty, with increased transmission and reduced firing evident at three weeks of age. In control female mice, mild (sub-male) elevation of androgens increases both GnRH neuron firing rate and GABA transmission, similar to that observed in adult PNA mice. We hypothesized that increased GnRH neuron activity of adult PNA mice was due to dysregulated ovarian feedback and that removal of ovarian inputs would reduce GnRH activity levels to that observed in control mice during diestrus. PNA mice were generated by injecting dams with 225µg dihydrotestosterone on days 16-18 of gestation. To test if GnRH neuron firing is elevated by ovarian factors in PNA mice, extracellular recordings (1hr duration) were made from GFP-identified GnRH neurons in acute brain slices 5-7 days post ovariectomy (OVX). Firing rate in PNA-OVX mice is lower than previous recordings in adult PNA females ($p<0.01$) but did not differ from adult ovary-intact diestrous controls (n=7-11 cells/group, one-way ANOVA/Tukey's post hoc). This preliminary finding suggests a role for ovarian factors, likely androgens, in elevating GnRH neuron firing in adult PNA mice. NIH-HDP5028934

PS1.0013 MATERNALLY INVOLVED GALANIN NEURONS IN THE PREOPTIC AREA AND THEIR INPUTS IN THE RAT

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Recent selective stimulation and ablation of galanin neurons in the preoptic area of the hypothalamus established their critical role in control of maternal behaviors. Here, we identified a group of galanin neurons in the anterior commissural nucleus (ACN), and a distinct group in the medial preoptic area (MPA). Galanin neurons in ACN but not the MPA co-expressed oxytocin. We used immunodetection of phosphorylated STAT5 (pSTAT5) involved in prolactin receptor signal transduction to evaluate the effects of suckling-induced prolactin release and found that 76 % of galanin cells in ACN but only 12% in MPA were prolactin-responsive. In turn, nerve terminals containing tuberoinfundibular peptide 39 (TIP39), a neuropeptide we showed is induced in mothers and mediates the effects of suckling on maternal motivation via thalamo-hypothalamic projections, were abundant around galanin neurons in both preoptic regions. Perisomatic innervation of galanin neurons was demonstrated using correlated light and electron microscopy. The connection was excitatory based on glutamate content of TIP39 terminals demonstrated by post-embedding immunogold electron microscopy. Injection of the anterograde tracer biotinylated dextrane amine into the TIP39-expressing posterior intralaminar complex of the thalamus (PIL) demonstrated that preoptic TIP39 fibers originate in PIL activated by suckling. Thus, galanin neurons in the preoptic area of mother rats are innervated by an excitatory neuronal pathway that conveys suckling-related information. In turn, they can be topographically and neurochemically divided into 2 distinct cell groups, of which only one is affected by prolactin. Support: NKFIH KTIA_NAP_13-2-2015-0003, NKFIH-4300-1/2017-NKP_17, OTKA K116538 research grants.

PS1.00130 MORPHOLOGICAL PLASTICITY OF HYPOTHALAMIC DOPAMINERGIC NEURONS DURING ESTROUS CYCLE AND LACTATION

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The hypothalamic tuberoinfundibular dopaminergic (TIDA) neurons play a key role in regulating prolactin secretion and are known to exhibit remarkable plasticity during lactation, switching from dopamine to enkephalin release. This study investigates if this functional plasticity is accompanied by morphological changes. The arcuate nuclei of adult female, tyrosine hydroxylase (TH):Cre, rats were unilaterally injected with Cre-dependent AAV expressing Brainbow markers. Two weeks later Brainbow expression was compared between reproductive stages. The effect of 17β-estradiol (E_2) on ovarectomized animals was examined in a separate cohort. Immunohistochemistry revealed that approximately 90% of Brainbow-expressing cells were TH-positive with about 50% of the TIDA neurons transfected. This moderate transfection efficiency facilitated morphological examination of individual TIDA neurons. Neuronal spine density was measured at the cell soma and proximal dendrite (30-60 μm) of 5 animals at each stage (total of 73 - 93 cells per group). Dendritic spine density remained constant, but soma

density decreased towards estrous, falling from 0.088 ± 0.006 spines/µm at diestrus to 0.059 ± 0.004 by estrous (*P<0.001*). A role for estrogens was suggested by the finding that E2 treatment of ovarectomized animals caused a decline in somatic spine density (from 0.140 ± 0.011 spines/µm to 0.099 ± 0.012, *P<0.05*). Interestingly, somatic spine density was also elevated during lactation, being significantly greater than that during proestrous and estrous. These data revealed that TIDA neurons undergo morphological plasticity across the reproductive cycle. Intriguingly, the rise in somatic spine density during lactation is suggestive of increased TIDA neuronal activity at this time.

PS1.00131 CHANGING DYNAMICS OF PROLACTIN AND LEPTIN TRANSPORT INTO THE BRAIN DURING PREGNANCY AND WITH AGING

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The blood brain barrier (BBB) modulates the ability of large circulating hormones to access the brain, and represents an important regulatory site in the control of hormonal action in the brain. During pregnancy, mothers undergo numerous metabolic changes, including altered responses to leptin and prolactin but the mechanism by which the brain changes sensitivity to these hormones is unclear. We investigated the transport of ^{125}I-labelled prolactin (^{125}I-prolactin) and leptin (^{125}I-leptin) into the brain of female mice. Co-administration of unlabeled leptin with ^{125}I-prolactin in virgin mice revealed that leptin competes with prolactin transport, while unlabeled prolactin failed to impair leptin transport into the brain. During pregnancy, serum leptin and placental lactogen (a prolactin analogue) concentrations are chronically elevated, however, there was no change in ^{125}I-prolactin transport into the brain during pregnancy. Surprisingly during pregnancy, leptin clearance from the blood and transport into the brain was completely suppressed, indicating a different regulatory transport mechanism for these two hormones. We also investigated changes in prolactin transport into the aged brain of male mice. The emergence of hyperprolactinaemia with aging suggests that prolactin regulation of the arcuate nucleus may be disrupted. We found that prolactin is transported into the brain more efficiently in young 3 month-old male compared to aged-matched female mice. In aged 22-24 month-old mice, however, prolactin transport into the brain was reduced. These data demonstrate how changes in hormone action in the may be closely related to a change in the ability of these hormones to access the brain.

PS1.00132 INSULIN-LIKE GROWTH FACTOR 2, A PROLACTIN-RESPONSIVE GENE IN THE ADULT MOUSE CHOROID PLEXUS

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Prolactin receptors (PRLR) are highly expressed in the choroid plexus and up regulated during lactation. Previously PRLR in choroid plexus were thought to provide receptor-mediated transport of prolactin from the blood into cerebrospinal fluid but recently we demonstrated that PRLR are not required for prolactin transport across this blood-brain barrier. Consequently, the function of PRLR in choroid plexus is unknown. To gain insight into what role PRLR might have we used RNA-seq and Nanostring techniques to characterise transcriptional changes in response to different levels of prolactin. For RNA-seq we included adult female mice in, diestrus, diestrus with prolactin (5 mg/kg/i.p. given 4 hours before sacrifice); lactation (days 7-10, with pups continuously suckling) and lactation (days 7-10) treated with bromocriptine (5 mg/kg/s.c., two doses 18 and 4 hours before sacrifice, pups remained suckling but prolactin secretion and milk production were suppressed). Total RNA was extracted from choroid plexus and enriched for mRNA that was used as template for cDNA library synthesis. Barcoded cDNA libraries were pooled and run on the Ion Proton sequencing platform. Sequence reads were analysed through the Cufflinks pipeline. Transcripts of interest were validated and further characterised using Nanostring in choroid plexus tissue collected from mice in early and late pregnancy and during lactation (days 7-10). Insulin-like growth factor 2 (*Igf2*), the third highest expressed gene in the choroid plexus during lactation showed a 6-fold increase at lactation that returned back to baseline on suppression of prolactin. Thus prolactin may markedly elevate *Igf2* in cerebrospinal fluid.

PS1.00133 OPTOGENETIC STIMULATION OF KNDY NEURONES AND MATHEMATICAL MODELLING OF THE GNRH PULSE GENERATOR

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Gonadotrophin-releasing hormone (GnRH) is secreted in a pulsatile manner under the control of the neural oscillator known as the hypothalamic GnRH pulse generator. Kisspeptin neurones in the arcuate nucleus (ARC) of the hypothalamus that co-express neurokinin B (NKB) and Dynorphin (Dyn), and known as KNDy, stimulate GnRH release and are thought to comprise the GnRH pulse generator. What initiates and maintains the rhythmic activation of the KNDy neural network to drive pulsatile secretion of GnRH is unknown. We developed a mathematical model of the KNDy neural network, which revealed that the level of network excitability, controlled by

the parameter *basal activity*, is a sufficient modulator of the network oscillatory dynamics in terms of pulse frequency. Mathematical analysis predicts that alterations in the level of *continuous* basal activity in the KNDy neurones increases pulse generator *frequency*. This model prediction is eminently testable using optogenetics. A viral vector carrying channelrhodopsin was injected into the ARC of female Kiss-Cre mice. Implanted fibre-optic canulae in the ARC allows for optic stimulation of KNDy neurones. Serial blood samples (5µl, tail-tip) were collected at 5min intervals for 2.5h for measurement of LH. Continuous optic stimulation at 1Hz (473nm, 5ms) for 90min increased LH pulse frequency, and 5Hz stimulation further increased pulse frequency. These data demonstrate that a continuous low frequency (1 or 5Hz) stimulation of KNDy neurones evokes a continuous pulsatile mode of LH secretion. These observations which aligns with the model predictions provide a novel first step towards understanding the intrinsic mechanisms underlying pulse generation.

PS1.00134 ROLE OF AMP-ACTIVATED PROTEIN KINASE (AMPK) IN GNRH NEURONS IN THE METABOLIC CONTROL OF REPRODUCTION

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GnRH neurons are a key regulatory hub for the control of reproduction and its modulation by metabolic signals. However, the mechanisms whereby metabolic cues finely control GnRH neurosecretion remain unknown. AMP-activated protein kinase (AMPK), a key cellular sensor that becomes activated in conditions of energy deficit, plays a major role in whole-body energy homeostasis. Fragmentary evidence suggests that AMPK may participate also in the regulation of the reproductive axis, driving inhibitory signals in situations of energy insufficiency. Notably, prenatal androgenization, as model of polycystic ovary syndrome (PCOS), is known to enhance GnRH neuronal firing, which can be normalized by the AMPK activator, metformin. Moreover, metformin inhibits the release of GnRH in vitro. Yet, the putative physiological role of AMPK signaling in GnRH neurons remains unfolded. We report herein the generation of the first mouse line, named GAMKO, with conditional ablation of a1-AMPK in GnRH neurons. Congenital elimination of AMPK from GnRH neurons significantly advanced puberty onset in female, but not male mice. In addition, GAMKO females submitted to chronic subnutrition showed a faster recovery of estrus cyclicity after re-feeding. Both features are compatible with unrestrained GnRH secretion in the absence of AMPK, which might be reminiscent of neurosecretory alterations in PCOS. Moreover, GAMKO females showed exaggerated LH responses to GnRH, and higher body weight and fat mass content. Further characterization of LH pulsatility and ovarian morphology is currently in progress to fully define the reproductive phenotype of GAMKO mice and, thereby, the pathophysiological roles of AMPK signaling in GnRH neurons.

PS1.00135 ESTRADIOL LEVELS THAT INDUCE AN LH SURGE ALSO INCREASE GLUTAMATERGIC INPUTS ONTO KNDY CELLS IN EWES

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Neurons in the arcuate nucleus co-expressing kisspeptin, neurokinin B (NKB) and dynorphin (KNDy) play a critical role in control of gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) secretion. In sheep, KNDy cells mediate both steroid-negative and –positive feedback during pulsatile and preovulatory surge secretions of GnRH/LH. In addition, KNDy cells receive glutamatergic inputs expressing vGlut2, both from KNDy cells and from other populations of glutamatergic neurons. Previously we showed higher numbers of vGlut2-positive axonal inputs onto KNDy neurons during the LH surge than in luteal phase ewes. In the present study, we further examined the effects of ovarian steroids progesterone (P) and estradiol (E) on glutamatergic inputs to KNDy cells. Ovariectomized (OVX) ewes received either control (OVX) or steroid treatments that mimicked the luteal phase (low E+P), and early (low E) or late follicular phases (high E) of the estrous cycle (n=4/group). Brain sections were processed for triple-label immunofluorescent detection of NKB/vGlut2/synaptophysin and analyzed using confocal microscopy. Results showed higher numbers of vGlut2 inputs and total inputs onto KNDy cells in high E compared to OVX controls. Low E and E+P did not differ from OVX controls. These results suggest that synaptic plasticity of glutamatergic inputs onto KNDy cells during the ovine follicular phase is dependent on increasing levels of E required for the preovulatory GnRH/surge. These synaptic changes may contribute to the switch between estrogen negative and positive feedback on GnRH/LH secretion and thus the generation of the preovulatory surge.

PS1.00136 GNRH PULSE GENERATOR ACTIVITY ACROSS THE ESTROUS CYCLE IN FEMALE MICE

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The pulsatile secretion of luteinizing hormone (LH) from the pituitary gland is required for the maintenance of fertility and is critically dependent upon kisspeptin signaling in the brain. Recent work in our laboratory demonstrated that the arcuate nucleus kisspeptin (ARN^{KISS}) neurons are the hypothalamic GnRH pulse generator (Clarkson *et al*. 2017). Those studies included the use of GCaMP6 fiber photometry which allowed the calcium levels of the ARN^{KISS} neuron population to be measured in real-time in male mice. In female mice, LH pulsatile secretion shows variation across the estrus cycle. In the present studies we used

GCaMP photometry to examine activity of the GnRH pulse generator in female mice across the estrous cycle. Cre-dependent adeno-associated viral vectors (AAVs) encoding the calcium reporter GCaMP6s were bilaterally injected into the ARN of female KISS1-Cre mice, specifically targeting the ARN ^{KISS} neurons. GCaMP fluorescence was recorded between 9 am – 12 pm via an indwelling 400-µm optical fiber positioned immediately above the mid-caudal ARN and connected to a fiber photometry system. To confirm the correlation between synchronized ARN ^{KISS} neuron calcium activity and pulsatile LH secretion, sequential blood sampling was paired with photometry recordings. Average pulse generator frequencies were 1.0, 1.20, 1.4 and 0.4 per 60 minutes during the morning of metestrus, diestrus, proestrus and estrus respectively. Our data provide further evidence of the ARN ^{KISS} neurons as the GnRH pulse generator in mice and highlight a substantial reduction in activity during estrus.

PS1.00137 ANALYSIS OF GNRH PULSE GENERATOR ACTIVITY IN INTACT MALE MICE

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Recent work from our laboratory demonstrated that arcuate nucleus kisspeptin (ARN ^{KISS}) neurons represent the gonadotropin-releasing hormone (GnRH) pulse generator (Clarkson *et al*., 2017). Using fiber photometry in freely behaving mice, we showed that ARN ^{KISS} neurons exhibit intermittent synchronous activity with an interval of ~9min in gonadectomized male mice, with near-perfect correlation with luteinizing hormone (LH) pulses. In the present study we continued to utilize fiber photometry to analyze the activity pattern of ARN ^{KISS} neurons in intact males. We targeted an adeno-associated virus encoding a calcium indicator GCaMP6s to the ARN ^{KISS} population of Kiss-Cre mice, and implanted an optical fiber (400µM) immediately above the mid-caudal ARN. The fiber was connected to a photometry system and the GCaMP signal was recorded for 24 h with 12:12 h light cycle. To examine the relationship of synchronized calcium events of ARN ^{KISS} neurons to LH pulses, serial blood sampling was undertaken during a separate 4-h photometry recording. In intact male mice, ARN ^{KISS} neurons exhibited episodes of synchronous activity with intervals of 3.0±0.5 h with no noticeable change in the frequency between day and night (3.4±0.6 and 2.7±0.2 h intervals respectively). Paired photometry and blood sampling in four mice revealed a perfect correlation with synchronized calcium events always preceding LH pulses. No LH pulse was detected without a preceding calcium event. This study reveals the endogenous pattern of ARN ^{KISS} neuron synchronization in intact male mice and supports the recent finding of ARN ^{KISS} neurons as the GnRH pulse generator.

PS1.00138 IN VIVO POPULATION ACTIVITY OF RP3V KISSPEPTIN NEURONS ACROSS THE MOUSE ESTROUS CYCLE

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Kisspeptin signaling through its receptor Kiss1r is essential for reproductive function. Kisspeptin neurons residing in the rostral periventricular area of the third ventricle (RP3V) of the rodent are highly implicated in the generation of the preovulatory gonadotropin-releasing hormone and luteinising hormone surges. During the preovulatory surges many RP3V kisspeptin neurons express cFos, indicating recent activity, yet the activity patterns of RP3V kisspeptin neurons throughout the estrous cycle remains unknown. In the present experiments we have used GCaMP fibre photometry to record the activity patterns of RP3V kisspeptin neurons as a population in awake, freely-behaving mice on different days of the estrous cycle. Adeno-associated viral vectors (AAVs) were injected unilaterally into the RP3V of kisspeptin-Cre mice to specifically and exclusively target the expression of GCaMP and the excitatory DREADD hM3D to RP3V kisspeptin neurons. A fiberoptic cannula was implanted adjacent to the RP3V at the time of AAV injection. All recordings were made in the home cage with mice connected to a fibre photometry system via a fibre optic patch cord. Expression of GCaMP and correct fibre placement was confirmed by detection of an increased GCaMP signal upon stimulation of intracellular Ca^{2+} levels by CNO activation of hM3D signaling. GCaMP activity was recorded for up to 8-hours encompassing periods prior to and following lights-out on each day of the estrous cycle. We observe that populations of RP3V kisspeptin neurons exhibit a range of activity patterns throughout the estrous cycle in the mouse.

PS1.00139 DELAY IN PUBERTY ONSET AFTER NEONATAL UNDERFEEDING CAN BE REVERSED BY SILENCING AGRP NEURONS

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Agouti-related peptide (AgRP) is co-expressed with neuropeptide-Y and gamma-aminobutyric acid in arcuate neurons. The activity of these neurons is modulated by metabolic hormones such as leptin, ghrelin, and insulin and therefore act to convey metabolic cues to the Hypothalamic-Pituitary-Gonadal axis. However the effect of AgRP neuronal modulation on reproductive function and puberty onset has yet to be clearly elucidated. Using 'Designer Receptors Exclusively Activated by Designer Drugs' technology, we selectively silenced AgRP neurons non-invasively by administering the synthetic ligand CNO into drinking water of male mice from postnatal day 26 to 30 (~2 mg/day/mouse). The age at preputial separation (an anatomical marker of puberty) was significantly advanced (by 2.1 days) in AgRP neuron-inhibited mice compared to controls ($p<0.05$). We wondered whether this effect could counteract the delay in puberty onset observed under neonatal underfeeding. Pups mice were fostering within 3 days after birth into large litters (12 pups) to create underfeeding conditions,

or into normal litters (6 pups), and received the same treatments as described above. As expected, the age at preputial separation was delayed in controls from large litters compared to controls from normal litters ($p<0.05$), but this effect was reversed in AgRP neuron-inhibited mice, with a similar age at preputial separation compared to mice from normal litters. Additional studies are underway to look at the effects on puberty onset in females. Whether this effect occurred in response to AgRP itself or one of the other secreted products of these neurons also remains to be determined.

PS1.0014 GESTATIONAL BISPHENOL A EXPOSURE ACCELERATES HYPOTHALAMIC NEUROGENESIS AND ALTERS BEHAVIOUR.

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The hypothalamus is a key brain region that regulates and influences homeostatic body processes, multiple endocrine axes, mood, and behavior. Previously, we highlighted the unique susceptibility of the developing zebrafish hypothalamus to endocrine disruption by low-dose bisphenol A (BPA), observing precocious hypothalamic neurogenesis with associated hyperactivity. Here we ask whether mammalian hypothalami are susceptible to environmentally-relevant, low-dose BPA, and if so, what is the underlying mechanism? We found gestational BPA exposure (50 µg/kg diet) accelerates hypothalamic neurogenesis from E9.5-E15.5 in developing mouse embryos and that these BPA-exposed offspring show altered social behavior (three chamber test, novel animal test) and increased hyperactivity (open field test, elevated plus maze) with no defects in learning, memory, or spatiomotor skills. Additionally we tested their circadian regulation by modulation of the light-dark cycle, observing an altered period and activity onset in offspring of BPA-fed dams. To understand the mechanism linking BPA exposure to altered neurogenesis, we utilized the neurosphere assay, to study E12.5 hypothalamic neural stem and progenitor cells (NSPCs) in vitro. We found that E12.5 fetal hypothalamic NSPCs isolated from BPA-fed dams exhibit increased proliferation and fate commitment. We found this same result using direct BPA treatment (10µM and 100nM) of E12.5 NSPCs from untreated dams. To elucidate the responsible steroid signaling pathways, we used the neurosphere assay to test pharmacological steroid receptor inhibitors (fulvestrant and flutamide, 10uM dose), identifying both estrogen and androgen receptors as mediators. Together these data indicate that embryonic hypothalamic NSPCs are specifically susceptible to neurodevelopmental disruption by BPA.

PS1.00140 ALTERED CENTRAL CERAMIDE SIGNALING AS A NOVEL MECHANISM FOR OBESITY-INDUCED PRECOCIOUS PUBERTY.

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Childhood obesity has been linked to different adverse outcomes, including metabolic and pubertal disorders. Yet, the mechanisms underlying this association remain unknown. Here, we analyze the role of hypothalamic ceramides, a family of sphingolipids that mediates the central actions of key regulators of metabolism and puberty, in the control of puberty and its alterations due to early-onset obesity. Early overnutrition increased hypothalamic ceramide content and advanced puberty in female rats. Pharmacological stimulation of central ceramide signaling in lean female rats partially mimicked the advancement of puberty onset caused by overnutrition, whereas its chronic inhibition delayed puberty, both in lean and obese females. Blockade of ceramide signaling also prevented the permissive actions of the puberty-activating neuropeptide, kisspeptin, on puberty onset, but failed to alter hypothalamic Kiss1 expression and both basal and kisspeptin-stimulated GnRH/LH secretion, thus suggesting alternative pathways. We describe here a novel kisspeptin-ceramide pathway involving the paraventricular nucleus (PVN) and ovarian sympathetic innervation: (i) PVN received abundant kisspeptin fibers in pubertal rats, (ii) obesity-induced precocious puberty was linked to advanced maturation of the ovarian sympathetic tone and, (iii) PVN-specific knockdown of ceramide synthesis partially normalized ovarian sympathetic activity and the timing of puberty onset in obese rats. Overall, our data are the first (i) to document the involvement of central ceramide signaling in the control of pubertal timing and its alterations due to early-onset obesity and, (ii) to propose an alternative pathway, linking PVN ceramide synthesis and sympathetic ovarian innervation, as an underlying mechanism for obesity-induced precocious puberty.

PS1.00141 AGE DEPENDENT CHANGES IN REPRODUCTIVE AXIS RESPONSIVENESS TO KP-10 ADMINISTRATION IN HEALTHY MEN

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Present study was designed to assess the responsiveness of hypothalamic pituitary gonadal axis to kisspeptin administration with increasing age in men. Human kisspeptin-10 was administered in single iv bolus dose (1μg/kg BW) to healthy adult (BMI 22.06±0.63 Kg/m^2; age 25.80±0.37 year; n=5), middle age (BMI 21.78±1.21 Kg/m^2; age 47.00±0.77 year; n=5) and advance age men (BMI 21.26±0.70 Kg/m^2; age 73.20±0.91 year; n=5) without any known comorbidity. Serial blood samples were collected for 30 min pre and 120 min post-kisspeptin injection periods at 30 min interval. Changes in plasma LH and testosterone levels were determined by using specific enzyme immunoassay. Paired t-test on log transformed data showed that mean plasma LH level of all the three groups were significantly ($P<0.05$) elevated after kisspeptin-10 administration. Post kisspeptin mean LH levels were comparable in all the age groups. In case of testosterone, paired t-test on log transformed data showed that administration of kisspeptin-10 significantly increased ($P<0.05$) mean plasma testosterone level of adult men while no significant effect was observed on plasma testosterone levels of middle and advance age men. In summary, this study describes that iv bolus administration of kisspeptin-10 significantly increases plasma LH secretion in adult age, middle age and advanced aged men equally. While, it causes a significant increase in plasma testosterone concentration of adult age men only. Present results suggest that in men central hypothalamic pituitary axis remains active and shows responsiveness to kisspeptin stimulation throughout life. However, Leydig cell responsiveness to kisspeptin induced LH decreases with age in men.

PS1.00142 LESIONS OF NK3R-CONTAINING NEURONS IN THE RETROCHIASMATIC AREA (RCH) BLUNTS THE LH SURGE IN EWES

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Neurokinin B (NKB) actions in the RCh appear to be important for the estrogen-induced luteinizing hormone (LH) surge in ewes because local implants of an NK3R antagonist decreased the amplitude of the surge by ~50%. Here, we tested this hypothesis by lesioning NK3R-containing RCh neurons using a saporin conjugate (NK3-SAP). Breeding season ewes were ovariectomized and immediately given an estradiol (E) implant sc and two progesterone implants (CIDRs) intravaginally that produced luteal phase levels of these steroids. Ewes then received bilateral one uL injections of either NK3-SAP (n=5) or Blank-SAP (n=3). Three weeks later, an “artificial follicular phase” was produced by inserting 4 long E implants 24 hrs after CIDR removal. LH pulses were monitored just before CIDR removal and before insertion of E

implants; blood samples were then taken every 2-4 hrs for 48 hrs to monitor the LH surge. NK3-SAP injection dramatically decreased numbers of NK3R-containing neurons/section in the RCh in 4 of 5 ewes (1.8 ± 1.1), compared to controls (16.3 ± 3.2). There was no significant difference in episodic LH secretion between the two groups, but the amplitude of the LH surge in lesioned ewes was 50% lower (20.5 ± 2.5 ng/mL) than in control ewes (48.7 ± 2.8 ng/mL) and in the ineffectively-lesioned ewe (44.4 ng/mL). These data support an important role for RCh NK3R-containing neurons in the LH surge in ewes, and the similarity in effects with icv infusion of a KISS1R antagonist is consistent with the proposal that these neurons act via kisspeptin.

PS1.00143 ACTIVATION OF ARN AGRP/NPY NEURONS SLOWS LH PULSATILITY IN CONTROL AND PCOS MICE

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Homeostatic processes like metabolism and reproduction are tightly regulated by neural circuits, and dysregulation can cause energy imbalance or infertility, respectively. Furthermore, disruption of energy homeostasis can also lead to infertility, as seen in anorexia. Agouti related peptide (AgRP)/Neuropeptide Y (NPY) expressing neurons in the arcuate hypothalamus (ARN) are known to play a crucial role in energy homeostasis and are implicated in fertility regulation, suggesting that they may serve as important integrators of metabolic and reproductive neural circuits. However it is still unclear how specific activation of this neuronal population regulates gonadotropin-releasing hormone (GnRH) neurons or luteinising hormone (LH) secretion in vivo. We used the stimulatory DREADD hM3dq in AgRP-cre mice to activate Agrp/NPY neurons and measured pulsatile LH secretion by ELISA, as a readout of GnRH neruonal activity. We found that activation of NPY neurons by injection of DREADD ligand (clozapine-N-oxide), decreased post-castration LH secretion and pulse frequency, which suggests a slowing of GnRH pulse generation. These results led us to investigate AgRP/NPY neuron activation in a prenatally androgenized model of PCOS, known to exhibit high LH pulsatility. AgRP/NPY neuron activation in these mice also decreased LH pulsatility, indicating a potential therapeutic target for slowing the hyperactive GnRH neural network in PCOS. Preliminary evidence from direct optogenetic stimulation of AgRP/NPY terminals surrounding GnRH neurons also demonstrated decreased LH pulsatility. Overall, our findings clearly identify a direct role for AgRP/NPY neurons in fertility, expanding its importance beyond its well-known role in energy homeostasis.

PS1.00144 RESCUE OF FUNCTION OF INACTIVATING MUTATIONS IN THE HUMAN NK3R RECEPTOR

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Inactivating mutations of GPCRs of the hypothalamic-pituitary-gonadal axis can result in a failure to progress through puberty. The majority give rise to misfolding of the receptors with intracellular retention and a failure to traffic to the cell surface. Cell-permeant small molecule agonists and antagonists have been indentified which stabilize mutant luteinizing hormone receptors and gonadotropin-releasing hormone receptors and chaperone them to the cell surface thus restoring function. We have examined whether function can be restored to the human neurokinin B receptor (NK3R) with inactivating mutations, resulting in intracellular retention. Seven inactivating mutations in the human NK3R which resulted in infertility were examined. Five caused intracellular retention and a failure to traffic to the cell surface (G93D, H148L, Y256H, Y267N and P353S). We investigated whether a cell-permeant small molecule antagonist with high specificity for NK3R, M8, could rescue cell surface expression of these mutant receptors. The mutagenized cloned receptors tagged with HA at the N-terminus were expressed in HEK 293-T cells which were exposed to M8 for 24 hours. Cell surface receptor was quantified in intact cells by ELISA using a mouse anti-HA antibody. Cell surface expression was restored to wild type values in all except G93D which was increased 53%. The restoration of cell surface expression was accompanied by a restoration of signaling, as measured by stimulation with a peptide agonist of NK3R, senktide, and measurement of inositol phosphate production. These findings indicate that human inactivating mutations causing poor cell surface expression may be treatable using NK3R small molecule analogs.

PS1.00145 THE EFFECTS OF PROGESTERONE ON REGULATION OF THE LUTEINISING HORMONE SURGE IN ADULT FEMALE RAT

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Premature LH surges are a major cause for cycle cancellation during controlled ovarian stimulation (COH) in women undergoing in-vitro fertilization (IVF). Efforts to minimize the occurrence of premature LH surges have mainly relied on the use of GnRH agonist/antagonist, which exert greater risk of side effects. Clinical studies have shown that progesterone acts as an effective alternative for blocking premature LH surges in COH. Early studies indicate the hypothalamus mediates the feedback effect of progesterone on regulation of LH level. However, the underlying mechanisms remain unknown. Here, we tested the hypothesis that progesterone may prevent premature LH surges via progesterone receptor in the hypothalamic arcuate (ARC) and/or anteroventral periventricular (AVPV) nuclei; loci known to be involved in LH pulse and LH surge generation. Also, the follicle development and embryological result were

examined. Adult female Sprague-Dawley rats were given a single intraperitoneal injection of pregnant mare serum gonadotropin (PMSG,150IU/kg) to induce follicle development followed by either progesterone (5mg/kg, bid) or oil injection for two days. Progesterone administration prolonged the estrous cycle ($P<0.05$). Onset of the LH surge was significantly delayed when progesterone was administrated with PMSG($P<0.05$). Furthermore, bilateral microinjections of anti-progestin RU486 (5ng/µl, 0.8 µl) into the AVPV and ARC affect LH levels. Our results indicated that progesterone prevents premature LH surges during superovulation in rats without affect the number of oocyte retrieved and further embryonic development, which coincide with previous clinical observation. The hypothalamic AVPV and ARC nuclei may be targeted for progesterone regulation of LH surges in the rat.

PS1.00146 SEXUALLY DIMORPHIC RESPONSE OF SELECTIVE MODULATION OF AMYGDALA KISS NEURONS ON REPRODUCTIVE AXIS

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Kisspeptins (Kiss) are a family of neurohormones that serve as potent regulators of the reproductive axis through actions in the hypothalamus. However, Kiss neurons also reside in the medial amygdala (MeA) but whether they directly contribute to reproductive function is unknown. Combining stereotaxic delivery of Cre-dependent adenoassociated virus (AAV) vectors (M3 muscarinic DREADD receptor or κ–opioid DREADD receptor) into the MeA of *Kiss1*-CreGFP mice, we have stimulated and inhibited MeA kiss circuits *in vivo* to measure how reproductive and stress centres in the hypothalamus respond. Although the amygdala shows sexually dimorphic anatomical organization and expression of sex steroid receptors, our work directly implicates MeA Kiss neurons in triggering a sexually dimorphic reproductive hormone response. We demonstrate that MeA Kiss neurons in female mice innervate a number of hypothalamic brain centres, with approximately 11% of GnRH neurons receiving MeA Kiss neuron appositions, similar to the 15% observed in males. Strikingly, acute chemogenetic stimulation of MeA Kiss neurons in male mice resulted in a surge in circulating LH but this response was lacking in female mice. Opposite-sex pheromone exposure further raised the plasma LH levels in male mice whereas even chronic stimulation of MeA Kiss neurons in female mice failed to generate an LH response. These results establish the connectivity of MeA Kiss neurons to the HPG axis while highlighting some key functional differences between male and female reproductive physiology.

PS1.00147 COMPLETE KISSPEPTIN RECEPTOR INACTIVATION DOES NOT IMPEDE EXOGENOUS GNRH-INDUCED LH SURGE IN HUMANS

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Mutations in Kisspeptin receptor (*KISS1R)* have been reported in a few patients with normosmic congenital hypogonadotropic hypogonadism (nCHH). Exogenous GnRH treatment has been used to induce an ovulation in very few KISS1R mutated women. In rodents, kisspeptin signaling is mandatory for induction of the estradiol-induced LH surge. By analogy, kisspeptin may be involved in estradiol-induced positive feedback on the LH surge in humans, although its exact site of action remains unclear. Here we took the advantage of the description of a new mutation of *KISS1R* to document the role of Kisspeptin signaling on the GnRH-induced LH surge. A novel homozygous c.953T>C variant in *KISS1R* was identified in a woman who was refereed for a primary amenorrhea due a partial gonadotropic deficiency without anosmia. This mutation led to substitution of a proline for leucine 318 (p.Leu318Pro) in the seventh transmembrane domain of KISS1R. Functional analysis revealed that the mutated receptor was completely inactivated and not located at the cell surface of HEK293 cells. After several pulsatile GnRH therapy cycles, an LH surge with persistent GnRH pulses leading to an ovulation and a pregnancy was obtained, despite complete KISS1R inactivation. A GnRH pulsatile therapy can thus induce an LH surge independently of KISS1R signaling in women.

PS1.00148 THE EVOLUTION OF THE MAMMALIAN GNRH RECEPTOR SHAPED GNRH'S ACTIONS ON GONADOTROPINS

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The mammalian GnRH receptor (GnRHR) is unusual among G protein coupled receptors in lacking an intracellular C-terminus (C-tail). This specialization prevents homologous desensitization and slows receptor internalization. To understand the *in vivo* significance of the loss of the C-tail, we generated knock-in mice expressing a chimeric GnRHR, where the chicken GnRHR C-tail was fused in-frame to the C-terminus of the endogenous murine GnRHR. Neither serum LH nor pituitary *Lhb* subunit mRNA levels differed between adult C-tail and wild-type males or females. In contrast, both serum FSH and pituitary *Fshb* subunit expression were decreased in C-tail males. Gonadotropin α subunit and *Gnrhr* mRNAs were also decreased. Gonadotropin subunit and *Gnrhr* expression did not differ between genotypes in females; however, serum FSH was reduced in C-tail animals. These females also exhibited abnormal estrous cyclicity and were subfertile. These data suggest that the addition of a C-tail impairs GnRH regulation of FSH. In heterologous cells, the chimeric GnRHR retained the ability to

mediate ERK1/2 phosphorylation in response to GnRH, but intracellular calcium mobilization was greatly reduced. This stemmed from an impairment in the coupling of the chimeric receptor to Gq/11 proteins. When calcium mobilization was inhibited in immortalized gonadotrope-like cells, LbetaT2, *Fshb* mRNA induction by GnRH was abolished, suggesting a role for calcium signalling in GnRH regulation of *Fshb*/FSH. Based on the available data, we propose that the loss of the C-tail in mammalian GnRHR evolution conferred a reproductive advantage by enhancing GnRH's regulation of FSH rather than by enabling the LH surge.

PS1.00149 ESTRADIOL-DEPENDENT AND -INDEPENDENT UPREGULATION OF KISS1 IN THE AMYGDALA, BNST, AND SEPTUM OF MICE

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Kisspeptin, encoded by *Kiss1*, stimulates GnRH release. *Kiss1* neurons reside in the hypothalamic AVPV/PeN and ARC nuclei, and in smaller numbers in extra-hypothalamic areas, including the medial amygdala (MeA), bed nucleus of the stria terminalis (BnST), and lateral septum (LS). We previously demonstrated that: 1) MeA *Kiss1* expression is essentially undetectable in the absence of sex steroids and stimulated by estradiol (E_2) acting via ERα; and 2) gonad-intact $GABA_BR$KO mice have much greater *Kiss1* expression in the MeA, BnST, and LS than WT littermates. However, it is unknown which estrogen receptor subtype mediates E_2's upregulation of BnST and LS *Kiss1* expression or if *Kiss1* levels in the MeA, BnST, and LS are still elevated in $GABA_BR$KO mice even without sex steroids. Here we used *in situ*hybridization to compare *Kiss1* expression in the MeA, BnST, and LS of gonadectomized and gonadectomized+E_2 ERαKO, βERKO, $GABA_BR$KO, and WT mice. We found that E_2 treatment increases BnST and LS *Kiss1* expression in WT and βERKO mice, but not in ERαKOs, indicating *Kiss1* expression in these regions is upregulated specifically via ERα pathways. Next, we found that, unlike gonadectomized WTs, gonadectomized $GABA_BR$KOs still have notable *Kiss1* expression in the MeA, BnST, and LS, indicating that absent $GABA_BR$ signaling upregulates *Kiss1* in these regions even in the absence of sex steroids. In both genotypes, E_2 further increases *Kiss1* in the MeA, BnST, and LS, with higher levels in the $GABA_BR$KOs than WTs. Thus, E_2 (stimulatory) and GABA (inhibitory) can independently regulate *Kiss1* in extra-hypothalamic regions.

PS1.0015 MEDIATION OF RECOGNITION MEMORY BY THE INTERACTION BETWEEN THE RAPID ESTROGENIC EFFECTS AND OXYTOCIN

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Social recognition (SR) is the ability to distinguish between conspecifics. Estrogens and oxytocin have been implicated in mediating SR. Studies with long term manipulations have shown a critical role for oxytocin, the oxytocin receptor, and estrogen receptors in SR. Recently, we also showed very rapid enhancements of SR with 17β-estradiol or estrogen receptor-specific agonists in the dorsal hippocampus and medial amygdala. Estrogens are known to regulate oxytocin. Thus, we suggest oxytocin may mediate estrogens' rapid facilitation of SR. To test this, we infused 17β-estradiol into the paraventricular nucleus (PVN), where the majority of oxytocin is produced in the brain, and found that SR was facilitated within 40 minutes of administration. To determine whether this rapid facilitation of SR by estrogens in the PVN occurred through an interaction with oxytocin we infused a dose of an oxytocin receptor antagonist that by itself would not block SR into the medial amygdala, a region that has been found to be important for SR, as well as 17β-estradiol into the PVN. We found that the oxytocin receptor antagonist in the medial amygdala blocked the facilitative effect of 17β-estradiol in the PVN, suggesting that estrogens' rapid effects on SR occur through an interaction with oxytocin. We are also examining whether other types of memory, like object recognition are similarly facilitated by 17β-estradiol in the PVN or inhibited by an oxytocin receptor antagonist in the medial amygdala. Our work demonstrates the interplay of two brain regions in estrogenic/oxytocin rapid enhancement of SR. Funded by NSERC.

PS1.00150 SIM-1 NEURONS ARE SUFFICIENT FOR MC4R-MEDIATED SEXUAL FUNCTION IN MICE

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Sexual dysfunction is a poorly understood condition that affects both men and women. Previous studies have shown that central melanocortins, which are released by pro-opiomelanocortin neurons in the arcuate nucleus of the hypothalamus, can lead to male erection and increased libido in men and women. Several studies specifically implicate the melanocortin 4 receptor (MC4R) in the central control of sexual function, but the specific neural circuitry involved is unknown. We hypothesized that single-minded homolog 1 (Sim1) neurons play an important role in the melanocortin-mediated regulation of male sexual behavior. To test this hypothesis, we examined the sexual behavior of mice expressing MC4R only on Sim1-positive neurons (tbMC4Rsim1 mice) in comparison with tbMC4R null mice and wild-type controls. In tbMC4Rsim1 mice, MC4R reexpression was found in the medial amygdala and paraventricular nucleus of the hypothalamus. These mice were paired with sexually

experienced partners, and their sexual function and behavior was scored. tbMC4R null male mice showed a longer latency to mount, a reduced intromission efficiency, and an inability to reach ejaculation. tbMC4R null female mice showed a substantially reduced lordosis quotient. Expression of MC4R only on Sim1 neurons reversed the sexual deficits seen in tbMC4R null mice. This study implicates melanocortin signaling via the MC4R on Sim1 neurons in the central control of sexual behavior.

PS1.00151 EARLY AND LATE EFFECTS OF MOTHERHOOD ON HIPPOCAMPAL NEUROGENESIS, MICROGLIA, AND THE CYTOKINE MILIEU

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Motherhood is accompanied by a host of physiological and behavioural adaptations. The maternal brain displays considerable plasticity, and motherhood is associated with changes in affective and cognitive function. Emerging evidence also indicates that motherhood can alter the trajectory of brain ageing, including modifications to neuroplasticity and cognition. Here, we investigated the short- and long-term effects of motherhood on hippocampal neurogenesis, microglial morphology, and circulating cytokine levels; domains known to be altered with age and implicated in cognition and mood. Female Sprague-Dawley rats were bred, then euthanized at gestation day 13 (GD13), or postpartum day (PPD) 8, 30, 90, or 180. Nulliparous rats were assigned as age-matched controls to each primiparous condition. We report that hippocampal neurogenesis, assessed via the expression of the immature neuronal marker doublecortin, was significantly suppressed during gestation and the entire postpartum period. Interestingly, while neurogenesis declined significantly in middle-aged nulliparous rats, it increased in primiparous rats across the same period of time. Further, transient postpartum adaptations to the neuroimmune environment of the hippocampus were evidenced, as Iba-1-immunoreactive microglia assumed a de-ramified morphology. Intriguingly, ageing-related changes in cytokine levels were dependent on parity, suggesting that motherhood can modify certain aspects of immunosenescence. Collectively, these data indicate that maternal experience has early and late effects on hippocampal neurogenesis, microglia, and the peripheral cytokine profile. The reported adaptations in neurogenic and immune processes may have ramifications for maternal mood and cognition across the peripartum period and beyond.

PS1.00152 SELECTIVE ACTIVATION OF ARCUATE NUCLEUS GABA NEURONS PROMOTES LUTEINIZING HORMONE SECRETION IN MICE

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Neuroanatomical studies indicate that GABA neurons originating in the arcuate nucleus (ARN) densely contact gonadotropin-releasing hormone (GnRH) neurons. Plastic changes in this circuit are evident in a prenatally androgenized (PNA) mouse model of PCOS, which is associated with elevated luteinizing hormone (LH) secretion suggesting an important role in fertility yet the biological relevance of this circuit remains unknown. The current work aimed to investigate the impact of selective activation of arcuate GABA (ARN GABA) neurons on LH secretion using *in vivo* optogenetic activation of these neurons in normal and PNA mice. Cre-dependent expression of channelrhodopsin-2 E123T accelerated variant (ChETA) was targeted to ARN GABA neurons in vesicular GABA transporter (VGAT)-Cre mice. Cell-attached voltage-clamp recordings showed that ChETA-transduced ARN GABA neurons can respond to light pulses with high spike fidelity (100%) up 50 Hz (N = 19 GABA neurons). *In vivo* studies in isoflurane-anesthetized VGAT-Cre$^{+/-}$ male (N = 6), diestrus female (N = 10) and PNA (N = 5) mice revealed that 20-Hz light stimulation evoked robust LH release in male and diestrus female which lasted for over 60 minutes after stimulation ($P < 0.05$). Interestingly, 20 Hz-optogenetic activation in PNA mice induced smaller changes in LH levels when compared to male and diestrus female groups ($P < 0.05$). These data indicate that ARN GABA neurons can activate the reproductive axis and that altered LH release in PNA mice might reflect a decreased pituitary LH releasable pool due to the high GnRH pulse frequency in a PCOS-like condition.

PS1.00153 TRPV REGULATION OF VASOPRESSIN NEURON FIRING RATE IN PREGNANT RATS IN VIVO

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Vasopressin, a hormone that regulates plasma osmolality by promoting water reabsorption at the kidney collecting duct, is secreted into the circulation by supraoptic nucleus (SON) and paraventricular nucleus neurons following neuronal firing. Vasopressin neurons are directly osmosensitive, whereby reduced osmolality inhibits stretch-inactivated transient receptor potential vanilloid (TRPV) channels to reduce vasopressin neuron activity, thus regulating fluid balance. During pregnancy, there is a reduction in the threshold for vasopressin release, leading to lower osmolality than in the non-pregnant state. However, the mechanism that causes this reduction in the threshold for vasopressin release remains unclear. We recorded from vasopressin neurons in urethane-anaesthetised female virgin and late-pregnant (18 – 20 days

post-conception) rats *in vivo*. The TRPV antagonist, ruthenium red, was administered into the SON through a microdialysis probe for up to 1 h. Preliminary results show ruthenium red reduced the firing rate of vasopressin neurons from 5.4 ± 0.8 Hz to 3.4 ± 0.9 Hz at 50 – 60 min in virgin rats (n = 11). However, ruthenium red did not reduce the firing rate of vasopressin neurons in late-pregnant rats (7.4 ± 0.7 Hz and 7.9 ± 2.2 Hz; n = 4). Plasma osmolality was lower in pregnant rats (314.3 ± 2.5 mosmol kg^{-1}) compared to virgin rats (326.9 ± 1.9 mosmol kg^{-1}). These results suggest that there is less activation of stretch-inactivated TRPV channels in pregnancy. Hence, TRPV upregulation is unlikely to contribute to the reduced osmotic threshold for vasopressin secretion in pregnancy.

PS1.00154 PREOPTIC NEUROKININ 3 RECEPTOR-EXPRESSING NEURONS MODULATE THERMOREGULATORY PATHWAYS IN THE MOUSE

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We have hypothesized that KNDy neurons activate heat dissipation effectors via projections to neurokinin 3 receptor (NK_3R)-expressing neurons in the median preoptic nucleus (MnPO). These studies have shed light on the etiology of hot flushes and led to clinical trials that have successfully treated hot flushes with NK_3R antagonists. We recently ablated NK_3R neurons in the MnPO and adjacent medial preoptic area (MPOA) in the mouse using saporin conjugated to an NK_3R agonist. Ablated animals exhibited a regulated elevation in core temperature during the light phase that was independent of estrogen treatment. To determine if these neurons are sensitive to warm stimuli, *Tacr3*-EGFP mice (with GFP expressed in NK_3R neurons) were exposed to ambient temperature regimens designed to stimulate skin thermosensors or elevate core temperature. Although both procedures increased preoptic fos-activation, there was no co-expression of fos in NK_3R neurons in either the MnPO or MPOA. Finally, we used RNAscope to determine if NK_3R mRNA was co-expressed with *vglut2 or vgat* mRNA, markers of glutamatergic or GABAergic neurotransmission, respectively. Interestingly, 94% of NK_3R neurons in the MnPO were glutamatergic, whereas in the adjacent MPOA, 97% of NK_3R neurons were GABAergic. Thus, our studies show that NK_3R neurons in the MnPO are glutamatergic but do not respond to warm thermal challenges. Combined with previous studies, we propose that KNDy neurons modulate heat defense pathways via glutamatergic NK_3R neurons in the MnPO.

PS1.00155 IS MRI SUITABLE TO STUDY THE CENTRAL EFFECT OF GONADAL STEROIDS DURING THE OESTRUS CYCLE IN EWES?

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Neuroendocrine control of reproduction results in permanent interactions between central nervous system and gonadal steroids feedback. In female, gonadal steroids (estradiol, progesterone) play an important role in the control of the preovulatory surge of GnRH, through their actions on different neuronal populations. The aim of this work is to use *in vivo* Magnetic Resonance Imaging (MRI) to examine modifications of neuronal networks induced by gonadal steroids. Indeed, MRI techniques make it possible to have various types of *in vivo* image contrasts, providing several information on structural, functional and metabolic aspects of the brain. We conducted a study in ovariectomized ewes treated with steroids implants to mimic artificial oestrus cycle (n=9). Four MRI sessions were performed during the oestrus cycle: before treatment, before and after preovulatory surge and at the end of the luteal phase. This study includes the use of anatomical high-resolution T1-weighted imaging to investigate morphology and texture analyses, multi-contrast imaging for T1 and T2 mapping. We also used single voxel Magnetic Resonance Spectroscopy (MRS) and Diffusion-Weighted MRI (DWI) acquisitions to examine metabolic and structural changes. Our first results show specific variations of MRI parameters in different structures known to play a role in the control of the preovulatory surge like the mediobasal hypothalamus and the preoptic area, in agreement with previous immunohistochemical data. This study demonstrates that *in vivo* MRI methods are suitable to detect subtle changes of the neuronal networks involved during oestrus cycle in ewes.

PS1.00156 SINGLE MOTHERS COMPENSATE TO CARE FOR OFFSPRING IN THE BIPARENTAL SPECIES, COLUMBA LIVIA

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Biparental care is a parental investment strategy used to care for offspring as a way to maximize fitness. The majority of bird species studied are reported to exhibit biparental care. However, unpredictable situations such as disease or predation can result in the loss of a partner. If the single surviving parent continues to care for their current offspring, how might they compensate behaviorally and physiologically after the loss of their partner? In the biparental species of the rock dove, *Columba livia,* we removed male partners on the first day of chicks hatching and then measured parenting behaviors 4 days post partner removal. We found that single parent females compensated for the loss of a partner by spending more time brooding and feeding their chicks as compared to control female parents still partnered with a male. Currently, we are evaluating the effects of our parental manipulation on males as well as on changes in neuroendocrine and immune function, and we will report these findings. Our

eventual goal is to yield insight into potential sex-biased selection pressures surrounding parental care, complementing and advancing parental investment theory and the study of eco-evolutionary tradeoffs more broadly.

PS1.00157 RAPID EFFECT OF ESTRADIOL ON DIFFUSION DYNAMICS OF GLUTAMATE RECEPTORS

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Changes in diffusion properties of glutamate receptors (AMPAR and mGluR1) play pivotal role in synaptic plasticity. Among many different factors, controlling the synaptic plasticity, gonadal steroid estradiol (E2) is an essential factor. In spite of the well established genomic effect of E2 on synaptic plasticity little if any attention has been given to the rapid action of E2 on the glutamate receptor diffusion. Using our unique single molecule live cell imaging technique we examined the rapid effects of E2 on the lateral diffusion of AMPAR and mGluR1 molecules in the plasma membrane of neurons differentiated from PC12 cells. Single AMPAR or mGluR1 molecule trajectories were individually tracked and analyzed both on the membrane of soma and neurits and mean square displacement as well diffusion coefficient (D: μm^2/sec) was determined. Both AMPAR and mGuR1 molecules showed restrictive and area specific movements along the neurits and somas with multimodal trajectories. The administration of 100pM and 100nM of E2 evoked a rapid (<10 min) clear dose-dependent effect on the D of AMPAR molecules with limited or no effect on the D of mGluR1 molecules. This effect was mimicked by G1, membrane estrogen receptor agonist. Our findings provide first evidence that E2 rapidly alters membrane diffusion of glutamate receptors in living neurons possibly through the membrane estrogen receptor. These datas suggest that E2 rapidly tunes the synaptic plasticity via altering the surface movement of AMPAR and mGluR1 receptors.

PS1.00158 SEASONAL BREEDING IS RELATED TO THE PLASTICITY OF GNRH EXPRESSION AND CELL PROLIFERATION

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Seasonal brain plasticity contributes to physiological and behavioral processes including seasonal breeding. We hypothesized that seasonal variations of brain plasticity facilitated the life-history trait of seasonal breeding. To test this hypothesis, we took seasonal sampling in April, July and September in natural population, manipulated food-induced hoarding in breeding season and tested the role of photoperiod in inducing seasonal reproduction and brain plasticity in Mongolian gerbils (*Meriones unguiculatus*). We found that gerbils captured in

April and July had better sexual development and stronger exploratory capacity, and preferred much more to novelty than those in September. We also found that male gerbils had lower GnRH content in September than in April and July, and food-induced hoarding behavior suppressed GnRH content in summer. In addition, the gerbils both in subadult and adult but not in young captured in April and July had a higher level of cell proliferation in several brain areas such as SVZ, hypothalamus and amygdala compared with those in September. However, adult gerbils captured in September preferred to investigate the familiar object, and no seasonal differences in cell proliferation were found in DG among three seasons. The laboratory study showed that a single photoperiod cue did not alter reproductive traits, behaviors and cell proliferation and survival in the brain. These findings suggest that the structural changes in GnRH content and cell proliferation are associated with seasonal breeding and food hoarding in gerbils, but a single photoperiod cue may not be critical to induce these seasonal life-history traits.

PS1.00159 ΔFOSB IS INCREASED IN THE NUCLEUS ACCUMBENS OF FEMALE MICE FOLLOWING A HORMONE-SIMULATED PREGNANCY

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Pregnancy and the surrounding peripartum period is a time of profound physiological and behavioral changes. Whereas some changes in behavior are adaptive, many women experience unwanted peripartum behavioral changes that are indicative of depression and/or anxiety. During pregnancy, the ovarian hormones estrogen and progesterone increase precipitously; following delivery, however, estrogen and progesterone levels decrease abruptly. Although it is assumed that the dramatic drop in ovarian hormones following birth is related to the symptoms of peripartum mood disorders, how this hormone withdrawal impacts the brain is poorly understood. The nucleus accumbens (NAc) is a candidate site of hormone-mediated neural and behavioral plasticity during the peripartum period. We hypothesized that the accumulation of ΔFosB, a transcription factor associated with long-term neural plasticity, would be altered in female mice following a hormone-simulated pregnancy. Females were ovarictomized and administered daily injections of estrogen and progesterone that approximate early and late pregnancy. Following this hormone-simulated pregnancy, one group of females was withdrawn from estrogen, simulating postpartum hormone withdrawal, while the other group continued to receive estrogen injections. Using immunohistochemistry, we found that ΔFosB was increased the NAc of estrogen withdrawn female mice. Further, estrogen withdrawn females showed a decrease in anxiety-like behaviors. These data suggest that ΔFosB accumulation in the NAc may be part of a typical mechanism of anxiety reduction in new mothers, and importantly, may be dysregulated in those females who experience peripartum mood disorders. Ongoing experiments are testing whether changes in ΔFosB are specific to neuronal subtypes in the NAc.

PS1.0016 IS DIET-INDUCED GHRELIN RESISTANCE A CAUSAL FACTOR IN STRESS-RELATED MENTAL DISORDERS?

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Post-traumatic stress disorder is associated with an increased risk for obesity. Diet-induced obesity elicits ghrelin resistance or the inability of ghrelin to efficiently activate its receptor. Interestingly, ghrelin resistance has been proposed to alter stress-related behaviors. We therefore explored whether ghrelin resistance is a causal factor in stress-related mental disorders. Adolescent male C57BL6/J mice were placed on a standard or high-fat diet for a total period of two months. In the first experiment, mice were subjected to auditory fear conditioning prior to diet onset and tested for fear extinction, anxiety and anhedonia. In a second experiment, mice were subjected to fear conditioning one month after diet onset and tested for fear extinction, anxiety and anhedonia. Mice placed on a high-fat diet gained significantly more abdominal fat and showed ghrelin resistance. Ghrelin resistance was evidenced by lower food intake following administration of a ghrelin receptor agonist. Fear memory was resistant to extinction training only in mice that were conditioned one month after high-fat diet onset. High-fat diet did not affect anxiety but lowered saccharin preference. Interestingly, ghrelin receptor knockout mice displayed no abnormalities in fear processing or anxiety, but showed anhedonia in the saccharin preference test. Taken together, our data suggest that ghrelin resistance per se does not cause fear processing or anxiety. However, the observation of abnormal reward processing suggests that ghrelin resistance may aggravate anhedonia in stress-related disorders.

PS1.00160 EFFECT OF PREDATOR STRESS ON PUBERTY ONSET AND OESTROUS CYCLICITY IN RAT: ROLE OF CRF SIGNALLING

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The onset of puberty may be influenced by several factors including psychosocial stress. The present study investigated cat-odour stress and its interaction with corticotropin releasing factor (CRF) signalling in modulating puberty onset and oestrous cyclicity in rat. Female weanling rat were exposed to cat odour for 10 days (from pnd 21 to 30) and monitored daily for vaginal opening and first oestrus from pnd 28 to determine puberty onset. Vaginal smear was taken for 12 consecutive days to determine oestrous cyclicity. Brains were collected to determine CRF, CRF-R1 and CRF-R2 mRNA in the PVN and amygdala (CeA and MeA) 14 days post vaginal opening. Cat odour led to a significant delay in vaginal opening, day of first oestrus and caused abnormal oestrous cycle. Cat odour-treated animals showed a significant increase in relative mRNA expression of CRF in the PVN compared to control animals. Conversely, CRF

mRNA expression in the CeA was similar between the two groups. Relative CRF-R1 mRNA levels in the PVN were comparable between control and cat odour-treated groups. However, in the MeA, a significant decrease in CRF-R1 mRNA levels was seen in cat odour-treated group compared to control. CRF-R2 mRNA level was not significantly different in control and cat odour-treated groups in either the PVN, CeA or MeA. These data suggest that the delay in puberty timing in odour-treated rats may be related to CRF signalling in the PVN and the odorous cue altering CRF R1 expression suggest the involvement of the MeA in the pubertal delay.

PS1.00161 DIFFERENTIAL EXPRESSION OF HYPOTHALAMIC GENES IN A SONGBIRD DURING GONADAL DEVELOPMENT

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Seasonally breeding birds must time their reproduction to match optimal environmental conditions. The transition into breeding condition and gonadal growth are regulated by the hypothalamic-pituitary-gonadal (HPG) axis at multiple levels. Our study focuses on the level of the hypothalamus and examines expression of candidate genes in relation to three potential mechanisms regulating gonadal recrudescence: 1) top-down stimulation and inhibition of the HPG-axis via gonadotropin-releasing hormone (GnRH) and gonadotropin-inhibitory hormone (GnIH), 2) sex steroid negative feedback sensitivity via androgen receptor (AR) and estrogen receptor alpha (ERα), and 3) sensitivity to stress hormones via glucocorticoid receptor (GR) and mineralocorticoid receptor (MR). We measured hypothalamic mRNA expression of these genes via qPCR in captive male Dark-eyed Juncos (*Junco hyemalis*) held under the same conditions but expressing different stages of gonadal recrudescence in early spring. All males were captured from the same overwintering population, but males from a resident subspecies were in a more advanced stage of gonadal recrudescence than males from a migratory subspecies. We found that residents had significantly higher mRNA expression levels of *GnRH*, lower levels of *AR* and *ERα*, and similar levels of *GR* and *MR*. These results suggest decreased hypothalamic sensitivity to sex steroid negative feedback and increased GnRH production as factors promoting gonadal recrudescence.

PS1.00162 SEX DIFFERENCES IN THE ROLES OF KISSPEPTIN AND NEUROKININ B IN PUBERTY

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To understand sex differences in the roles of kisspeptin and NKB in puberty, we conducted a series of experiments with direct measurements of GnRH and kisspeptin: 1) GnRH and kisspeptin levels in pubertal females and males were higher than those in prepubertal counterparts. 2) Kisspeptin 10 (hKP10), stimulated GnRH release in a dose-responsive manner in males and females at the prepubertal and pubertal stages along with developmental amplification in both sexes, although the GnRH responses in females were ~10-fold greater. 3) Senktide (NKB agonist), stimulated GnRH release in males and females at both developmental stages, but developmental amplification of the senktide-induced GnRH release was only observed in females. 4) Senktide also stimulated kisspeptin release in both sexes at both stages, but the dose response and developmental amplification were only seen in females. 5) Combination experiments with agonists and antagonists indicated that while in prepubertal females kisspeptin and NKB signaling independently stimulated GnRH release, in pubertal females the formation of a collaborative kisspeptin and NKB network further accelerates the pubertal increase in GnRH release. 6) In prepubertal males, however, a low-level cooperative mechanism between kisspeptin and NKB signaling was present already, but in pubertal males NKB was mediated through kisspeptin neurons. Collectively, the results are interpreted that kisspeptin and NKB signaling are both indispensable to facilitate the pubertal increase in GnRH in both sexes, but in females, reciprocal signaling pathways between kisspeptin and NKB neurons are necessary to provide efficiency and flexibility for GnRH stimulation to ensure complex reproductive functions.

PS1.00163 BDNF PROMOTES RESILIENCE TO SOCIAL DEFEAT: COMPARATIVE AND TRANSLATIONAL RESULTS

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Ethologically relevant rodent models of social defeat are used to investigate human neuropsychiatric disorders that are precipitated or exacerbated by social stress. Brain derived neurotrophic factor (BDNF) in the nucleus accumbens has been shown to promote the depression- and anxiety-like behavioral responses that are observed following social stress in rodents. Paradoxically, there are also abundant data suggesting that BDNF signaling is necessary for the therapeutic effects of antidepressants and that BDNF may prevent behavioral responses to social stress. The purpose of this study was to determine if BDNF promotes or prevents depression-like changes in behavior (i.e., increases in submission and social avoidance) following acute social defeat stress in Syrian hamsters and in mice. We found that BDNF given in the nucleus accumbens did not significantly alter behavioral responses to acute social defeat. Administration of BDNF into the basolateral amygdala (BLA) prior to social defeat, however, significantly reduced defeat-induced behavioral responses. Congruently, inactivating TrkB receptors within the BLA increased defeat-induced social avoidance in mice. For increased translational relevance, we also demonstrated that peripherally administered TrkB agonists reduce and TrkB antagonists enhance defeat-induced social avoidance in hamsters and mice. Together, these data suggest that BDNF signaling can promote resilience to acute social stress

and highlight a promising target for combatting stress-related neuropsychiatric illnesses in humans.

PS1.00164 CRF-R1 ACTIVATION IN THE MPOA IMPAIRS MATERNAL BEHAVIOR AND TRIGGERS OXYTOCIN RELEASE IN RAT MOTHERS

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The brain corticotropin-releasing factor (CRF) system is a potent modulator of maternal behavior and maternal anxiety. Thus, their appropriate appearance postpartum strongly depends on dampened CRF receptor (CRF-R) activity as recently shown for limbic brain regions. Here, we studied the arguably most important brain region for maternal behavior, i.e. the medial preoptic area (MPOA). While expression of CRF receptor subtype 1 mRNA (*Crfr1)* in the MPOA was higher than for *Crfr2*, there were no differences between virgin and lactating rats. Subtype-specific activation of CRF-R1 or CRF-R2 in the MPOA decreased arched back nursing and total nursing under non-stress conditions. Following acute stressor exposure, inhibition of CRF-R1, but not CRF-R2, rescued a stress-induced reduction in arched back nursing. Furthermore, inhibition of CRF-R1 heightened maternal aggression towards a virgin female intruder. Surprisingly, maternal motivation, a key maternal behavior regulated within the MPOA, was not affected by any treatment. Interestingly, maternal anxiety on the elevated plus-maze was increased by CRF-R1 activation. Further experiments using local intracerebral microdialysis revealed that activation of CRF-R1, by central as well as by local infusion of CRF in the MPOA, increased local oxytocin release. In conclusion, intra-MPOA CRF-R signaling, particularly of subtype 1, impaired behavioral and emotional postpartum adaptations and, therefore, needs to be dampened during lactation. Furthermore, to counteract the negative impact of CRF-R activation on maternal behavior, oxytocin release in response to CRF-R1 activation may provide a regulatory mechanism.

PS1.00165 RFP-BASED MOLECULAR TOOLS TO DETECT AND UTILIZE GLUCOCORTICOID RECEPTOR ACTIVATION

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Glucocorticoid receptors (GRs) are widely involved in stress responses by acting as transcription factors to regulate gene expression. GRs are found in the cytoplasm, but after ligand binding

they translocate to the nucleus and then form dimers for interaction with DNA. GFP has been used to observe nuclear translocation of GRs, but its detection in living animals is still difficult. In this study, we used dimer-dependent RFPs (ddRFPs) in order to detect dimerization of GRs by fluorescence intensity change. ddRFPs are fluorgenic proteins that emit red fluorescence upon its dimerization. We also generated RFP-dependent Cre recombinase by employing split Cre fragments fused with nanobodies or designed ankyrin repeat proteins (DARPins) that specifically bind to mCherry and other variant RFPs. We found that functional binding units are different between monomeric mCherry and dimeric tdTomato. We also found that cellular localization of target RFPs affected recombinase activity of RFP-dependent Cre. Luciferase assays showed that dimerization and nuclear translocation of GRs induced by dexamethasone increase recombinase activity of RFP-dependent Cre targeting RFP-tagged GRs. Using adeno-associated virus vectors, we confirmed selective FLEX switching induced by RFP-dependent Cre in mRFP1-expressing neurons *in vivo*. These results suggest a new method to detect GR activation and/or utilize dimerization and nuclear translocation of GR for selective gene expression in living animals.

PS1.00166 REGULATION OF CRH NEURONAL NETWORK ACTIVITY BY NORADRENERGIC STRESS SIGNALS.

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Stress relevant information is relayed from multiple brain areas to the corticotropin-releasing hormone (CRH) neurons within the paraventricular nucleus (PVN) of the hypothalamus in order to control the stress response. One neurotransmitter that is essential for the activation of CRH neurons following stress is noradrenaline (NA). However, the effects of NA on the activity patterns of CRH neurons are unknown. To reveal this, we have performed GCaMP6f calcium imaging and electrical recordings from CRH neurons in brain slices. We find that bath application of NA (10 uM) induces a robust enhancement in CRH neuron network excitability with most CRH neurons switching into a bursting pattern of activity. Ca^{2+} bursts were tightly synchronized with action potential bursts within neurons, but no coordination of Ca^{2+} bursting activity was observed between different neurons. NA induced excitation required $alpha_1$ adrenergic receptors but not glutamatergic or GABAergic fast synaptic transmission. Surprisingly, Ca^{2+} bursts persisted after blocking action potentials with tetrodotoxin (1uM). However, Ca^{2+} bursts were inhibited with bath application of voltage gated calcium channel blockers (100uM $CdCl_2$ or 100uM nifedipine). Release of Ca^{2+} from internal stores was not found to contribute to NA induced Ca^{2+} bursts. Together these data demonstrate that NA drives CRH neurons into a bursting pattern of activity with large underlying Ca^{2+} oscillations. This pattern of activity may be important for induction of plasticity in this neural circuit as well as driving efficient secretion of CRH peptide during periods of stress.

PS1.00167 LONG-TERM EFFECTS OF SYNTHETIC GLUCOCORTICOID THERAPY ON THE ADRENAL GLAND STEROIDOGENIC PATHWAY

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Pharmacological treatment with synthetic glucocorticoids, which are widely prescribed for the treatment of numerous inflammatory and autoimmune diseases, can also affect the way the adrenal gland produces cortisol. Indeed, patients undergoing synthetic glucocorticoid treatment can develop adrenal insufficiency. This condition is characterised by reduced responsiveness of the adrenal to ACTH stimulation, and adrenal crisis/shock can occur in response to acute physiological stress (e.g. surgical or inflammatory stress). Here we have investigated the effects of prolonged treatment with the synthetic glucocorticoid methylprednisolone on HPA axis dynamics and on the adrenal steroidogenic pathway. We have found that 5 days of treatment with methylprednisolone not only suppresses basal ACTH and corticosterone secretion, as well as corticosterone secretion in response to a high dose of ACTH, but also down-regulates key genes in the adrenal steroidogenic pathway, including StAR, MRAP, CYP11A1 and CYP11B1. Importantly, 5 days after withdrawal of the treatment, ACTH levels are restored, yet basal levels of corticosterone, as well as some key steroidogenic genes, including MC2R and HSL, remain down regulated. Our data suggests that the steroidogenic pathway is directly affected by synthetic glucocorticoid treatment in the long-term, presumably via a mechanism involving activation of the glucocorticoid receptor.

PS1.00168 STRESS FAMILIARITY BUT NOT FAST NEGATIVE FEEDBACK ROBUSTLY TUNES STRESS NEURON OUTPUT

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Corticotropin-releasing hormone (CRH) neurons are the critical final output cells that drive the neuroendocrine stress response. Using fibre photometry in freely behaving mice, we recorded the natural CRH neuron activity dynamics to understand how their excitability is modulated. We observed that CRH neurons are tonically active and their excitability can be rapidly modulated, revealing distinct patterns of activity at rest, during, and after stress (loud white noise). After stress, the majority of CRH neuron “shut-off” (return to baseline) occurred within minutes and was too fast to be explained by CORT negative feedback. Consistent with this, termination of post-stress excitability was unaffected by metyrapone-induced inhibition of CORT synthesis. We then tested the CRH neuron stress response in the presence of CORT feedback, within the timeframe of non-genomic actions. Using either endogenously released or exogenously administered CORT, both experiments resulted in a very modest suppression of the CRH

response. Interestingly, a far stronger inhibition of ACTH release was observed, indicating that fast CORT feedback favours pituitary inhibition and that CRH neuron shut-off dynamics do not require CORT feedback after an acute stress. Independently of CORT milieu, stress familiarity dramatically reduced the CRH stress response. Habituation to sequential homotypic stress was observed as early as 30min. A strong habituation was observed across 4 consecutive days of single homotypic stress, which could then be completely extinguished following 3-weeks of stressor abstinence. These data reveal that CRH neurons are surprigingly vigilant, respond rapidly in response to threat, and exhibit robust plasticity.

PS1.00169 THE EFFECTS OF CHRONIC PERI-ADOLESCENT ASTHMA ON ACUTE BRAIN AND PERIPHERAL IMMUNE RESPONSES.

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Asthma is a common adolescent chronic health challenge affecting 9% of U.S. adolescents and is often comorbid with anxiety and depression. Little is known about the neurobehavioral impacts of chronic adolescent asthma. Microglia, the immune cells of the brain, become activated after peripheral insult, and their over-activation is implicated in development of neuropsychiatric disorders. The mechanism underlying asthma and internalizing disorder comorbidity, and the involvement of microglia, are unknown. To determine these mechanisms, we developed a BALB/c mouse model of chronic developmental asthma to individually manipulate airway inflammation (via repeated exposure to house dust mite extract, HDM) and labored breathing (via repeated exposure to methacholine, MCH). We have previously demonstrated that mice exposed to adolescent MCH had significantly higher adult anxiety-related neurobiological and behavioral symptoms than unexposed mice. Here, we examined the acute effects of HDM and MCH on lung immune function, circulating corticosterone concentration, and microglia activation at postnatal day (P) 56 at 0, 1, 2, 4, 8, or 24 hours after final asthma treatment (HDM or MCH). At P56, lung *IL-1β* and *IL-5* gene expression peaked at 4 hours after final HDM treatment. Alveolar macrophages were significantly higher and serum corticosterone concentration was blunted in HDM animals compared to other groups. Based on *Cd11b* expression, hippocampal microglia did not respond acutely in a time-dependent manner. However, preliminary data suggest that there may be a chronic impact on baseline microglia activity following this asthma paradigm. These results provide preliminary background on the potential role of microglia in asthma-anxiety comorbidity.

PS1.0017 SEXUALLY DIMORPHIC REGULATION OF LOCAL PROTEIN SYNTHESIS BY ESTRADIOL

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Estrogens, particularly 17beta-estradiol (estradiol), have repeatedly been shown to have long-lasting influences over learning and memory. The mechanisms underlying estrogenic-facilitation of memory are driven partly through activation of signalling cascades, resulting in modulation of synaptic structure and function. However, growing evidence indicates that estradiol can also rapidly modulate local protein synthesis; the ability to produce nascent proteins near or at synapses, independent of gene transcription. However, the molecular and cellular mechanisms that underlie estradiol's ability to regulate local protein synthesis and subsequently, the consequence of modulating local protein translation of synaptic function and whether male and female brains utilise the same signalling mechanism to regulate this process are unknown. Using Surface Sensing of Translation (SUnSET) and fluorescent non–canonical amino acid tagging (FUNCAT) assays, we demonstrate that estradiol increases protein synthesis in acute hippocampal slices prepared from 10-12 week old male and ovariectomized female mice. Critically, underlying signalling pathways were different between males and females. Interestingly we find that the result of this increased local translation is an increase in a subset of synaptic proteins in both sexes. Furthermore, using superresolution imaging we are determining whether these nascent proteins are targeted to synapses. Taken together, our study suggests that the rapid modulation of local protein synthesis by estradiol may result in an increase in synaptic function in male and females and thus, underlie the facilitation of cognition offered by estrogens; this however, occurs via distinct signalling mechanisms between sexes.

PS1.00170 GHRELIN'S ROLE IN THE HPA AXIS RESPONSE TO IMMUNE CHALLENGE

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Ghrelin is known to regulate HPA axis responses to psychological stress at the level of the brain and pituitary. However, its role in the hypothalamic-pituitary-adrenal (HPA) axis response to immune challenge is still imprecisely described. We hypothesized ghrelin's anti-inflammatory actions on lipopolysaccharide (LPS)-induced cytokine release are mediated by activation of the HPA axis. We gave the rats a single injection of acylated (AG) or desacylated (DAG; 1 mg/kg sc), with a concomitant injection of LPS (*E. coli*, 100 µg/kg i.p.) or pyrogen-free saline. We assessed circulating cytokines and paraventricular nucleus of the hypothalamus (PVN) neuronal

activation (c-Fos) as well as *in vitro* pituitary and adrenal responses. Our data suggest that high AG stimulates release of adrenocorticotropic hormone (ACTH) from the anterior pituitary immediately upon exposure. At the same time, LPS activates the PVN. The AG-induced corticosterone response is not sufficient to prevent LPS-induced activation of the PVN. However, it is sufficient to suppression of cytokine transcription that persists for 2 hr despite AG being very quickly metabolized to DAG. DAG alone does not affect HPA axis or cytokine transcription at 2 hr, supporting the idea that the effect of AG on cytokines is one that is very early in the response. Despite evidence for a potentiating effect of DAG on ACTH-induced corticosterone release from the adrenal *in vitro*, exogenous DAG does not influence either HPA axis or cytokine transcription. These data reveal an important modulatory role for the different forms of ghrelin responses to immune challenge involving the HPA axis.

PS1.00171 OXYTOCIN RECEPTOR ANTAGONISM, SOCIAL BEHAVIOUR, & CORTICOSTERONE RELEASE IN ADOLESCENT-STRESSED RATS

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Social instability stress (SS) from postnatal day 30 to 45 in adolescent rats increased oxytocin receptor (OTR) densities in the lateral septum and nucleus accumbens. Here, we investigated the effects of the OTR antagonist L-368,899 (OTRa) on social behaviour and corticosterone release in SS and non-stressed control (CTL) rats. Rats were injected with 1 mg/kg of L-368,899 or saline 40 min before behaviour testing. Blood samples were collected 20 min after injection, and immediately and 60 min after the social interaction test. Rats had greater corticosterone concentrations immediately after social interaction compared to before or 60 min after social interaction ($p<0.001$). OTRa rats had higher corticosterone than saline rats ($p=0.035$), and CTL rats had higher corticosterone concentrations than SS rats ($p=0.045$). The corticosterone increase in OTRa versus saline rats was significant in CTL ($p=0.034$) and not in SS rats ($p=0.359$), such that the difference in corticosterone between CTL and SS was driven by those receiving OTRa ($p=0.068$; saline, $p=0.575$). Consistent with our previous reports, SS rats spent greater time in social approach ($p=0.001$) and reduced time spent in social interaction ($p=0.026$) than did CTL rats. SS injected with OTRa spent less time interacting with an unfamiliar peer compared with saline-injected SS, but the difference was not statistically significant ($p=0.09$). In sum, CTL rats have heightened corticosterone release than do SS rats because of a greater sensitivity to OTRa. The effect of OTRa on social behaviour in SS rats may be diminished because of their low social interaction (floor effect).

PS1.00172 EFFECTS OF EARLY-LIFE STRESS ON METABOLISM, THE HYPOTHALAMIC FEEDING CIRCUITRY AND FOOD PREFERENCES

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Exposure to early-life stress (ES) is associated with increased vulnerability to metabolic disorders such as obesity. We have shown that ES in mice leads to an acute and lasting reduction in adipose tissue and leptin levels, thus to a seemingly leaner phenotype. In addition, we showed that ES-exposed mice, although starting from a leaner phenotype, accumulate more fat in response to a moderate obesogenic diet, and that ES leads to alterations in the hypothalamic circuitry early in development. These data lead us to hypothesize that the metabolic alterations might contribute to persistent alterations in morphology and function of the hypothalamic feeding circuitry, possibly affecting food preference, and that these changes in concert might lead to the increased vulnerability of ES-exposed individuals to develop obesity later in life. We used an established ES mouse model, in which we provide limited nesting and bedding material from postnatal day 2-9, and characterized NPY, AgRP and POMC expression in the hypothalamus in adult animals as well as food preference with a free choice high-fat high-sugar diet. In addition, we further investigated metabolic programming by ES by studying liver lipid and glucose metabolism, and showed that (liver and brain) lipid composition and PPARa levels are affected by ES. We conclude that ES exposure lastingly affects metabolism and levels of metabolic hormones. To what extent the regulation of energy metabolism by the hypothalamic circuitry is affected, and whether these metabolic alterations are involved in the vulnerability to develop obesity later in life needs further investigation.

PS1.00173 MILD BLAST TBI DIFFERENTIALLY DISRUPTS THE CRF-UCN STRESS SYSTEM IN MALES AND FEMALES

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Traumatic brain injury (TBI) affects 10 million people world-wide. In recent wars, a significant portion are induced by explosive devices. Mild blast TBI (mbTBI) has a unique signature, increasing susceptibility to psychological disorders. Corticotropin releasing factor (CRF) is associated with endocrine, autonomic and behavioral stress responses. CRF and the related peptide, urocorotin (UCN), modulate anxiety-related behaviors via binding to CRF receptors (CRFR1/2). Not only is there limited information on how mbTBI results in anxiety-like behaviors, but sex differences are understudied. Therefore, we examined the effect of mbTBI on the CRF-UCN system. Although both males and females had increased anxiety-like behaviors in the elevated plus maze ($p<0.05$), they had different patterns of gene expression after mbTBI. In the

anterior bed nucleus of the stria terminalis (aBNST), mbTBI decreased baseline CRF, increased restraint-induced CRF and decreased restraint-induced CRFR2 in males ($p<0.05$). In addition, mbTBI decreased restraint-induced CRFR2 in the amygdala ($p<0.05$). In females, mbTBI increased baseline UCNI and restraint-induced UCNIII in the posterior (p)BNST ($p<0.05$). In the amygdala, mbTBI decreased baseline and restraint-induced UCNII ($p<0.05$). Overall mbTBI altered CRF, hnCRF and CRFR2 expression in males and UCNI,II,III expression in females. Within the BNST and amygdala, CRF neurons may play a stronger role in regulating the behavioral stress response, while UCN populations may modulate the autonomic stress response, ultimately both manifesting as anxiety-like behaviors. Targeting the projections from specific neuronal populations within these structures may provide insight into sex dependent mechanisms of stress regulation after TBI.

PS1.00174 INVESTIGATING THE NATURAL RHYTHMS OF CRH NEURON EXCITABILITY OVER THE DAY

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Corticotropin-releasing hormone (CRH) neurons are the final regulators of the neuroendocrine stress response and corticosterone (CORT) secretion. CRH as well as CORT secretion is known to follow a circadian and ultradian pattern of release. Plasma CORT levels are also hypothesised to influence the responsiveness of CRH neurons during stress. However, the natural activity patterns of the CRH neuron population have never been recorded in vivo across the day-night cycle. Using GCaMP6s fiber photometry, we have optically measured the natural activity patterns of the CRH neuron population in awake behaving adult male mice over the 24-hour day. In addition, we tested if the CRH neural responses to stress differed at the circadian peak or nadir of CORT (evening versus morning). Our preliminary results reveal distinct oscillating bursts of activity in the CRH neuron population across the day with no obvious circadian rhythmicity (n = 8 mice). Intervals between bursts were variable both within and between animals. In response to a 5-minute white noise stress, preliminary results (n=3 mice) indicate that the CRH population was 24%±34% less active during circadian peak levels of CORT compared to trough levels. On-going experiments will increase the sample size of this experiment. Together these data reveal natural ultradian rhythms in CRH neuron excitability across the day. While spontaneous population activity did not vary between morning and evening, CRH neuron responsiveness to stress may be regulated in a circadian rhythm.

PS1.00175 EFFECTS OF CHRONIC CORTICOSTERONE ADMINISTRATION ON MALE SEXUAL FUNCTION AND ITCH SENSATION IN RATS

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Chronic psychological stress causes various physiological dysfunctions such as erectile dysfunction and chronic itch symptom (atopic dermatitis), but little is known about the mechanisms. In our study, chronic-stress rat model produced by the repeated administration of exogenous corticosterone for 3 weeks were used to analyze the effects of chronic psychological stress on these dysfunctions. The chronic administration of corticosterone decreased the body weight, plasma testosterone level, genital weights, semen volume, and erectile ability in male rats. More importantly, the chronic corticosterone treatment increased the scratching behavior as a marker of itch sensation induced by peripheral itch mediator, serotonin, but did not change the tactile sensitivity and noxious heat sensitivity. We further focused on two different gastrin-releasing peptide (GRP) systems in the spinal cord. GRP system involved in the male sexual function is located in the lumbar spinal autonomic and motor systems, and the other GRP system related to itch transmission is in the spinal somatosensory system. The chronic corticosterone administration increased GRP mRNA expression in the spinal cord specifically involved in the itch transmission. Taken together, these results showed that the male sexual dysfunction and itch hypersensitivity due to the chronic stress were observed in this rat model as well as humans, and suggest that two different GRP systems in the spinal cord which control the male sexual function or itch transmission respond independently to chronic stress.

PS1.00176 TEMPERATURE AND TACTILE STIMULATION ALTER STRESS-RELATED GENE EXPRESSION IN THE NEONATAL RAT BRAIN

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Early life experiences can affect stress response in later life. In rat pups, maternal licking/grooming in the first week of life can regulate adult stress reactivity. However, we do not know whether the temperature drop pups experience during the mother's absence facilitate the effects of licking/grooming when the mother returns. To study this potential interaction, we briefly separated pups in the first week of life at room temperature (19-21º C) or nest temperature (33-35º C) and provided half the animals in a litter with supplemental tactile stimulation (a proxy for licking/grooming). Results indicate that temperature conditions affected corticotropin-releasing factor and oxytocin gene expression in the week-old rat paraventricular nucleus of the hypothalamus. In addition, tactile stimulation decreased paraventricular corticotropin-releasing factor, oxytocin, and arginine vasopressin gene expression. These findings suggest that both temperature and tactile stimulation affect gene

expression related to the stress response early in life. We are currently investigating thyroid hormone, implicated in both temperature and tactile stimulation, as a mechanism for these differences.

PS1.00177 ASTHMA DURING DEVELOPMENT LEADS TO LONG-TERM CHANGES IN STRESS-REGULATORY GENE EXPRESSION

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In developing youth, allergic asthma is the most common, chronic condition, with approximately 10% of US children affected. Asthma is associated with additional health issues, in particular psychiatric conditions that are associated with altered stress regulation. To understand causal mechanisms by which developmental asthma may lead to these health conditions, we used a mouse model to test long-term effects of asthma symptoms on adult gene expression in brain areas associated with stress regulation. We manipulated airway inflammation and labored breathing in young male and female BALB/cJ mice, then measured gene expression in the brain and lungs three months after final experimental asthma manipulations. Results indicate that allergen exposure, used to cause airway inflammation, led to persistent airway inflammation, increased collagen, and elevated asthma-related gene expression in the lungs three months after final allergen exposure. At this same age, experimentally-induced airway inflammation led to altered expression of genes related to stress regulation (i.e. prefrontal *corticotropin releasing hormone receptor 1* and hippocampal *glucocorticoid receptor*), whereas labored breathing led to altered expression of brainstem *serotonin transporter* gene. Importantly, pre-asthma fear-related behavior (ultrasonic vocalizations) and young adult housing conditions (group vs. individual) modulated these adult outcomes. For example, a previously documented short-term result of developmental asthma (i.e. decreased brainstem *serotonin transporter* gene expression) persisted for three months post-asthma in individually-housed mice, whereas this effect was reversed in group-housed mice. Developmental asthma may have long-term impacts on stress regulation, which may be moderated by social conditions and pre-asthma behavioral phenotype.

PS1.00178 PERINATAL HIGH FAT DIET EFFECTS ON THE DEVELOPMENT OF THE HPA AXIS IN NEONATAL RATS

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The maternal environment has a profound effect on the development of offspring, including responses to stress mediated by the hypothalamic-pituitary-adrenal (HPA) axis. Maternal diet programs the HPA axis in a manner that persists throughout adulthood, however studies of its effects on stress-related behaviors and physiology in neonatal life are limited. This study investigates the effects of perinatal high fat diet (HFD) on developmental trajectory of the HPA axis at two developmental time points by measuring neonatal stress physiology, ultrasonic vocalization, and gene expression. First, during the stress hyporesponsive period, postnatal day (PND) 7, when animals do not respond to many stressors known to elicit behavioral and physiological response in mature animals, and second as neonates emerge from this hyporesponsive period at PND 13. PND7 HFD pups produced fewer USVs and showed higher corticosterone levels in response to adult male odor as a stressor. Further, HFD pups had an upregulation in corticotropin-releasing hormone transcript levels in the paraventricular nucleus of the hypothalamus. PND13 HFD pups on the other hand exhibited increased anxiety-like behaviors represented by increased USVs and immobility, along with higher adrenocorticotropic hormone in response to adult male odor. This was associated with lower glucocorticoid receptor transcript levels in the ventral hippocampus, indicating a disinhibited HPA axis negative feedback. These results indicate an alteration in the typical responses to stress during the hyporesponsive period of the HPA axis as a function of perinatal HFD exposure, which may involve changes in the regulation of genes mediating the HPA axis.

PS1.00179 SEASONAL CHANGES IN PITUITARY FEEDBACK SENSITIVITY AND PLASMA CORTICOSTERONE IN SPARROWS

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Environmental conditions are known to influence avian stress physiology with higher corticosterone secretion often observed in birds coping with harsh environments. Circulating corticosterone is regulated by negative feedback through mineralocorticoid (MR) and glucocorticoid (GR) receptors, although the amount of hormone that reaches the receptors is also regulated by 11βHydroxysteroid dehydrogenase (11βHSD1) which reactivates and 11βHSD2 which can deactivate the hormone. The pituitary gland has been proposed as one site of stress axis regulation. Here we compared plasma corticosterone concentrations and pituitary

mRNA expression using qPCR in two sub-species of free-living White-crowned sparrow (*Zonotrichia leucophrys)* during breeding, molt, and wintering stages. *Z. l. gambelii*, migrates from temperate California to breed in the Alaskan Arctic and *Z. l. Nuttalli* is a non-migratory resident of California. Corticosterone was highest in migrants during breeding compared to molt and wintering while residents showed lower corticosterone levels during molt compared to breeding and winter. During breeding, migrant males had higher corticosterone compared to residents while no difference were found for females. Pituitary MR and GR mRNA for both sexes of migrants and male residents were lowest during breeding when plasma corticosterone was elevated. supporting a reduced negative feedback mechanism. Concordantly, to reduce local availability of hormone for negative feedback, 11βHSD1 expression was lower and 11βHSD2 higher during breeding compared to wintering in male migrants. Together these data evidence exquisite plasticity of pituitary cellular feedback control as necessitated by unique stress profiles between resident and migrant white-crowned sparrows at key stages of the annual cycle.

PS1.0018 SEX-SPECIFIC MODULATION OF JUVENILE MIDBRAIN DOPAMINE EXPRESSION BY PERINATAL SEROTONIN

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The developmental hyperserotonemia rat model of autism spectrum disorder (ASD) induces a number of neural changes that help explain symptomology, particularly deficits in social behavior. However, ASD is also characterized by stereotypical and repetitive behaviors, and although developmental manipulations of serotonin can also induce these behaviors, the underlying neurobiology is not well understood. In this experiment, we hypothesized that developmental hyperserotonemia could alter midbrain dopamine expression in juveniles. We injected pregnant dams and subsequently pups with a serotonin agonist, 5-methoxytryptamine, from embryonic day 12 to postnatal day 20. At postnatal day 30–32, we perfused subjects, collected and sectioned brain tissue, and processed it immunohistochemically for tyrosine hydroxylase. We then counted the number of dopaminergic neurons in both the substantia nigra and the ventral tegmental area. Surprisingly, we found a female-biased sex difference in the number of tyrosine hydroxylase-immunoreactive neurons in control subjects in the substantia nigra. Furthermore, the effect 5-MT treatment was male-specific such that it increased the number of dopaminergic neurons to the level of females. This is particularly interesting given that repetitive behaviors are typically more extreme in males, and they decrease after puberty. An increased sensitivity to developmental perturbations in males could explain this susceptibility. In contrast, no sex difference was observed in the VTA, nor was there any effect of treatment. We are now exploring whether this sex-specific effect of hyperserotonemia is limited to the substantia nigra by analyzing tyrosine hydroxylase expression in other dopamine-rich areas, such as the AVPV and the zona incerta.

PS1.00180 IMMIGRATION STRESS, CORTISOL AND IMMUNE FUNCTIONING IN LATINO IMMIGRANT FARMWORKERS

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The stress of immigration necessitates that we better understand how adversity, in particular, socioeconomic challenge and discrimination by the dominant culture, compromises neuroendocrine functioning and, in turn, health. We investigated immigration-linked stress in a sample of N=61 Latino immigrant farmworkers living in Oregon (*M* age = 37.2, *SD* = 10.3) an average of 9.7 years (*SD* = 7.2). Participants provided saliva samples 3 times daily for 2 consecutive days, which we used to model salivary cortisol area under the curve with respect to ground (AUCg). Dried blood spots yielded two measures of immune functioning: C-reactive protein (CRP) and Epstein Barr Virus (EBV) antibodies. Our hypothesis: greater socioeconomic adversity (as measured an income-to-need ratio) and self-reported discriminatory stress would explain greater cortisol dysregulation (AUCg), greater inflammation (CRP), and elevated immunosuppression (EBV). Reflecting evidence that HPA dysregulation provides a pathway linking stress and poor health, we also tested whether individual differences in AUCg mediated the link between immigration stress and the health biomarkers. Adjusting for age, gender, BMI, regression models suggest that discrimination (*b* = -.41, *p* = .001) but not income-to-need (*b* = -.06, *p* = .27) predicts lower AUCg, which we interpret as cortisol blunting. We find mixed support for a mediated pathway. For men, a significant link between discrimination and elevated CRP and EBV appears partially mediated by cortisol blunting (R^2 reduction of 2.9% and 4.4%, respectively). For women, a significant link between discrimination and greater CRP appears mediated by higher not lower diurnal cortisol (R^2 reduction of 5.1%).

PS1.00181 AN EXAMINATION OF HIPPOCAMPAL MICROGLIA AND ANXIETY BEHAVIOR AFTER CHRONIC PERI-ADOLESCENT ASTHMA.

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Asthma is a chronic allergic disease that affects over 22 million people in the U.S. including 9% of adolescents. Asthma is also often comorbid with anxiety, depression and behavioral disorders. Importantly, adolescence is a period of substantial neurological development, which coincides with asthma and associated conditions. The hippocampus, which is implicated in mental health function, has a high density of microglia, the resident immune cells of the brain that can become activated due to peripheral immune challenges. Asthma is characterized by an influx of eosinophils, which are attracted by the chemokine CCL11. Previous research

demonstrates that microglia express CCR3, the main receptor for CCL11. In the current study, we determined whether peripheral asthma symptoms (airway inflammation, labored breathing) during development led to increased microglial activation in the hippocampus. In a mouse model, we used house dust mite extract, the most common human allergen, and methacholine, a muscarinic receptor agonist, to induce airway inflammation and labored breathing, respectively. Hippocampal samples were collected at 0, 1, 2, 4, 8, and 24 hours after the final asthma exposure on postnatal day 56 and stained for *Iba1*, a microglia marker. *CD11b,* an eosinophil marker*,* and *CCR3* expression were measured with rtPCR. Preliminary evidence indicates that these genes are upregulated after developmental labored breathing. One cohort of mice was tested on the elevated plus maze, and data indicate that methacholine-induced labored breathing also caused increased anxiety-like behavior. These data suggest that developmental labored breathing may alter microglia function to influence asthma-anxiety comorbidity.

PS1.00182 EARLY-LIFE STRESS AND NERVE INJURY IN ADULTHOOD ALTER CRF-R & ACTH-R EXPRESSION IN THE HPA AXIS

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Background and Aims: Despite triggering similar increases in thermal and mechanical sensitivity in all rats, chronic constriction of the sciatic nerve (CCI) evokes disturbances in social behaviour and emotional coping style in only a subgroup (30%) of injured rats. This subgroup also shows altered HPA-axis regulation. A possible contributor to the heterogeneity of complex behavioural and neuroendocrine responses to injury may be early-life experiences. We investigated this possibility by examining, in a rat model of neuropathic pain, whether an early-life stress (ELS) might drive an altered hormone-receptor expression profile in key loci of the HPA axis that may underpin the altered regulation of the HPA. **Methods:** On post-natal days 3 and 5, male, Sprague-Dawley rats received *i.p.*, injections of lipopolysaccharide (0.05mg/kg: *ELS*). *Control* litters received equi-volume sterile saline injections. In adulthood, all rats underwent social-interactions testing 6 days before, and 6 days after a CCI. Adrenal and pituitary glands from rats were sectioned and labelled immunohistochemically for ACTH and CRF receptors. **Results:** Compared to uninjured rats, CRF-receptor immunoreactivity in the right anterior pituitary was increased in ELS rats experiencing disturbances in social interactions post-CCI. ACTH-receptor immunoreactivity in the right adrenal gland was decreased in rats experiencing social disturbances compared to rats that showed no behavioural change post-CCI. **Conclusion:** Nerve injury alone, and when coupled with ELS alters the expression of key HPA-axis hormone-receptors in the pituitary and adrenal glands. These changes occur in a manner, which depends on an individual's emotional coping style in response to nerve injury.

PS1.00183 ADOLESCENT SOCIAL STRESS LEADS TO LASTING, ALTERED RESPONSES TO IMMUNE CHALLENGE IN FEMALE RATS.

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We investigated how adolescent social instability stress (SS; from postnatal day [P] 30-45, daily 1 hour isolation + new cage partner) influenced responding to an immune challenge either soon after SS or several weeks later, compared with non-stress controls (CTL) in Long Evans male and female rats. At either P46 or P70, rats were injected with a low dose of lipopolysaccharide (LPS 0.1 mg/kg) or vehicle (saline). Sickness behaviour and plasma corticosterone concentrations were determined at 1, 2, 4, 6, and 24 h after injection. In brief, the main results (all $p < 0.05$) indicated that males displayed sickness behaviour earlier and for longer than did females after LPS, and SS males showed more sickness behaviour than did CTL males at P46. Among LPS-treated, CTL females had higher corticosterone concentrations at 1 hr and 6 hr than did SS rats, whereas SS had higher corticosterone concentrations at 2 hr than did CTL rats. In males, SS rats had higher corticosterone concentrations than CTL rats irrespective of treatment. Saline-treated rats showed no sickness behaviour and had lower corticosterone concentrations than did LPS-treated rats. These results indicate that stress in adolescence can lead to sex-specific, long-lasting, altered responses to an immune challenge. Thus, stress in adolescence may influence health outcomes in adulthood.

PS1.00184 INTERACTIONS BETWEEN PGE2- AND E2-SIGNALING PATHWAYS ARE NEUROPROTECTIVE IN THE INJURED BRAIN

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Estrogens are profound modulators of brain and behavior across the lifespan. In the brain, aromatase (estrogen synthase) is expressed constitutively in neurons but is induced in astrocytes following brain injury. The resultant increase in neural estradiol (E_2) is neuroprotective. In the songbird, injury-associated COX-activity is necessary for the induction of astrocytic aromatase, and injury-induced E_2 decreases neuroinflammation. The mechanisms underlying these effects are completely unknown. We first documented dramatic changes in the prostaglandin E2 (PGE2)-receptors EP3 and EP4, and the estrogen receptors ERα and ERβ following brain injury. We hypothesized that EP3 and EP4 were involved in induction of E_2-synthesis, and ERα and ERβ were responsible for the anti-inflammatory actions of E_2. To test this, adult birds received bilateral injections of vehicle or the appropriate receptor antagonist into contralateral telencephalic lobes. EP-antagonists (L-798,106 (EP3) or BGC 20-1531 hydrochloride (EP4)) and the ER-antagonists (MPP-9 (ERα) or PHTPP (ERβ)) decreased receptor-dependent downstream signaling but not the availability of the ligands PGE2 and E_2. Antagonism of EP3 and EP4 prevented injury-induced increases in aromatase expression and

E_2 content in males and females respectively, suggesting sex-specific, PGE2-dependent induction of E_2 synthesis following brain injury in the songbird. Antagonism of ERα but not ERβ, caused an exaggerated increase of PGE2 relative to the contralateral lobe, suggesting that ERα-dependent signaling pathways may be responsible for the anti-inflammatory effects of injury-induced aromatization in both sexes. Taken together, the data suggest an elegant feedback loop between inflammatory and steroidogenic pathways in the traumatized vertebrate brain.

PS1.00185 SEXUALLY DIMORPHIC CORTICOTROPIN-RELEASING FACTOR RECEPTOR 1 NUCLEI IN THE MOUSE FOREBRAIN

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Sex-dependent exposure to gonadal hormones and influences on neural circuitry are generally believed to underlie sex differences in the prevalence of stress/mood disorders (females>males) although the specific brain areas involved are largely unknown. We have identified sex differences in corticotropin releasing factor receptor 1 (CRFR1) distribution within 3 forebrain structures. The medial preoptic nucleus (MPN) and the paraventricular nucleus of the hypothalamus (PVN) both express higher levels of CRFR1 in the male, whereas higher levels of expression were seen in the female rostral/anteroventral periventricular nucleus (AVPV/PeN). The AVPV/PeN sex difference is apparent in the early neonatal period and is regulated by perinatal and not adult gonadal hormones. A single injection of testosterone on the day of birth reverses the AVPV/PeN sex difference, while adult gonadectomy has no effect. On the contrary, PVH and MPN populations do not become sexually dimorphic until puberty or adulthood and both show decreases in CRFR1 following adult male gonadectomy. We further demonstrate that estrogen receptor alpha is highly co-expressed with CRFR1 in all three hypothalamic regions, indicating a receptor site through which gonadal hormones can affect CRFR1 function and expression. Glucocorticoid receptor is also highly co-expressed in CRFR1 cells in all three regions, suggesting they may be influenced by circulating glucocorticoid levels. Following acute and chronic stressors, AVPV/PeN, PVH, and MPN CRFR1 cell groups show sex specific alterations in expression and neural activation patterns. Given the known role of CRFR1 in anxiety and depression, these dimorphic structures may mediate observed sex differences.

PS1.00186 OXYTOCIN IN THE BED NUCLEUS OF THE STRIA TERMINALIS PROMOTES SOCIAL AVOIDANCE IN NOVEL CONTEXTS

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Oxytocin (OT) is usually considered a prosocial hormone, but growing evidence suggests that the behavioral effects of OT are context and sex-specific. It has been proposed that OT enhances the salience of positive and negative social stimuli; nonetheless, the underlying mechanisms are not understood. We previously showed that social defeat induces social avoidance and social vigilance in female but not male California mice. This response is accompanied by hyperactivity in OT neurons in bed nucleus of the stria terminalis (BNST). In stressed females, one systemic dose of OT receptor antagonist (OTA) reversed the effects of stress on social avoidance and vigilance, and infusion of OTA into the BNST had identical effects. Here, we show that morpholino knockdown of OT in the BNST prevents stress-induced phenotype in females, and furthermore, infusion of OT into the BNST induces social avoidance and vigilance in females naïve to social defat. Preliminary data suggests that in males, OT in BNST also induces social avoidance, which is surprising considering that intranasal OT increases male social interaction. Together, our results suggest that OT signaling within BNST enhances the salience of unknown social cues, and may partly explain how OT can have such diverse effects depending on the context.

PS1.00187 OXYTOCIN AND OXYTOCIN RECEPTOR METHYLATION ASSOCIATIONS WITH ETHNIC DISCRIMINATION IN LATINAS

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Latinas represent the largest minority group in the United States, with the highest fertility rate among all ethnic groups. Latina mothers are exposed to a multitude of stressors, including chronic discrimination which adversely affects psychological health. This combination of stressors, particularly the accumulation of lifetime stress, can contribute to biological vulnerability and impair the overall health of Latina mothers and their children. For example, the prevalence of perinatal depression in US Latinas is an estimated three times higher than that of the general US population. One key neurohormone involved in both maternal care and the stress response is oxytocin (OXT). The objective of this project was to assess associations between perceived ethnic discrimination, plasma oxytocin levels, and oxytocin receptor (OXTR) methylation. It was hypothesized that oxytocin measures would be negatively associated with ethnic discrimination. Pregnant Latina women (n=150) living in North Carolina were enrolled and data collection was completed during the prenatal visit at 24-32 weeks gestation (T1) and at 4-6 weeks postpartum (T2). Blood was collected at T1, and the Everyday Discrimination Scale was administered at T1 and T2. Plasma OXT levels were negatively associated with ethnic discrimination at both T1 and T2. In contrast, OXTR methylation was positively associated with discrimination at T2. In addition, plasma OXT was negatively correlated with methylation of the glucocorticoid receptor gene. It is concluded that OXT variables represent reliable indicators of

exposure to ethnic discrimination in Latina mothers, and this neurohormone may also be an effective treatment target in this neglected population.

PS1.00188 TH17 CELLS INDUCE DOPAMINERGIC NEURONAL DEATH VIA LFA-1/ICAM-1 INTERACTION IN PARKINSON'S DISEASE

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T helper (Th)17 cells, a subset of $CD4^+$ T lymphocytes, have strong pro-inflammatory property and appear to be essential in pathogenesis of many inflammatory diseases. However, the involvement of Th17 cells in Parkinson's disease (PD) that is characterized by a progressive degeneration of dopaminergic (DAergic) neurons in the nigrostriatal system is unclear. Here we aimed to demonstrate that Th17 cells infiltrate into brain parenchyma and induce neuroinflammation and DAergic neuronal death in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)- or 1-methyl-4-phenylpyridinium (MPP^+)-induced PD models. Blood-brain barrier (BBB) disruption in the substantia nigra (SN) was assessed by the signal of FITC-labeled albumin that was injected into blood circulation via the ascending aorta. Live cell imaging system was used to observe a direct contact of Th17 cells with neurons by staining these cells using the two adhesion molecules, leukocyte function-associated antigen (LFA)-1 and intercellular adhesion molecule (ICAM)-1, respectively. Th17 cells invaded into the SN where BBB was disrupted in MPTP-induced PD mice. Th17 cells exacerbated DAergic neuronal loss and pro-inflammatory/neurotrophic factor disorders in MPP^+-treated ventral mesencephalic (VM) cell cultures. A direct contact of LFA-1-stained Th17 cells with ICAM-1-stained VM neurons was dynamically captured. Either blocking LFA-1 in Th17 cells or blocking ICAM-1 in VM neurons with neutralizing antibodies abolished Th17-induced DAergic neuronal death. These results establish that Th17 cells infiltrate into brain parenchyma of PD mice through lesioned BBB and exert neurotoxic property by promoting glial activation and importantly by a direct damage to neurons depending on LFA-1/ICAM-1 interaction.

PS1.00189 NOREPINEPHRINE INHIBITS TH17 CELLS VIA BETA2-ADRENORECEPTOR SIGNALING IN COLLAGEN-INDUCED ARTHRITIS

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Background: Norepinephrine (NE), a neurotransmitter released from the sympathetic nerves, has been involved in rheumatoid arthritis (RA). However, role of the sympathetic nervous system in RA is divergent. Herein, we demonstrated that the sympathetic neurotransmitter NE

exerts an anti-inflammatory property in collagen-induced arthritis (CIA), a mouse model of RA, by inhibiting T-helper (Th)17 cell differentiation and function via β2-adrenoreceptor (β2-AR) signaling. **Material/Methods:** CIA was prepared by intradermal injection of collagen type II in tail base of DBA1/J mice. On the 41st day post-immunization, the mice were used as CIA models. $CD4^+$ T cells from the spleen were purified using magnetic cell sorting and activated with anti-CD3 anti-CD28 antibodies. Th17 cells were polarized from the $CD4^+$ T cells using various antibodies and cytokines. **Results:** A co-expression of CD4 and β2-AR was observed in spleens of both intact and CIA mice. The β2-AR expression in the ankle and spleen was downregulated in CIA mice. CIA induced increases in production of interleukin (IL)-17 and IL-22, $CD25^-IL\text{-}17^+$ cell percentage, and ROR-γt exprssion in $CD4^+$ T cells. Importantly, NE reduced the CIA-induced $CD4^+$ T cell shift towards Th17 phenotype and the β2-AR antagonist ICI118551 blocked the NE effect. Moreover, the β2-AR agonist terbutaline (Terb) inhibited CIA-induced $CD4^+$ T cell proliferation and shift towards Th17 phenotype, and the protein kinase A (PKA) inhibitor H-89 abolished the agonist effect. Terb also reduced CIA-induced Th17 enhancement, and H-89 impaired the Terb effect. **Conclusion:** NE inhibits Th17 cell differentiation and function in CIA condition by activation of β2-AR/PKA signaling.

PS1.0019 EFFECTS OF A MATERNAL HIGH-SUCROSE DIET ON OFFSPRING BEHAVIOUR AND NEUROENDOCRINOLOGY IN A RAT MODEL

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Adult female rats were placed on either a high-sucrose diet (25% kCal from sucrose) or a matched isocaloric diet (0% sucrose) prior to mating, during gestation, and during lactation. At weaning (postnatal day 21), all offspring were placed on a standard rat chow (3.3% sucrose). In adulthood (3-4 months old), offspring underwent a food preference test. They also underwent a progressive ratio test in operant conditioning chambers to measure their motivation to work for a sugar reward. In the food preference test, sucrose-exposed males showed an increased preference for high-sucrose diet and high-fat diet, relative to control males. In the progressive ratio test, sucrose-exposed males showed greater motivation to obtain a sugar reward, relative to control males. Sucrose exposure had no effects on behaviour of female offspring. To assess neuroendocrine correlates, we used qPCR to measure the expression of genes involved in steroid and dopamine signaling in the mesocorticolimbic system. In the mesocorticolimbic system of the offspring, early-life exposure to sucrose produced long-lasting changes in the expression of steroid and dopamine receptors in the ventral tegmental area and medial prefrontal cortex. These data suggest that a maternal diet high in sucrose has long-lasting effects on the behaviour and brain of the offspring.

PS1.00190 LUMAN REGULATES THE GLUCOCORTICOID MEDIATED STRESS RESPONSE

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Aberrant glucocorticoid signalling is implicated in many stress-related mental disorders. Glucocorticoids are released from the adrenal gland as the product of the Hypothalamic-Pituitary-Adrenal (HPA) axis activation, which in turn regulates the HPA via a negative feedback loop. Recently, Luman/CREB3 has been implicated as a key regulator of glucocorticoid-mediated stress responses. CREB3 is an endoplasmic reticulum-transmembrane protein; during the Unfolded Protein Response including stress in the Golgi, CREB3 translocates to the Golgi where the cytosolic fragment is liberated by proteolytic cleavage to act as a transcription factor in the nucleus. CREB3 deficient mice exhibit a blunted stress response as well as altered steroid hormone levels. Specifically, CREB3 deficient mice have decreased glucocorticoid and sex steroid hormone levels (androgens and estrogens), while the mice also exhibit "daredevil" behaviours when presented with stress stimuli. In addition, these mice have higher glucocorticoid receptor (GR) protein levels and activity. We took a molecular approach to characterize the functional interaction between CREB3 and GR. Our findings suggest that CREB3 may regulate the glucocorticoid signaling, both as a potent coactivator of GR and as a transcription factor acting on GR downstream genes directly.

PS1.00191 PITUITARY VOLUME MEDIATES THE EFFECT OF PRENATAL STRESS ON INTERNALIZING SYMPTOMS: PROJECT ICE STORM

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RATIONALE. Animal and human studies have shown that prenatal maternal stress (PNMS) affects the function of the hypothalamic-pituitary axes and is associated with more behavioural problems in the offspring. In our prospective longitudinal study of women exposed to a natural disaster during pregnancy (Project Ice Storm), we have found that more objective hardship (e.g., number of days without electricity) is associated with more internalizing problems in children. Neuroimaging studies have reported larger pituitary volumes to be associated with more internalizing symptoms during adolescence. **AIM.** The goal of this study was to determine whether pituitary volume mediates the association between objective PNMS and internalizing symptoms in Project Ice Storm youth. **METHODS:** Participants were 31 (17M, 14F) 18-year old youth exposed to the January 1998 ice storm prenatally (ICE) and 28 (13M, 15F) community

controls born in 1997. Maternal objective hardship experienced during the ice storm was measured by questionnaire. Self-report internalizing symptoms were obtained at 18-years old using the Adult Self-Report. Pituitary volume at 18-years old was manually delineated using T1 and T2 weighted images acquired on a 3T Siemens magnetic resonance imaging scanner. **RESULTS:** Mediation analyses revealed that higher objective hardship was associated with more internalizing symptoms via smaller pituitary volumes in the ICE cohort, but not in controls. **CONCLUSION:** Our data suggest that PNMS leads to smaller pituitary volume, which in turn explains increased internalizing symptoms, more than 18 years after PNMS exposure. Results are discussed in light of contradictory findings relating pituitary volume to internalizing symptoms.

PS1.00192 HORMONAL REGULATION OF AMYGDALAR CORTICOTROPIN RELEASING HORMONE IN THE PERIPARTUM PERIOD.

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Postpartum depression (PPD) affects 10-20% of women and exerts adverse consequences on mother and child. Although the pathogenesis of PPD is unknown, perinatal changes in reproductive hormones and abnormalities in hypothalamic-pituitary-adrenal (HPA) axis are thought to play key roles in PPD. Here, we measure the effects of reproductive hormone fluctuations during the peripartum period on corticotropin-releasing hormone (CRH) and on glucocorticoid receptor (GR) signaling associated proteins, modulators of the HPA aixs. C57Bl/6 mice have a significant increase in corticotropin releasing hormone (CRH) mRNA in the central nucleus of the amygdala (CeA) from postpartum day 2 (PP2) to PP7 compared to virgin controls. Although no changes in GR expression are observed, upregulation of CRH is associated with an increase in PPID and FKBP51, co-chaperones known to regulate GR transcriptional activity. To understand if the increase in CeA CRH is mediated by changes in estrogen (E2) and progesterone (P4) experienced during the transition from pregnancy to postpartum stage, we measured changes in CRH in an exogeneous E2-P4 model that mimics hormonal levels seen in the peripartum period. There is a significant increase in CeA CRH mRNA following E2-P4 withdrawal when compared to controls. These results provide evidence of changes in CeA neural circuitry following E2-P4 withdrawal and suggest a role for GR signaling in mediating affective dysregulation in the postpartum period. Behavioral effects following chronic gestational stress as well as the mechanistic role of GR in mediating these alterations are currently being investigated by selective spatiotemporal modulation of GR using Cre-loxP technology.

PS1.00193 CLINICAL NEUROENDOCRINOLOGY OF PHOSPHOETHANOLAMINE AS MDD THERAPEUTIC RESPONSE INDICATOR

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Background: Major depressive disorder (MDD) is a long dueling, relapsing condition associated with high levels of disability and mortality. The neurobiology of MDD is characterized by both functional and structural dysregulation in the CNS and peripheral neuroendocrine systems. We recently reported in a metabolomics study that plasma phosphoethanolamine (PEA) levels were significantly decreased in Japanese MDD patients compared to healthy controls. **Methods:** Using targeted metabolomics, the present study investigated the effects of antidepressant treatment on plasma phosphoethanolamine levels in Japanese MDD patients and controls; and sought to understand the neuroendocrine impact of plasma PEA changes on MDD course in patients treated with the antidepressants selective serotonin re-uptake inhibitors (SSRI) and selective norepinephrine reuptake inhibitors (SNRI). 17 depressive or anxiety patients were treated with a single SSRI only; and 20 depressive patients were treated with a single SNRI only. **Results:** Treatment with SSRIs decreased, while SNRIs increased plasma PEA levels respectively. Specifically, SSRI decreased plasma PEA levels in patients with depression compounded by anxiety disorder, resulting in suppression of anxiety. On the other hand, SNRI treatment increased plasma PEA level significantly in MDD patients without anxiety disorder, resulting in remission of the depressive state. **Conclusion:** These results indicate that pharmacological treatment modulates PEA signaling which in turn dampen or augment the neuroendocrine response to stress, and recovery of homeostasis. Plasma PEA levels is a novel metabolite for monitoring MDD therapeutic progress and response.

PS1.00194 OPTOGENETIC STIMULATION OF VASOPRESSIN RETINAL GANGLION CELL AXONS IN THE SUPRACHIASMATIC NUCLEUS

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Physiological circadian rhythms are orchestrated by the hypothalamic suprachiasmatic nucleus (SCN). The activity of SCN cells is synchronised by environmental signals, including light information from the retina. However, it remains unclear how light-responsive retinal cells entrain the rhythm of the SCN. Recently a population of vasopressin-expressing retinal ganglion cells (VP-RGC) have been identified that project to the SCN and secrete vasopressin in response to light (Tsuji *et al* 2017 J Physiol). To determine whether vasopressin secreted from these VP-RGC influences the activity of SCN cells we used optogenetic tools to specifically activate VP-RGC axons in the SCN and recorded changes in electrical activity of SCN cells using *in*

vitro electrophysiology. Rats were subjected to intravitreal injections of A90VP-ChR2mCherry (2µl; titre10^{-13} genomic copies/ml) to express ChR2 under the vasopressin promoter in VP-RGS. After 4-6 weeks of viral expression, acute brain slices were made. SCN cells were recorded from in a loose patch configuration and slices were stimulated with pulses of blue light. The effect of blocking SCN vasopressin receptors was determined by adding a vasopressin 1a receptor antagonist to the extracellular solution ($(d(CH_2)_5{}^1Tyr(Me)^2Arg^8)$-vasopressin; 1-5µM). SCN cells responded to optogenetic stimulation with a general increase in firing rate. This activation was prevented or attenuated by the application of a vasopressin 1a receptor antagonist, suggesting that VP-RGC directly regulate the electrical activity of SCN neurons in a vasopressin dependent manner.

PS1.00195 LOW GLUCOSE ENHANCES THE CIRCADIAN AMPLITUDE OF HYPOTHALAMIC TANYCYTES

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Hypothalamic tanycytes are specialized ependymal cells lining the third ventricle of the brain and sending a single protrusion into the brain parenchyma. Recent evidence suggests that tanycytes are capable of detecting and signaling fluctuations in glucose and amino acids in the cerebrospinal fluid composition. In turn, this can alter the activity of neurons involved in feeding behavior such as NPY and POMC neurons, which are responsible for food intake and satiety, respectively. Feeding behavior is also significantly modulated by the circadian clock. In fact, most cell-types express cell-autonomous circadian clocks that regulate much of cellular physiology. These clocks are then synchronized by the master circadian oscillator, the suprachiasmatic nuclei (SCN) of the hypothalamus, which drive many overt hormonal and behavioural rhythms such as feeding patterns. Here, we were interested in confirming molecular circadian oscillations in tanycytes and investigating the interactions of such tanycyte clocks and nutrient levels. For this, we generated tanycytic primary cultures from circadian luciferase fusion reporter B6.129S6- *Per2*$^{tm1Jt/J}$ (*PER2::luc*) mice, and used bioluminescence assays to show that *Per2*robustly oscillates in tanycytes; suggesting a functional clock. Moreover, in cells exposed to low glucose concentration (1mM), the amplitude of Per2::luc was highest, compared to controls (standard DMEM, 4.5mM glucose) and high glucose (10mM) which showed a dampened amplitude. Similarly, 2mM lactate also elicited a lower amplitude than controls. The variation in amplitude of tanycyte circadian clock in response to changing nutrient availability suggests an important link between the clock and nutrient sensing in these key hypothalamic cells.

PS1.00196 NEUROPHYSIOLOGICAL ALTERATIONS IN CELLS OF THE 'MASTER CLOCK' IN A MODEL OF ALZHEIMER'S DISEASE.

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In mammals, circadian rhythms (biological oscillations with ~24-hour period) are controlled by a 'master clock': the hypothalamic suprachiasmatic nuclei (SCN). SCN neurons receive light-driven synaptic input from the retina to generate a co-ordinated electrical output, driving circadian rhythmicity in multiple aspects of physiology and behaviour. In dementia, including Alzheimer's disease (AD), 'non-cognitive' symptoms are prevalent: including changes in daily activity and sleep cycles. Such circadian and sleep disturbances are observed at early disease stages in AD sufferers and animal models of dementia: potentially, playing a role in AD pathogenesis. Understanding the mechanisms underlying these effects will be invaluable for aiding early diagnosis and developing new therapeutic interventions. Since one of the major hallmarks of AD is progressive central nervous system accumulation of amyloid β (Aβ) protein, this study was performed in the Aβ-overproducing J20 mouse model of amyloidopathy (overexpression of human amyloid precursor protein gene harbouring Indiana: V717F and Swedish: K670N/M671L mutations). Coronal hypothalamic slices were prepared from male J20 heterozygous (HET) mice at early-to-mild disease stage (3-6 month old) and wild-type (WT) littermates. Whole-cell patch clamp recordings were made from SCN cells to assess electrical membrane (electrogenic/electrotonic) properties. We found early alterations in intrinsic membrane properties of SCN neurons in J20 HET mice. Specifically, an increase in input resistance and decreased hyperpolarisation-activated sag was observed. This suggests a potential loss of hyperpolarisation-activated, cyclic nucleotide-gated channels (HCN) in J20 HET mice. This is the first study examining neurophysiological alterations of SCN cells in the face of AD-associated pathology, namely amyloidopathy.

PS1.00197 INFLUENCE OF CIRCADIAN RHYTHMS ON MEMORY IMPAIRMENTS INDUCED BY AN OBESOGENIC DIET

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In previous studies on rodents, our team showed that an obesogenic high-fat diet (HF) during adolescence induces specific memory impairments and involves the brain glucocorticoid receptor (GR). In this study, we hypothesize that an obesogenic diet during adolescence may affect GR signaling in hippocampus, thereby resulting in the observed memory deficits. Given that time-restricted feeding was shown to prevent the deleterious effects of HF on metabolism we tested if this was also true for the hippocampus alterations. We have compared 4 groups of mice that were submitted since weaning to either a normal chow (NC) or HF, provided either *ad*

libitum (NC ad lib, HF ad lib) for 12 weeks or under forced synchronization (i.e. food removed during the day, inactive period) for the last 4 weeks (NC fs, HF fs). The mice were then tested for hippocampus-dependent memory performances and later on, killed at 4 time points to study circadian gene expression in hippocampus. Despite similar calorie intake by HF ad lib and HF fs groups, impairments of recognition and location memory induced by HF ad lib is prevented by the time-restricted feeding. In the same mice, we observed a loss of oscillation of the GR and MR genes in hippocampus of mice fed a HF ad lib, restored at least partially by HF fs as well as disruption of oscillation of several GR target genes. Thus, circadian GR signaling is involved and chrononutrition may be a therapeutic strategy to alleviate hippocampus impairments induced by HF during adolescence.

PS1.002 CHEMOGENETIC ACTIVATION OF ENDOGENOUS VASOPRESSIN NEURONS INHIBIT FOOD INTAKE

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We established a novel transgenic rat line which expresses both human muscarinic acetylcholine receptors (hM3Dq), of which ligand is clozapine-N-oxide (CNO), and mCherry fluorescence specifically in AVP neurons. The mCherry neurons that indicate the expression of the hM3Dq gene were observed in the suprachiasmatic (SCN), supraoptic (SON), and paraventricular nuclei (PVN). hM3Dq-mCherry fluorescence was localized mainly in the membrane of the neurons. The mCherry neurons were co-localized with AVP-like immunoreactive (LI) neurons, but not with oxytocin-LI neurons. The induction of Fos, which is the indicator for neuronal activity, was observed in approximately 90 % of the AVP-LI neurons in the SCN, SON, and PVN at 90 min after intraperitoneal (i.p.) administration of CNO. Plasma AVP was significantly increased and food intake, water intake, and urine volume were significantly attenuated after i.p. administration of CNO. Locomotor activity and body temperature was disturbed after i.p. administration of CNO, suggesting that activation of endogenous AVP neurons may affect circadian rhythmicity. In non-transgenic rats, i.p. administration of CNO did not increase Fos-LI neurons in the SCN, SON, and PVN, did not elevate plasma AVP level, and did not affect food intake, water intake, and urine volume. Although the detailed mechanism should be clarified by further study, we demonstrated that the activation of endogenous AVP neurons affect circadian rhythmicity and decreased food intake. This novel transgenic rat line may provide a revolutionary insight into the neuronal mechanism regarding central AVP system responsible for various kinds of behavior.

PS1.0020 PROLACTIN DRIVES THE DECREASES IN VOLUNTARY PHYSICAL ACTIVITY IN EARLY PREGNANCY

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During pregnancy body weight increases not only due to the growth of the fetus and placenta, but also changes in the maternal body such as increased deposition of fat. Despite the increased energy requirements of pregnancy, a state of positive energy balance develops. The aim of this study was to investigate if decreased voluntary activity contributes to positive energy balance during pregnancy. Our data from mice demonstrate a remarkable reduction in running wheel activity as soon as mice become pregnant. This is observed on the first day after mating, even before there is implantation or increased body weight, suggesting that the early hormonal changes of pregnancy actively suppress this voluntary activity. One of the very early hormonal changes during rodent pregnancies is an increase in prolactin secretion, hence we investigated the effect of prolactin on running wheel activity in female mice. Acute prolactin treatment (i.p. injection 5mg/kg) just prior to the start of the dark phase results in a suppression of overnight running wheel activity, especially in the first four hours following treatment, compared to saline treatment. Furthermore, mice with a specific deletion of prolactin receptors in all forebrain neurons or in GABA neurons failed to show the immediate reduction in running wheel activity after mating, and displayed markedly increased running wheel activity during pregnancy compared to control pregnant mice. These surprising data suggest that prolactin mediates the suppression in voluntary physical activity normally seen in early pregnancy in mice, potentially contributing to the development of a positive energy balance.

PS1.0021 DOES CHRONIC PERIPUBERTAL GNRH AND TESTOSTERONE TREATMENT ALTER NOVELTY-PREFERENCES OF MALE SHEEP?

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Chronic gonadotropin-releasing hormone agonist (cGnRHa) treatment is used therapeutically to suppress the reproductive axis through down-regulation of GnRH receptors within the pituitary. However, cGnRHa may impact normal brain development and function as GnRH receptors are expressed in brain regions that regulate emotions, behaviors, motivations and memory. To investigate the role of peripubertal cGnRHa in these functions, an ovine model was used to test changes in novelty-preference behavior at 8 (pre-pubertal), 28 (post-pubertal, in breeding

season) and 42 (post-pubertal, non-breeding season) weeks of age. Rams were either untreated (Controls, n=60), received cGnRHa (n=55: s.c. goserelin acetate every 4 weeks, 8-52wks of age), or received both cGnRHa and testosterone replacement at physiological concentrations (cGnRHa+T, n=24: i.m. testosterone cypionate every 2 weeks, 10-52wks of age). Rams could approach both a familiar and a novel object for 5min during social isolation. The interactions and time spent near the objects were recorded together with emotional reactivity measures (i.e. vocalizations, escape attempts and urinations). As Control and GnRHa rams aged, they showed the expected shift in preference for familiarity (8wks) to novelty (28, 42wks). In contrast, cGnRHa+T rams increasingly preferred familiarity with older age (28, 42wks, $P<0.05$), which may reflect reduced activity in brain regions associated with information-processing and warrants further investigation. The disruption of GnRH signaling had no direct effect on novelty-preferences, but did modulate testosterone sensitive behaviors and functions (i.e. cGnRHa+T had contrasting effects to Controls). This has potential clinical relevance for peripubertal cGnRHa in conjunction with exogenous sex steroid treatment (e.g. gender dysphoria). Funded by the BBSRC (BB/K002821/1).

PS1.0022 LOCAL ACTIVATION OF RAP1 IS REQUIRED FOR ESTROGEN-INDUCED SPINE FORMATION.

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There is a growing appreciation that estrogens, in particular 17β-estradiol (estradiol) exert a positive effect on cognitive function within a rapid time frame (minutes to hours). These cognitive enhancing effects have been shown to be depedent on the activation of specific signalling pathways and are accompanied by increases in spine density in several areas of the brain. Interestingly, the initial formation of dendritic spines by estradiol is independent of protein synthesis and gene transcription and thus, wholly reliant on the activation of cytosolic signalling pathways. However, the direct observation of estradiol-induced signalling in dendrites or in dendritic spines has not been previously shown. The Ras-family of small GTPases, which includes Rap1, plays an important role in the morphogenesis of dendritic spines. Using a unimolecular FRET-sensor for Rap1, we have found activation of Rap1 in distal dendrites and dendritic spines following a 30 minute estradiol treatment. We find that active Rap1 accumulates within dendrites directly below and preceding the formation of nascent dendritic spines and subsequently, diffuses into newly formed spines, suggesting that Rap1 plays a significant role in co-ordinating the formation of estradiol-induced nascent spines. Critically, overexpression of a dominant-negative Rap1 mutant (Rap1 N17) blocks estradiol-induced spine formation. Additionally, Rap1 also plays a role in inducing an estradiol dependent internalisation of AMPA receptor subunit GluA1 within this time-frame. These data

demonstrate that estradiol specifically activates a Rap-dependent pathway locally within dendrites and dendritic spines, which orchestrates the formation of nascent dendritic spines.

PS1.0023 CENTRALLY ADMINISTERED KISSPEPTIN SUPPRESSES FEEDING VIA NESFATIN-1 IN MALE RATS

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Kisspeptin (KP) is a hypothalamic neuropeptide that plays a critical role in the regulation of not only reproduction but also food intake. A newly identified anorectic neuropeptide, nesfatin-1, is synthesized in both peripheral tissue and the central nervous system that includes the hypothalamus and brainstem. Here, we examined the effects of the intracerebroventricular (icv) administration of KP on feeding and nesfatin-1-immunoreactive (ir) neurons and oxytocin (OXT)-ir neurons in the rat hypothalamus, using double immunohistochemistry for Fos. Cumulative food intake was significantly decreased 0.5-3 h after icv administration of KP-10 (6.0 μg/rat) but not 3.8 μg/rat. Icv administration of KP-10 (6.0 μg/rat) significantly increased the number of nesfatin-1-ir neurons expressing Fos-ir in the supraoptic nucleus (SON), the paraventricular nucleus (PVN), the arcuate nucleus (ARC) and several nuclei in the brainstem. Moreover, icv administration of KP-10 (6.0 μg/rat) significantly increased the number of OXT-ir neurons expressing Fos-ir in the SON and the PVN. The decreased food intake induced by KP-10(6.0 μg/rat) was significantly attenuated by pretreatment with icv administration of antisense nesfatin-1. KP-10-induced anorexia was partially abolished by pretreatment with OXT receptor angatonist (OXTR-A). The number of nesfatin-1-ir neurons expressing Fos-ir in the ARC was also suppressed by OXTR-A pretreatment. These results indicated that icv administration of KP-10 activated nesfatin-1 and OXT neurons in the hypothalamus and brainstem and may play an important role in suppression of feeding in male rats.

PS1.0024 PERSONALITY AND GENETIC RISK FOR TESTOSTERONE-INDUCED AGGRESSION

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Contrary to folk wisdom and animal models, testosterone's effects on human aggression are weak and inconsistent, with little known about the neurobiological pathways through which, and people for whom, testosterone has its effects. Using a psychopharmacogenomic approach, we show that testosterone does not increase aggression indiscriminately, but rather exerts differential effects depending on personality and genotype: Testosterone increased aggression in men with personality profiles conferring high dominance, independent self-construal, and low self-control, and these conditional effects were further enhanced among men with fewer (vs more) CAG repeats in exon 1 of the androgen receptor (AR) gene, a polymorphism associated with increased AR efficiency. The effects on aggression were rapid, occurring within 30 minutes of administration, and mediated by participants' subjective feelings of reward (rather than anger) associated with aggression. These results provide novel evidence that testosterone may promote human aggression through an AR mediated mechanism, and that effects are enhanced among men with certain personality profiles because testosterone more strongly upregulates the subjective pleasure these men derive from aggression. Given previous research indicating that testosterone regulates reward through dopaminergic pathways, and that the sensitivity of such pathways may be enhanced among individuals with the described personality profiles, our findings also implicate dopaminergic processes in testosterone's heterogeneous effects on aggression, representing an important direction for future research.

PS1.0025 EFFECTS OF MELATONIN ON SEASONAL SHIFTS IN ANDROGEN LEVELS AND AGGRESSION IN MALE SIBERIAN HAMSTERS

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Classic neuroendocrine studies have demonstrated a positive correlation between gonadal steroids and territorial aggression during the breeding season. However, some species exhibit equivalent or increased levels of aggressive behavior during the short-day photoperiods of the non-breeding season, despite gonadal regression and reduced levels of circulating androgens. While the mechanisms underlying short-day increases in territorial aggression are not well understood, previous work from our group suggests that pineal melatonin and the adrenal androgen dehydroepiandrosterone (DHEA) are important in facilitating non-breeding aggression in Siberian hamsters (*Phodopus sungorus*). In the present study, we manipulated photoperiod and circulating melatonin levels to distinguish the role of melatonin in modulating seasonal changes in territorial aggression in male Siberian hamsters. Furthermore, we assessed circulating and neural hormone levels in these animals during the transition period between breeding and non-breeding condition. Males were housed in long or short days and treated with either timed exogenous melatonin or saline, and aggression, circulating androgens, and

neurosteroid levels in brain nuclei associated with aggression and reproduction were quantified following 3 weeks, 6 weeks, and 9 weeks of photoperiodic housing. Short-day hamsters showed increased aggression and exhibited reductions in paired testes and body mass relative to long-day hamsters across photoperiodic treatment. Interestingly, long-day hamsters administered melatonin exhibited intermediate levels of gonadal and body mass reduction and aggressive behavior. Neural and circulating androgen levels will also be presented. Collectively, this study provides insight into the roles of melatonin and DHEA in mediating seasonal aggression.

PS1.0026 PROSTAGLANDIN F SIGNALING CONTROLS FEMALE REPRODUCTION: INSIGHTS FROM CRISPR EDITING IN CICHLID FISH

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Cichlid fish are a family of >2000 species that display a wide range of fascinating innate behaviors. Male *Astatotilapia burtoni* cichlids exhibit stereotyped territorial behaviors within a social hierarchy. Females are keen observers of male behavior and actively select a mate, then show parental behavior toward offspring. Hormone-sensitive modules in the brain mediate each of these behaviors, enabling access to the underlying neural circuits. Importantly, numerous cichlid genomes have been sequenced, and we have developed transgenic and gene editing tools for *A. burtoni*. We used injections of hormones including progestin and prostaglandin F (PGF), alongside CRISPR gene editing to manipulate these pathways. Combining molecular genetics with behavioral analysis and other tools, we dissect the control of female behavior in *A. burtoni* to show that PGF signaling is necessary and sufficient for sexual behavior. Our results suggest that PGF signaling is initiated by mature eggs in the reproductive tract, and gates male courtship signals via a hypothalamic population of cells. Sexual selection by females is a likely driver of the explosive speciation of cichlids. Thus, analysis of the PGF-responsive neural circuit for mating will permit the interpretation of genetic changes that drive mate preference and evolution. CRISPR and high-throughput sequencing also now enable the genetic study of phenotypes exhibited across cichlid species, including parenting, monogamy, and social hierarchies.

PS1.0027 EFFECTS OF MUSCARINIC RECEPTOR BLOCKADE ON SOCIAL LEARNING IN OVARIECTOMIZED AND INTACT FEMALE MICE

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Social learning, acquiring information from conspecifics, is a highly adaptive common learning strategy in animals and humans, yet we know little about the underlying neurobiological mechanisms. Social learning can be studied in rodents using the social transmission of food preferences (STFP), in which an observer prefers a novel food it previously smelled on a demonstrator's breath, over other novel foods. Previous research in male rats indicates that muscarinic acetylcholine receptor (mAChR) signaling is particularly important for acquisition of a socially learned food preference (Boix-Trelis et al,2007,Neurobiol Learn Mem,87:659; Carballo-Márquez et al,2009,Hippocampus,19:446; Carballo-Márquez et al,2009,Neurobiol Learn Mem,91:98). The STFP is also affected by the estrous cycle of mice (Ervin et al,2015,Horm Behav,74:53) and estrogens improve social learning in the STFP and promote ACh signaling (Hammond et al,2011,Psychoneuroendocrinology,36:182; Mitsushima et al,2009,J Neuroendocrinol,21:400). Thus we investigated whether gonadal hormones interact with the ACh system to influence social learning in the STFP. Female observer mice received ovariectomy or sham surgery. Observers were treated intraperitoneally with saline, 0.1, 1, or 2mg/kg scopolamine, a general mAChR antagonist. Contrary to our predictions, ovariectomized and intact observer mice were similarly impaired by scopolamine in the STFP; in both groups, mice were impaired when given 1 or 2mg/kg scopolamine, and we observed no effects of the estrous cycle. Systemic treatment and dose range may have led to generalized effects; treatments directed at more specific brain regions or mACh subtypes may show an estrogen-ACh interaction. Our results, however, suggest gonadal hormones do not modulate cholinergic signaling involvement in social learning.

PS1.0028 EXAMINING THE IMPACT OF A TWO-HIT MODEL OF NEUROINFLAMMATION ON SOCIAL BEHAVIOR IN JUVENILE RATS

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Autism is characterized by impaired social interactions, inadequate verbal and nonverbal communication, restricted interests, and stereotyped behaviors. But, the biological correlates of these symptoms remain inconclusive. In addition to genetic factors, epidemiological data has indicated that environmental factors also contribute to the risk of autism. Neonatal exposure to infectious pathogens is one of these environmental factors, suggesting that activation of the neonatal immune system may contribute to autism pathology. Microglia, the resident immune cells of the brain, perform functions crucial for normal brain development and behavior. According to a "two-hit model of neuroinflammation," neonatal neuroimmune activation causes persistent deficits in microglial functioning, resulting in an exaggerated immune response and significant behavioral deficits following subsequent immune activation later in life. Furthermore, males are more likely than females to be diagnosed with autism. During early development, males and females exhibit different microglial phenotypes, possibly leaving males more susceptible to the negative outcomes associated with early-life neuroinflammation. Our goal was to better understand the impact of the two-hit model of neuroinflammation on the

development and expression of social behaviors in male and female rats. We first piloted behavioral paradigms to characterize the development of social behavior in juvenile rats, and then applied the two-hit model of neuroinflammation to determine how immune activation may affect the expression of these social behaviors. We concurrently measured cytokine expression in the male and female juvenile brain. These experiments may help to elucidate when and how a specific environmental risk factor contributes to behavioral outcomes associated with autism.

PS1.0029 DOMINANT VS SUBORDINATE STATUS IN THE REGULATION OF VASOPRESSIN, SEROTONIN AND OXYTOCIN RECEPTORS

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Dominance status in hamsters is driven by interactions between arginine-vasopressin (AVP), serotonin (5HT), and oxytocin (OT). In males, AVP and OT increase aggression by acting on V1a AVP and OT receptors in the anterior hypothalamus (AH). In females, however, AVP and OT decrease aggression. In contrast, activation of the 5HT1a receptor decreases aggression in males and increases aggression in females. It is unknown, however, how dominance status regulates receptor binding of V1a, 5HT1a and OT and if sex also influences binding. The following experiments investigated the binding of V1a, 5HT1a, and OT receptors in the AH of dominant versus subordinate male and female hamsters. Syrian hamsters were housed individually for 4 weeks. Male and female pairs were then weight-matched, and females were cycle-matched. Each pair was placed into one of the hamster's home cages nine times across 5 days for 5 min each time. Cage controls remained in their home cages but were moved from the animal housing room to the testing suite with the experimental hamsters. Brains were collected on the fifth day and processed for receptor binding using autoradiography. Results show that in the AH, OT binding was greater in dominant compared to subordinate hamsters. In addition, 5HT1a and OT binding was greater in males compared to females. There were no significant effects of dominance status or sex on V1a receptors. These results provide evidence that dominance status and sex play important roles in shaping receptor binding profiles in these animals. This work was supported by NIH-MH110212.

PS1.003 THE SUPRAMAMMILLARY NUCLEUS IS A NOVEL TARGET FOR GHRELIN IN RATS

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Ghrelin is a stomach-derived hormone that signals to many brain areas important in appetite control via a dedicated receptor, the growth hormone secretagogue receptor 1A (GHSR-1A). Prompted by studies demonstrating ghrelin binding in the supramammillary nucleus (SuM), we sought to determine whether ghrelin targets this brain area to affect the activity of SuM neurons and alter eating behaviour. Neuroanatomical studies using *in situ* hybridization in rats showed the presence of GHSR-1A in the SuM. Using *in vivo* extracellular recordings from single SuM cells in anaesthetized rats, we found that peripheral ghrelin administration altered the firing rate of SuM neurons. We also observed an increased number of SuM cells expressing Fos after i.p. injection of ghrelin or an overnight fast, and also in schedule-fed rats anticipating food. Lastly, acute ghrelin delivery to the SuM increased food intake. Collectively these data demonstrate that the SuM is activated when peripheral ghrelin levels are high, either after ghrelin injection, or when rats are hungry or anticipating food, and that ghrelin delivery targeting the SuM is sufficient to drive an orexigenic response.

PS1.0030 SOCIAL ENVIRONMENTS INFLUENCE THE EFFECT OF INTRANASAL OXYTOCIN ON MALE PRAIRIE VOLE BEHAVIOR.

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Social experiences at different life stages interact to shape brain and behavioral phenotypes. Parental composition before weaning (single mother vs. biparental) and the degree of social housing after weaning (isolated vs. group housing) shape neural oxytocin receptor and vasopressin 1a receptor expression. We explored the interaction between social environment and treatment with chronic intranasal (IN) oxytocin (OT) administration during post-weaning development. IN-OT is increasingly being used therapeutically in children without good knowledge of the long-term effects. Bales et al. (2013) showed that chronic IN-OT administration in prairie voles (*Microtus ochrogaster*) alters unmanipulated adult behavior under normal rearing conditions. If early-life social experience and chronic IN-OT over development have the capacity to alter nonapeptide receptor profiles, then we hypothesized that the consequences from intranasal OT at adulthood on prairie voles under typical rearing contexts would be systematically altered in animals reared under contexts with limited social interaction. We asked if rearing with single mothers and social isolation or with both parents and group housing would alter alloparental care and pairbonding as sub-adults and adults following chronic intranasal treatment (0.8 IU/kg of OT vs. saline) when young. We found that IN-OT increases affiliative behavior and that (surprisingly) being reared in limited social environments is associated with greater affiliative behavior. Aggression showed the opposite

pattern; subjects reared under typical social conditions receiving IN saline were most likely to attack pups. We conclude that the natal social environment profoundly affects the outcome of intranasal OT, a treatment that is commonly used therapeutically.

PS1.0031 THE GUT-BRAIN AXIS IN PRAIRIE VOLES: SOCIAL AND NEUROCHEMICAL EFFECTS OF LACTOBACILLUS REUTERI

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Accruing evidence suggests a role of gut microbiota on clinically relevant behaviors. One species of bacteria in the gut, *Lactobacillus reuteri*, has specifically been shown to alter a variety of behaviors, including social deficits – yet, the underlying neurochemical mechanisms are still largely unknown. The socially monogamous female prairie vole (*Microtus ochrogaster*) displays high levels of social behaviors and thus provides an opportunity to study influences by endogenous and environmental factors on these behaviors and the underlying mechanisms. Using this animal model, we tested the hypothesis that administration of *L. reuteri* alters social behaviors and brain neurochemical systems. Live or heated-killed (control) *L. reuteri* was administered into the drinking water of voles daily for 4 weeks. Subjects then went through a series of tests for locomotion, anxiety-like, and social affiliation behaviors. Our data show that the intake of live *L. reuteri* decreased social affiliation in female voles. Subject brains were processed for neurochemical expression in selected brain areas implicated in social affiliation and anxiety-like behaviors. We found that administration of live *L. reuteri* decreased corticotrophin releasing factor (CRF) and CRHR2 receptor levels in the nucleus accumbens (NAcc) and vasopressin 1a-receptor (V1aR) in the paraventricular nucleus of the hypothalamus (PVN), but increased CRF in the PVN. Together, these data demonstrate a behavior-, neurochemical-, and brain region-specific effect of live *L. reuteri* in prairie voles. We are currently examining the composition of stool samples collected pre- and post-administration of *L. reuteri* to determine specific alterations to microbiota.

PS1.0032 INVESTIGATION OF CALCIUM ACTIVITY AND PEPTIDE RELEASE OF NEURONS CONTACTING THE CEREBROSPINAL FLUID

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Kolmer and Agduhr described almost a century ago the cerebrospinal fluid contacting neurons (CSF-cNs), spinal sensory neurons in contact with the cerebrospinal fluid (CSF). Due to their

position at the interface between the cerebrospinal fluid and the central nervous system, these neurons are good candidate for sensing the CSF content and release factors in response. These GABAergic neurons express the transient receptor potential PKD2L1, and we recently had evidence that they respond to variations of CSF pH and flow, and modulate locomotion and posture via projections on spinal targets. CSF-cNs from different species have been shown to express neuromodulators and neuropeptides such as dopamine, serotonin or somatostatin. We confirmed these secreted factors were enriched and found new ones via a transcriptome analysis of zebrafish CSF-cNs validated by *in situ* hybridization combined with immunohistochemistry. Yet, how CSF-cNs are recruited during physiological contexts and release secreted factors into the CSF remains unknown. Here we perform calcium imaging *in vivo* and *in vitro* on CSF-cNs in order to identify how feeding, oxygenation, circadian rhythm and inflammation modulate CSF-cNs activity. In addition, we generated novel constructs to monitor release of dense granules in the apical extension and axonal terminals of CSF-cNs during physiological contexts that recruit these cells. Altogether, our data will reveal how CSF-cNs can release secreted factors in the cerebrospinal fluid that could regulate the molecular content of the CSF depending on the physiological context.

PS1.0033 SYNTHETIC ESTROGEN AND COGNITION: DOES TIME OF ORAL CONTRACEPTIVE INGESTION AFFECT WORKING MEMORY?

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Extensive research supports that 17-beta estradiol (E2), the main estrogen produced by the ovaries, plays a key role in cognition. Whether synthetic estrogens act in the brain remains largely unknown, and there is little consensus among the research that has investigated the impact of synthetic estrogens on cognitive performance. Studying women taking hormonal birth control presents a unique opportunity to examine whether synthetic estrogen affects learning and memory in a young, healthy population. Plasma concentrations of ethinylestradiol (EE), the main synthetic estrogen contained in oral contraceptives (OCs), typically peak 1-2 hours after pill ingestion, then gradually decline. To our knowledge, no study has considered the pharmacokinetics of EE when investigating cognition in OC users. By accounting for time of pill ingestion, the current study aims to characterize the effects of EE on working memory (WM) in women of reproductive age taking combined OCs as compared to normally cycling (NC) controls. OC users were tested at a peak EE state occurring 1-2 hours after pill ingestion, and at a low EE state just before pill ingestion. NC controls were tested at the early and late follicular phase, a low and high E2 menstrual cycle phase, respectively. No significant difference in WM performance was found between OC and NC women. However, NC women's performance was sensitive to estrogen condition, with better performance in high E2 phase, while OC women's performance was constant. These data suggest that active OC use does not affect WM performance.

PS1.0034 REPRODUCTIVE EXPERIENCE IMPROVES NEUROBEHAVIORAL CHANGES IN THE MOTHER'S BRAIN EXPOSED TO STRESS

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In mammals, behavioral and neurobiological changes occur throughout the female brain because of reproductive experience (RE). This functional modification remodels the neural circuits of the hippocampus and have a significant impact in cognition aspects. Changes in hippocampal neurogenesis and BDNF levels during RE and mother-pup separation, provide potential explanation for the changes in maternal cognitive performance during that period. Nulliparous (NP: none RE), Primiparous (PRI: one RE) and Multiparous (MULT: two RE) Wistar rats were use in the experiments. The PRI and MULT group were subjected to either animal facility rearing (C) or daily 4,5h of pups separation (S) from postpartum day (PPD) 1 to 21. Spatial memory was evaluated in the Barnes Maze (PPD 21-26). Cell survival and proliferation were evaluated by bromodeoxyuridine (BrdU) immunohistochemistry. Neurogenesis were also analyzed in age-matched NP females. Levels of BDNF were quantified in CA1, CA3 area of the dentate gyrus (DG). The results revealed that MULT and PRI-C dams showed a better performance in spatial memory demonstrated by less errors in their search ($p \leq 0.02$), PRI-C and MULT-C spent more time in the goal ($p < 0.04$), and showed a better progression from a random to serial search strategy. NP and PRI-S has less pokes on goal holes ($P \leq 0.008$), . Maternally-experienced dams showed an increase in cell proliferation ($p \leq 0.04$). The levels of BDNF were higher in CA1 area in MULT-C/S ($p \leq 0.05$). These data demonstrate that multiparity dampened the consequences of disrupting the natural dam-pup interaction in memory aspects, increased cell proliferation and BDNF in DG.

PS1.0035 PHYSIOLOGICAL RELEVANCE OF A NEUROSECRETORY SYSTEM IN CONTACT WITH THE CEREBROSPINAL FLUID

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Exploration is critical for animal survival as it enables to adjust locomotion to the inner physiological states reflecting food intake, metabolism, circadian rhythm and inflammation among others. The initiation of locomotion relies on descending commands from the brain as well as on the excitability of spinal circuits themselves. The mechanisms allowing the central nervous system to generate motor activity based on internal sensory inputs are not well understood. Our team investigates a novel interoceptive pathway relying on spinal sensory neurons called cerebrospinal fluid contacting neurons (CSF-cNs). We showed in zebrafish that these neurons constitute a sensorimotor loop in the spinal cord detecting changes in pH and

mechanical flow and modulating locomotion and posture via projections onto neuronal targets in spinal cord and hindbrain. We performed a transcriptome analysis of CSF-cNs and identified a wide repertoire of peptides and secreted proteins. Here we show that peptide expression was mainly localized in CSF-cNs in the spinal cord. To investigate the role of CSF-cN peptides, we generated mutants for peptides and key elements of the neurosecretory machine using CRISPR/Cas-mediated genome editing. We currently investigate the behavioral consequences of these mutations in the context of diverse physiological states of the zebrafish larva. Altogether, our results suggest that secreted peptides released by neurons contacting the cerebrospinal fluid could widely modulate physiology by regulating the chemical content of the cerebrospinal fluid.

PS1.0036 SEX AND BETA-ENDORPHIN INFLUENCE LIMBIC GABA-R SUBUNIT EXPRESSION IN A MOUSE BINGE DRINKING MODEL

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Binge drinking is linked to increased risk of alcohol use disorders (AUD). Sexually dimorphic alcohol effects are clinically well-characterized; however, the underlying mechanisms for sexual dimorphisms in the physiological and behavioral effects of alcohol remain unclear. Female mice lacking beta-endorphin (B-E), an endogenous opioid peptide, have an anxiety-enhanced phenotype. Alcohol-mediated anxiolysis depends upon B-E mediated stimulation of the inhibitory neurotransmitter, GABA. Therefore, we sought to determine whether B-E would affect sexually dimorphic binge drinking behavior via changes in GABA receptor alpha-2 subunit expression (GABRA-2). Adult male and female C57BL/6J controls (B6) and B-E deficient (KO; B6.129S2-Pomc^{tm1Low}/J) mice were provided with 20% ethanol (EtOH) and water (EtOH) or just water (Control) 3h into the dark cycle for 4 consecutive days. GABRA-2 mRNA expression in the Central Nucleus of the Amygdala (CeA) and the Bed Nucleus of the Stria Terminalis (BNST), Nucleus Accumbens (NAc) and Ventral tegmental area (VTA) was assessed via qRT-PCR. Alcohol naive B6 females expressed more GABRA-2 than their male counterparts, but this sex difference was absent in KO mice. Males exhibited similar alcohol intake and increases in GABRA-2 expression in response to alcohol, regardless of genotype. In contrast, alcohol consumption in B6 females decreased GABRA-2 expression, but increased it in B-E deficient females. Therefore, B-E has sexually dimorphic effects on binge-like EtOH consumption, perhaps to alleviate an exaggerated stress phenotype in B-E deficient females. Differential expression of GABRA-2 subunits in limbic structures in the brain may be a key factor in the sexually dimorphic effects of alcohol.

PS1.0037 ANDROGEN RECEPTORS AND HISTONE VARIANT H2A.Z INTERACT TO REGULATE FEAR MEMORY.

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Hormones have a significant effect on fear memory. While much is known about ovarian steroid hormones (e.g. estrogen) facilitating contextual fear conditioning in mice, much less is known about the role of androgens (e.g. testosterone) and the androgen receptor (AR) in memory formation. Previous literature shows mixed results, with testosterone leading to either a reduced or an enhanced contextual fear response. Using transgenic mice overexpressing AR, we showed that AR overexpression impairs fear memory. Gonadectomy eliminated group differences between AR-overexpressing and WT males, implicating testosterone as a negative regulator of fear memory through actions on AR. Further, treatment with the AR-blocker flutamide increased fear memory, suggesting that AR negatively regulates fear memory. In addition, we showed that expression of H2A.Z, a memory suppressor identified in our lab, is increased in AR overexpressing mice, prompting us to investigate AR regulation in H2A.Z conditional knockout mice. In contrast to AR overexpression, H2A.Z depletion results in increased fear memory and decreased AR expression in area CA1 of the hippocampus. Castration with DHT (dihydrotestosterone) replacement resulted in genotype-specific effects on fear memory, pointing to an interaction between H2A.z and AR. We also find corresponding changes in gene expression of genes encoding for synaptic proteins and memory-related genes. These results suggest a role of AR in modulating fear memory through interactions with nuclear histone proteins. This is a novel finding that we plan to expand to further understand the neuronal mechanisms through which this occurs.

PS1.0038 FEMALE SEXUAL RECEPTIVITY DEPENDS UPON EXTRA-OVARIAN ESTRADIOL SYNTHESIS IN MARMOSET MONKEYS

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Ovarian estradiol, E_2, supports the expression of female mammalian sexual behavior. In rodents, ovariectomy (OVX) abolishes female sexual behavior. In nonhuman primates, however, OVX decreases, but does not abolish female sexual behavior, serving as an example of behavioral emancipation from ovarian E_2. We hypothesize that extra-ovarian E_2 provides key physiological support for female sexual behavior in primates. We employed the use of an aromatase inhibitor, letrozole, to completely eliminate E_2 biosynthesis in a nonhuman primate model. Ten adult female marmosets were OVX and assigned to receive: subcutaneous E_2 containing silastic capsules (E_2; n=4), daily oral treatments of either vehicle (VEH, 1ml/kg,

n=3) or letrozole (LET, 1 mg/kg, n=3) for the entire study. Five months following treatment onset, females were separated from their male partners and singly-housed for 30 days before commencing 30 minute testing 3x/week for two weeks while singly housed. Intra- and inter-rater reliability was >80%. E_2-treated females displayed more sexual acceptance (p=0.004) and receptive head turns (p=0.01) than VEH or LET females. LET females exhibited the least receptive behaviors (p=0.002), while escalating rejections towards males (p=0.003), including aggressive hitting (p=0.002) not observed in VEH or E_2-treated females. Our findings provide the first evidence for extra-ovarian E_2, possibly neuroE_2, support of female sexual receptivity in a female primate. These findings also suggest the necessity of both ovarian and extra-ovarian E_2 in facilitating full expression of receptivity in the female marmoset. Such primate emancipation from complete dependence on ovarian E_2 may enable opportunistic female sexual engagement in complex social environments.

PS1.0039 SOCIAL TRANSMISSION OF MATERNAL BEHAVIOR BY OXYTOCIN

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Inexperienced virgin female mice can start responding maternally to pup distress calls - by retrieving isolated pups to the nest - after being co-housed with an experienced dam (mother) and her pups for three to seven days. To understand how the co-housing experience modulates activity in neural circuits to enable acquisition of maternal behavior, we built a system to continuously record synchronized behavioral and neuronal data throughout co-housing. Thus, we identified two spontaneous behaviors that contribute to the social transmission of maternal care in virgin mice. First, dams self-generate pup retrievals during co-housing with the virgin mouse. Wild-type but not oxytocin receptor knockout virgins that are exposed to repeated episodes of dam retrievals can start retrieving themselves, even outside of the co-housing context. Second, dams chase the virgins into the nest where they would spend more time caring for pups. To understand how these spontaneous dam behaviors might contribute to plasticity in virgins, we recorded single-unit neuronal data from the paraventricular nucleus of the hypothalamus (PVN), which contains oxytocinergic neurons. Oxytocin, a peptide important for social bonding and for maternal care, can induce cortical synaptic plasticity. We find that interactions with a dam increase firing of PVN neurons, including optogenetically-identified oxytocin neurons in virgins. Pharmacogenetic suppression of oxytocin neuron activity during co-housing prevents the acquisition of pup care in virgins, indicating an essential role for oxytocin in enabling the social transmission of maternal behavior.

PS1.004 RECRUITMENT OF VASOPRESSINERGIC AND OXYTOCINERGIC BRAIN REGIONS IN RESPONSE TO SOCIAL PLAY

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Social play is a highly rewarding and motivated behavior predominately displayed by juveniles and expressed by nearly all mammalian species. We recently demonstrated that the vasopressin (AVP) and oxytocin (OT) systems can regulate the expression of social play in sex-specific ways. Here we investigated whether there are sex differences in the recruitment of vasopressinergic and oxytocinergic brain regions following social play exposure in juvenile male and female rats. Exposure to social play did not increase recruitment of AVP or OT neurons in the supraoptic (SO) or paraventricular hypothalamic nuclei of either sex compared to the no-play control condition. However, a positive correlation was observed between recruitment of SO-OT neurons and the percentage of time spent engaged in social play. Interestingly, there was a robust sex difference in SO recruitment, irrespective of social play condition, with males exhibiting twice the recruitment of SO-AVP and SO-OT neurons compared to females. Lastly, exposure to social play increased recruitment of the posterior bed nuclei of the stria terminalis (pBST) and the posterodorsal medial amygdalar nucleus (MEApd) compared to the no-play control condition, and this effect was most pronounced in females. Our findings revealed sex differences in the recruitment of brain regions (i) independent of play condition (i.e., SO) possibly representing a sex difference in the baseline levels of AVP and OT signaling required for typical functioning and (ii) specific to play condition (i.e., pBST, MEApd). In sum, this study provides further evidence that the neural substrates underlying social play behavior are sex-specific.

PS1.0040 SEX DIFFERENCES IN ANDROGEN RECEPTOR ABUNDANCE IN THE VOCAL-MOTOR PATHWAY OF ALSTON'S SINGING MOUSE

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Differences between the sexes in brain and behavior are often among the most conspicuous forms of intraspecific variation. Although gonadal steroids often shape patterns of sexual dimorphism at the level of the limbic system, studies in a variety of vertebrates suggest that the sex-specific expression of steroid receptors may have direct consequences on motor outputs as well. The songs of Alston's singing mouse (*Scotinomys teguina*) are sexually dimorphic and are composed of a series of rapidly-repeated, broad-bandwidth, frequency-modulated notes. Males sing more often than females. Their songs are longer and are also androgen responsive –

castration makes songs softer, shorter, and less common. This decrease in performance can be rescued by the application of dihydrotestosterone, suggesting that androgen receptors play an important role. To further understand the neuronal mechanisms underlying their vocalizations, we first use a transynaptic pseudorabies virus to examine how the social behavior network connects to the motor control of the jaw. Next we map the sites of androgen sensitivity along a pathway that spans from limbic structures to vocal control regions, and then quantify differences in nuclear AR-like labeling between male and female brains. Preliminary data shows differences in limbic regions such as the septum and stria terminalis as well as differences in motor structures such as the periqueductal gray and nucleus ambiguus. Together these data provide a set of candidate brain nuclei that may underlie the androgen-sensitive vocal output of *S.teguina*, and suggest circuits for the integration of hormonal, social and motor function more broadly.

PS1.0041 A LONGITUDINAL STUDY OF COGNITIVE AGING IN THE MARMOSET

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Cognitive abilities decline significantly with age in humans but whether men and women follow different trajectories of cognitive decline remains unclear. Studies in nonhuman primate models of human aging may help clarify the effects of sex on cognitive aging and identify their biological correlates. With a relatively short lifespan (~ 10 years), sophisticated cognitive abilities and patterns of brain aging that resemble those of humans, the common marmoset (*Callithrix jacchus*) is uniquely suited for longitudinal studies of cognitive aging. We examined cognitive function and stress reactivity in male and female marmosets (age 4-5) followed longitudinally for 2 years. Monkeys were tested on a serial reversal learning task (CANTAB) and a test of stress reactivity (temporary social separation). There was little evidence for a decline in cognitive flexibility between the two time points. However, independent of year of testing, females took longer than males to reach criterion in reversal learning and were more reactive to the social stressor. Additional data points are needed to determine whether this sex difference is maintained with increasing age. Supported by NIH grant AG046266.

PS1.0042 SOCIAL ISOLATION FACILITATES MATERNAL CARE IN BOTH SEXUALLY NAÏVE MALE AND FEMALE DDN MICE

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Maternal care is an indispensable element of reproduction in mammalian species that feed offspring by lactation. When pups are present, lactating females immediately initiate maternal cares, such as licking around anogenital area, pup retrieving to the nest, and crouching over pups. These behaviors are female specific and are considered as one of sexually dimorphic behavior. We have previously reported that sexually naïve male mice show maternal behavior toward pups after social isolation (ISO). Social isolation, social exclusion, or feelings of social disconnection can lead to loneliness, which is a strongly aversive emotional state in human (House et al., 1988) and is also to rodent. The negative state of isolation can trigger the motivation to seek and engage in social contact. Here, we examined how social isolation affects the interaction of sexually naïve females with pups. Three weeks of isolation during puberty induced retrieving and crouching when exposed to pups, while group-housing (GRP) females were showed less responsive to pups. The number of mice in the 'Ignore' category was higher in the ISO males than in the ISO females. Crouching duration was significantly longer in the ISO female group than in the male group. These results demonstrate that social isolation prompts maternal care in both sexes and might be conserved mechanisms to maintain the neural circuits because of biological signification in social behavior. The effect of social stress during puberty can also cause long-lasting alterations in the brain substrate in both sexes and subsequently in adult parental behaviors.

PS1.0043 RAT MATERNAL BEHAVIOR IS IMPAIRED BY STIMULATION OF 5-HT1A RECEPTORS

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Previous work suggests that 5-HT_{1A} receptors play a role in rodent maternal aggression, but not in other aspects of maternal care (e.g. pup retrieval and nest building). The present study re-assessed the basic effects of 5-HT_{1A} activation or blockade on various maternal responses in postpartum female rats. Sprague–Dawley mother rats were injected with a 5-HT_{1A} agonist 8-OH-DPAT (0.1, 0.5 or 1.0 mg/kg, sc), a 5-HT_{1A} antagonist WAY-101405 (0.1, 0.5 or 1.0 mg/kg, sc) or 0.9% saline solution on postpartum days 3, 5, and 7. Maternal behavior was tested 30 min before, 30 min, 120 min, and 240 min after the injection. Acute and repeated 8-OH-DPAT treatment significantly disrupted maternal behavior in a dose-dependent fashion, whereas WAY-101405 had no effect at the tested doses. The 5-HT_{1A} receptor specificity of 8-OH-DPAT's action was confirmed as its maternal disruption effect was reversed by pretreatment of WAY-100635 (a highly selective 5-HT_{1A} receptor antagonist). Subsequent pup preference test found that 8-OH-DPAT did not decrease the pup preference over a novel object, thus no inhibition on maternal motivation or maternal affect. The pup separation test and pup retrieval on an elevated plus maze test also failed to find any motivational and motor impairment effect with 8-OH-DPAT. However, 8-OH-DPAT at the maternal disruptive dose did disrupt the prepulse inhibition (a measure of attentional function) of acoustic startle response and enhanced the basal startle response. These findings suggest that stimulation of 5-HT_{1A} receptors by 8-OH-

DPAT impairs maternal care by partially interfering with the attentional processing or basal anxiety.

PS1.0044 CEREBELLAR FASTIGIAL NUCLEAR PROJECTIONS TO THE HYPOTHALAMUS MODULATE IMMUNE FUNCTION

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Our previous work has shown that cerebellar fastigial nucleus (FN) modulates immune function, but pathways or mechanisms underlying this immunomodulation require clarification. In neuroimmunomodulation, the hypothalamus is a well-known crucial immunoregulatory center. In the present study, we firstly demonstrated direct cerebellohypothalamic γ-aminobutyric acid (GABA)-ergic and glutamatergic projections originating from cerebellar FN neurons and terminating mainly in the lateral hypothalamic area (LHA) by anterograde and retrograde tracing of nerve tracts combined with immunohistochemistry. We then microinjected vigabatrin, an inhibitor of GABA-transaminase that inhibits GABA degradation, or microinjected 3-mercaptopropionic acid (3-MP), a glutamic acid decarboxylase antagonist that reduces GABA synthesis, bilaterally into FN. Vigabatrin treatment in bilateral FN significantly reduced concanavalin A-induced T lymphocyte proliferation, anti-sheep red blood cell IgM antibody level, and natural killer cell cytotoxicity, while 3-MP treatment remarkably elevated these lymphocyte functions. Simultaneously, vigabatrin treatment enhanced but 3-MP impaired the FN-hypothalamic GABAergic transmission via altering both number of GABA-immunoreactive neurons in FN–hypothalamic projections and GABA content in the hypothalamus. On the other hand, 6-diazo-5-oxo-L-norleucine (DON), an inhibitor of glutaminase for glutamate synthesis, was microinjected in bilateral FN and D,L-threo-β-hydroxyaspartic acid (THA), an inhibitor of glutamate transporters on plasma membrane, was microinjected in both sides of LHA. Reducing FN-hypothalamic glutamatergic projections by DON inhibited T, B and natural killer cell functions, which were reduced by combined treatment with THA in the LHA. These findings show that the cerebellar FN has direct GABAergic and glutamatergic projections to the hypothalamus and that these projections actively participate in modulation of lymphocytes.

PS1.0045 TESTOSTERONE DURING PUBERTY IS NECESSARY FOR STEROID-INDEPENDENT MALE SEX BEHAVIOR IN B6D2F1 MICE

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Gonadal steroids, in particular, testosterone, play an essential role in male sexual behavior, and in most rodent models, this relationship is tightly coupled. However, many other species, including humans, continue to demonstrate male sexual behavior in the absence of gonadal steroids. An investigation into the mechanisms that regulate steroid-independent male sexual behavior is needed to further our understanding of individual variation in steroidal regulation of male sexual behavior, and one mouse model in which ~30% of castrated male B6D2F1 hybrid mice display male sexual behavior up to 26 weeks after castration provides this opportunity. During both the perinatal and pubertal critical periods, the organizational effects of gonadal steroids on sexual differentiation of the neural circuits controlling male sexual behavior are well-documented. Several factors can alter the normal range of gonadal steroids which may lead to the disruption of the normal processes of masculinization and defeminization, and it is unknown whether the organizational effects of gonadal hormones during puberty are necessary for steroid-independent male sexual behavior. Our results indicate that while gonadal steroids during puberty were not necessary for either testosterone or estradiol to activate male sexual behavior, exposure to gonadal steroids, specifically testosterone, during puberty was necessary for the expression of steroid-independent male sexual behavior in adulthood. The underlying mechanism by which testosterone organizes the normal maturation of neural circuitry during puberty that regulates steroid-independent male sexual behavior in adult castrated B6D2F1 male mice warrants further investigation.

PS1.0046 SEX DIFFERENCES IN OXYTOCIN MODULATION OF SOCIAL REWARD AND SOCIAL MOTIVATION IN SYRIAN HAMSTERS

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The rewarding and motivating properties of social interaction are critical in the expression of adaptive social behaviors and the development and maintenance of social relationships. Because social behavior evolved in response to different selective pressures in males and females, the neurobiological mechanisms mediating social reward are likely sex-dependent, and it seems likely that these sex differences may contribute to sex differences in the prevalence of psychiatric disorders. We have previously shown that activation of oxytocin (OT) receptors in the ventral tegmental area (VTA) is essential for social reward in male Syrian hamsters. With the Conditioned Place Preference (CPP) test and a novel Operant Social Preference (OSP) task, we hypothesized that OT in the VTA has sex specific effects on social reward. Social interaction increased the time spent in the non-preferred (social interaction) chamber in both males and females. OT (9 µM) and a highly selective OT receptor agonist (23µM) injected in the VTA increased time spent in the social interaction chamber in males, but decreased the time spent in the social interaction chamber in females compared to controls. An OT receptor antagonist (90µM) injected in the VTA decreased social reward in both males and females. These data

demonstrate that activation of OT receptors in the VTA plays a critical, but different role in modulating the rewarding properties of social interactions in males and females. Supported by NIH grant MH110212 to HEA and NIH predoctoral fellowship F31MH113367 to JMB

PS1.0047 OXYTOCIN INCREASES SOCIAL BEHAVIOUR DESPITE SEX-DEPENDENT EFFECTS ON ADULT HIPPOCAMPAL NEUROGENESIS

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Oxytocin regulates social behaviours and pair bonding. Adult hippocampal neurogenesis is increased by oxytocin in male rodents. Surprisingly, few studies have examined roles for oxytocin in both sexes. The aim of this study was to investigate the effects of oxytocin on social investigation and adult hippocampal neurogenesis in males and females. The use of systemic oxytocin is controversial as oxytocin has poor penetration of the blood-brain barrier. We used a novel nanoparticle drug delivery platform, TRIOZAN^{TM} (commercialized by Ovensa Inc.), that permits blood-brain-barrier penetration to deliver oxytocin in the brain. Adult male and female rats were injected daily for 10 days with oxytocin (in PBS) or oxytocin formulated with TRIOZAN^{TM} (0.5 or 1.0 mg/kg; i.p.) and tested for social behaviour in a three-chambered paradigm. Oxytocin significantly increased social investigation compared to control but males showed more investigation than females. In females, oxytocin (alone and delivered with TRIOZAN^{TM}) decreased the number of immature neurons (doublecortin-expressing cells) in the ventral hippocampus. In contrast, in males, oxytocin (alone and delivered with TRIOZAN^{TM}) increased the number of immature neurons in the dorsal and ventral hippocampus. Oxytocin and oxytocin delivered with TRIOZAN^{TM} resulted in similar levels of social investigation in both sexes but compared to oxytocin alone, oxytocin delivered with TRIOZAN^{TM} reduced sedation effects observed post-injection and increased the levels of oxytocin in the hypothalamus. The use of this nanomedicine platform may be a promising avenue as it eliminates some side effects of oxytocin while increasing central oxytocin levels and regulating neurogenesis.

PS1.0048 SEX DIFFERENCES IN EFFECTS OF LIGHT INTENSITY ON OREXINERGIC SYSTEM IN THE DIURNAL NILE GRASS RATS

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Seasonal fluctuation in ambient lighting has profound effects on mood and emotion, as seen in patients with seasonal affective disorder (SAD). Using a diurnal rodent model of SAD, our previous work involving chronic daytime light deficiency suggested a link between attenuated orexinergic output and increased depression- and anxiety-like behaviors in male *Nile* grass rats. The present study further examined how daytime light intensity modulates the orexinergic system in grass rats. Male and female animals were housed in either winter-like 12:12hr dim light/dark (dimLD) or a summer-like bright light/dark (brLD) conditions for 4 weeks. They were then examined for the number of orexin A immunoreactive (OXA-ir) neurons in the hypothalamus, and the level of orexin receptor (OX1R) protein in target regions. The number of hypothalamic OXA-ir neurons was significantly higher in brLD compared to dimLD condition in males, but did not differ between lighting conditions in females. Sex-specific modulation by daytime light intensity was also observed in OX1R expression in the prefrontal cortex (PFC) and the CA1 of the hippocampus, with OX1R significantly higher in brLD than dimLD in males, but the reverse was true for the PFC and no difference was found for the CA1 in females. In other brain regions including the dorsal raphe, amygdala and BNST, there was no daylight intensity effect on OX1R for either sex. The results collectively suggest sex differences in how the orexinergic system is involved in light-dependent changes in depression and anxiety.

PS1.0049 CONSEQUENCES OF PRENATAL EXPOSURE TO VALPROIC ACID IN THE SOCIALLY MONOGAMOUS PRAIRIE VOLE

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Environmental risk factors contribute to the etiology of autism spectrum disorders (ASD). In particular, prenatal exposure to the highly teratogenic anticonvulsant valproic acid (VPA) has been demonstrated to significantly increase the prevalence for developmental delay and ASD. Moreover, VPA-exposed rats and mice exhibit deficits in social behaviors that resemble some aspects of ASD. Although significant discoveries on the embryopathology of VPA have been proposed, its effects on social bonding, a complex behavior uncommonly displayed by rats and mice, remains unknown. In this study, we aimed at validating the socially monogamous prairie vole (*Microtus ochrogaster*) model for the study of the effects of prenatal VPA exposure. Despite receiving the same degree of bi-parental care, VPA-exposed male and female prairie voles maintain lower body weight throughout postnatal development, engage in fewer social affiliative behaviors in a familial context, exhibit less novel social interactions, and show enhanced anxiety-like behavior, compared to saline-exposed controls. A downregulation of cortical vasopressin receptor (V1aR) and methyl CpG-binding protein 2 (MECP2) mRNA expression coincide with these social impairments. Through chromatin immunoprecipitation, we confirm that reduced mRNA expression of V1aR and MECP2 occur through independent processes. Additionally, prenatal VPA exposure does not alter adult cortical total dendritic and spine-shape subtype densities. Remarkably, in spite of reduced levels of social affiliation with a

same-sex sibling, VPA-exposed males are still able to form a pair bond—as reflected by levels of partner preference and selective aggression similar to saline-exposed controls—following two weeks of cohabitation with a female.

PS1.005 VASOPRESSIN AND SOCIAL PLAY MODULATE LATERAL SEPTUM NEUROTRANSMITTER RELEASE IN SEX-SPECIFIC WAYS

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Social play is a rewarding behavior displayed by nearly all mammals and peaks during the juvenile period. We recently showed that arginine vasopressin (AVP) in the lateral septum (LS) regulates social play in opposite directions in male and female juvenile rats. Here, we sought to determine whether and how the LS-AVP system modulates the release of a wide array of neurotransmitters (NTs) in the LS. We used microdialysis with and without retrodialysis to quantify extracellular NT release in the LS of freely moving juvenile rats, while 1) AVP was applied into the LS, 2) rats were exposed to social play or 3) a vasopressin V1aR antagonist was administered into the LS. We observed a variety of dynamic release patterns of NTs that were sex-, and condition-specific. LS application of AVP caused an increase in the glutamate and dopamine release in females, while no change was seen in males. Other NTs did not change in sex-specific ways. Exposure to social play resulted in an increase in the release of all NTs in females, while in males, dopamine and norepinephrine remained unchanged. Finally, application of a V1aR antagonist in the LS caused sex differences in the release of glutamate and norepinephrine, with higher release in females. Interestingly, the sex differences in glutamate and dopamine release were eliminated with V1aR antagonist administration. These findings suggest a differential involvement of NTs in the LS of male and female juvenile rats exposed to social play, with potential roles of glutamate and dopamine in the sex-specific regulation of social play by the LS-AVP system.

PS1.0050 ADOLESCENT INCREASES IN REWARD-ASSOCIATED BEHAVIORS: IS PUBERTY TO BLAME?

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Adolescents exhibit marked increases in reward-associated behaviors such as risk-taking, novelty seeking, and social interaction. Whether these behavioral changes are driven by, or merely coincide with, elevated pubertal hormones has been difficult to discern. Seasonal breeders provide a unique opportunity to investigate this question. Siberian hamsters (*Phodopus sungorus*) born in long, summer-like day lengths (LDs) undergo rapid pubertal development, whereas those born in short, winter-like day lengths (SDs) delay puberty by 3-5 months to synchronize their breeding with the following spring. If pubertal hormones drive the adolescent behavioral changes, these changes should also be delayed in SD-reared hamsters. In the present experiment, male and female Siberian hamsters were reared in a LD or SD and tested in light/dark box, novel object, and social approach tests as either juveniles, early-adolescents, late adolescents, young adults, or adults to determine whether the SD-induced delay in puberty impacts developmental changes in exploration, novelty seeking, and social approach. For both male and female LD-reared hamsters, exploration, novelty seeking, and social approach increased across adolescence and subsequently levelled off or declined in young adulthood. SD-rearing did not alter the timing of their developmental rise, but instead extended this rise for exploration and novelty seeking (but not social approach) until the onset of puberty. These data indicate that the adolescent rise in reward-associated behaviors does not depend upon pubertal hormones. Instead, pubertal hormones likely bring about the end of this rise for non-social, reward-associated behaviors.

PS1.0052 MULTIGENERATIONAL EFFECTS OF CLOMIPRAMINE ON SOCIOEMOTIONAL BEHAVIOR AND SEROTONIN IN RATS

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Obsessive Compulsive Disorder (OCD) affects approximately 2% of adult Americans and involves repetitive, intrusive thoughts--obsessions--that lead to repetitive and ritualistic behaviors--compulsions. The postpartum period shows increased vulnerability to anxiety disorders including OCD. Anxiety disorders can be transferred to offspring through epigenetic effects, mediated by suboptimal maternal care. Clomipramine, a tricyclic antidepressant that targets serotonin and norepinephrine transporters, is normally used to treat adult OCD. However, exposure during the postnatal period can induce OCD in adulthood in male rats. We examined the effects of postnatal exposure to clomipramine on the development of OCD-like behavior in dams and their offspring. During postnatal days (PND) 9-16, females were treated with either saline or 15 mg/kg clomipramine. Around PND 90, females were mated and maternal behavior was observed during postpartum days (PPD) 1-6. On PPD 7, females were tested on the hole board to observe OCD-like behavior. Offspring were tested on the hole board on PND 30 and 90. During PPD 3-4, we observed that postnatal clomipramine-treated dams engaged in less active nursing compared to postnatal saline-treated dams. In addition, postnatal clomipramine-treated dams made more hole pokes compared to postnatal saline-treated dams. We also

found an epigenetic effect of clomipramine treatment, with peri-adolescent offspring of clomipramine-treated females making more hole pokes compared to offspring of saline-treated females. We are currently investigating the serotonin-related mechanisms behind clomipramine-induced epigenetic effects. These findings help to elucidate how development of OCD in offspring may be an indirect effect of early maternal exposure to clomipramine.

PS1.0053 GHRELIN RECEPTOR AGONISM SUPPRESSES SEIZURES IN THE D1R-MEDIATED KINDLING MODEL

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Epilepsy is a severe neurological disorder affecting millions worldwide. Despite the availability of numerous therapeutics, a staggering 30% of patients is not seizure-free. The ghrelin system has received considerable attention as a potential target for novel anti-seizure drugs. Ghrelin receptor (ghrelin-R) agonists exert anticonvulsive effects mediated via the ghrelin-R in various epilepsy models. This receptor forms heterodimeric complexes with the dopamine 1-receptor (D1R) in hippocampus, shifting canonical D1R-Gαs signaling towards Gαq signaling. Interestingly, D1R-activation is proconvulsive and repeated administration of the D1R agonist SKF81297 was shown to gradually induce a chronic kindled state in mice. Therefore, we aimed to investigate the effect of ghrelin receptor modulation on excitability exerted via D1R agonism. 9 to 11-week-old mice received five SKF81297 (5mg/kg i.p.) injections to induce a kindled state. Mice were pre-treated with macimorelin (5mg/kg i.p.), a potent ghrelin-R agonist, or saline prior to every SKF81297 injection. We observed a lower seizure severity in macimorelin-treated mice compared to saline treated control mice ($P<0.001$), with none of the macimorelin-treated mice reaching the most severe seizure score (61% vs. 0%; $P<0.01$). The intensity and duration of behavioral manifestations corresponded to hippocampal epileptiform discharges and EEG recordings revealed a lower number of seizures in the macimorelin-treated group (7.11 vs. 1.4; $P<0.001$). Our findings demonstrate that macimorelin treatment lowers the number and intensity of seizures in the D1R-kindling model, confirming similar observations in other seizure models. Further investigations will clarify whether ghrelin-R modulation is merely anticonvulsive or whether these effects are established via interfering with ghrelin-R:D1R interactions.

PS1.0054 PRENATAL EXPOSURE TO ENDOCRINE DISRUPTING CHEMICALS DISRUPTS MATE PREFERENCE IN ADULT RATS

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Endocrine disrupting chemicals are a class of compounds capable of interfering with the hormone systems of the body. They are a major concern for human and wildlife populations due to their global contamination and capacity to disrupt a broad range of biological processes. We have focused on two particular chemicals with differing mechanisms of action. The polychlorinated biphenyl mixture Aroclor 1221 (A1221) mimics some effects of endogenous estrogens; the fungicide vinclozolin blocks androgenic activity. In a model of prenatal exposure in rats, we show here that both A1221 and vinclozolin act in a sex-specific manner to disrupt typical mate preference behavior in adulthood. Pregnant dams were injected daily from embryonic day 8-18 with 1mg/kg A1221, 1mg/kg vinclozolin, or the vehicle 6% DMSO in sesame oil. Resulting offspring were tested in a mate preference paradigm at postnatal day 90. Experimental rats explored two opposite sex stimulus animals, one with circulating sex steroid hormones (gonadectomized and hormone replaced) and one without (gonadectomized only). Vehicle treated male and female rats showed a typical pattern of behavior, spending more time investigating the stimulus animal with hormone replacement (males: n=19, $p<0.05$; females: n=27, $p<0.001$). Male and female animals exposed to A1221, however, spent similar times investigating both stimulus options (males: n=16, p=0.44; females n=30, p=0.58). Vinclozolin treatment produced the same effect in males (n=21, p=0.99), but no effect in females. Additional experiments to test the contribution of olfactory dysfunction to these behavioral phenotypes are underway.

PS1.0055 THE IMPORTANCE OF INFANTILE NEURONAL NOS-DERIVED NO SIGNALING FOR FERTILITY, OLFACTION AND COGNITION

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The gonadotrophin-releasing hormone (GnRH) neurons are the key players in a complex neural network that is controlling sexual maturation, puberty onset and adult fertility in mammals. Interestingly, these GnRH neurons are not born inside the brain. They find their origin in the olfactory placode and migrate into the hypothalamic preoptic region during further embryogenesis. Defects in the migration of these GnRH neurons or the capacity to secrete their neurohormone result in a delay in puberty onset and fertility problems, and are related to genetic disorders like congenital hypogonadotropic hypogonadism (CHH) and Kallmann syndrome (KS). Neuronal nitric oxide (NO) synthase (nNOS or NOS1)-containing neurons have

been suggested to interact with GnRH neurons and regulate their activity and neurosecretory capacity. Here, we report that mutations in the *Nos1* human gene have been discovered in patients with CHH and KS, and it appears that several of them are associated with comorbidities. Similar to human, *Nos1* deficient mice do not only show impaired minipuberty, pubertal delay and infertility, but also cognitive, auditory and olfactory impairments. Moreover, we demonstrate that the administration of inhaled NO during the late infantile period can reverse the defects in sexual maturation, olfaction and cognition in this mouse model. Our results identify a critical time window for Nos1 action and suggest a potential therapeutic for human.

PS1.0056 A ROLE FOR THE HYPOTHALAMUS IN VITAMIN A HOMEOSTASIS

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Vitamin A is an essential lipid-soluble dietary micronutrient. It is stored as retinyl esters in the liver, released in the circulation primarily as retinol while retinoic acid (RA) is the active metabolite that regulates gene transcription. It is well established that circulatory plasma retinol levels are under tight homeostatic control maintained at 1µM concentration. This homeostasis is essential for life, however, how this homeostasis is controlled is unknown. We hypothesise that homeostatic regulation of plasma retinol involves sensing of retinol by hypothalamic tanycytes, cells that line the third ventricle positioned to sense retinol in the cerebrospinal fluid. To study this, rats were grouped randomly and 5µl of 10µM RA, retinol, or DMSO (vehicle) was stereotaxically injected into the third ventricle of a rat brain. This is predicted to disrupt homeostasis if retinol sensors were present in hypothalamic tanycytes. 24 hours after injection, samples were obtained for HPLC and qPCR analysis of genes involved in homeostasis. Response of tanycytes in the hypothalamus to the injected RA was demonstrated by a significant downregulation in *Raldh1*. Several *mRNA* transcripts of genes involved in homeostasis in the liver (*Crbp1*, *Rbp4*, *Cyp26a1* and *Lrat*) showed suggestive but non-significant change. HPLC quantification of retinoids showed significantly increased storage of retinyl palmitate in the liver, liver retinol and circulatory serum retinol in RA injected rats. In retinol injected rats, only circulatory plasma retinol was significantly altered. These results support our hypothesis that hypothalamic tanycytes detect the retinol metabolite RA as part of a retinol homeostatic regulatory system.

PS1.0057 GLUCOCORTICOIDS RAPIDLY INDUCE HYPERPHAGIA AND INSULIN RESISTANCE, BUT OBESITY IS DELAYED IN MICE

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Glucocorticoids (Gcs) are widely used therapeutic agents, but often cause adverse effects leading to obesity and diabetes. This study aimed to investigate the central and peripheral Gc effects in the development of these metabolic complications. Mice administered corticosterone (Cort, 75µg/ml in drinking water) developed hyperphagia from 24h and hypothalamic *Agrp* was increased 2-fold. Neither *Pomc* nor *Npy* were altered at this timepoint, despite the predicted effects of Gc treatment. This may be related to early increases in circulating insulin (10-fold) and leptin (5-fold). After 48h, the hyperinsulinaemia was associated with early widespread insulin resistance in tissues including skeletal muscle, liver and adipose tissues, indicated by changes in the expression of *Irs1*, *P85a* and/or *P110b*. After 3 weeks continued Cort treatment, leptin and insulin were markedly increased (22-fold and 36-fold respectively). In the hypothalamus, *Agrp* continued to be elevated but *Pomc* was still not altered and the small decrease in *Npy* expression, was assumed to be compensation for the continued elevation in food intake. Nine other hypothalamic factors related to food intake were also not altered. Insulin resistance was still present at 3 weeks, and was now accompanied by hyperglycaemia, elevated adiposity and increased body weight. Exogenous Gcs cause early changes in *Agrp,* but few other hypothalamic factors are altered. There are marked peripheral metabolic effects of chronic Gc treatment which could be influenced by these hypothalamic actions of Gcs but it is not clear whether central or peripheral mechanisms are the culprits.

PS1.0058 GLUCOCORTICOID-INDUCED OBESITY AND HYPERPHAGIA: IS THE BRAIN TO BLAME?

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Glucocorticoid (Gc) excess, resulting from exogenous Gcs given as medical therapy for inflammatory diseases such as rheumatoid arthritis, is recognised to cause a myriad of adverse metabolic side effects. In a mouse model of Gc treatment, Gcs increase food intake and chronically elevate hypothalamic Gc levels, which are associated with increased agouti-related peptide (*Agrp)* expression. The aim of this study was to determine the role of *Agrp* in contributing to Gc-induced hyperphagia and obesity. CRISPR technology was used to generate a

novel global AgRP knockout mouse (AgRP-KO), with the three coding exons of the AgRP gene deleted. Knockout was confirmed at the mRNA level through both *Agrp in situ* hybridisation and qRT-PCR of the hypothalamus. In female mice, knockout of AgRP alone did not affect food intake. However, when AgRP-KO mice were treated with corticosterone (75µg/ml, AgRP-KO-Cort) in drinking water for three weeks, they were partially protected against the Gc-induced hyperphagia. In addition, female AgRP-KO-Cort mice had a delayed increase in body weight compared to Cort treated wild-type mice. In contrast, 3-weeks Cort treatment in male AgRP-KO mice identified that the protective effect of AgRP KO was gender specific. While Gc-induced hyperphagia and obesity are mediated in part by AgRP in female mice, this data emphasizes that other mechanisms are also responsible. It will therefore be important to understand the role of other hypothalamic neuropeptides in the development of these metabolic abnormalities.

PS1.0059 INVESTIGATION OF MELANOCORTIN SYSTEM PEPTIDES IN THE DEVELOPING HUMAN BRAIN

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Neurons of the hypothalamic melanocortin system contribute to regulation of body weight, yet their characterization during human fetal development are limited. To determine when markers of melanocortin system function are expressed during development, we labelled human fetal brains from 9 to 14 gestational weeks (GW) of development. We hypothesized that pro-opiomelanocortin (POMC) expressing neurons would be observed earlier in development, followed by expression of neuropeptide Y (NPY), as a subpopulation of NPY neurons derive from POMC progenitor neurons in mice. In the developing human hypothalamus from GW9 to GW14, no staining was observed for alpha-MSH or beta-endorphin, indicating lack of POMC expression. NPY staining was not observed in the developing arcuate nucleus. However, a small population of NPY-immunoreactive cells was observed in the developing dorsomedial hypothalamic nucleus. This is a region known to contain NPY neurons, particularly in adult female mice. While markers for POMC were not observed in the developing human hypothalamus until the latest fetal stage that we could analyze, GW14, strong staining for ACTH, alpha-MSH, and beta-endorphin were observed in developing anterior pituitary corticotropes from GW9 to GW14. Moreover, expression of plasmalemma vesicle associated protein (PLVAP), a marker of fenestrated capillaries and vasculogenesis, was observed only in the developing fetal brain at GW14 but not the pituitary. This indicates that corticotropes appear even before capillary network formation in the human pituitary. This study sheds new light on the spatio-temporal expression of hypothalamic and pituitary markers in the human fetal brain.

PS1.006 ACTIN POLYMERIZATION INHIBITION BLOCKS RAPID ESTROGEN-FACILITATED SOCIAL RECOGNITION IN FEMALE MICE

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Estrogens can rapidly facilitate social recognition (SR) in ovariectomized female mice. SR is facilitated within 40min of systemic (Phan et al., 2012) or dorsal hippocampal (Phan et al., 2015) administration of 17β-estradiol (E2). Within the same timeframe, E2 increases dendritic spine density in CA1 hippocampal neurons (Phan et al., 2012; 2015). Modifications to dendritic spine morphology occur through remodeling of the actin cytoskeleton. Estradiol rapidly (30-60min) stimulates changes in actin cytoskeletal dynamics through rapid enhancement of actin polymerization (Briz & Baudry, 2014). Whether actin cytoskeletal remodeling is required for the rapid facilitation of SR by E2 has yet to be established. Here we first determined the highest dose of actin polymerization inhibitor latrunculin A (LAT-A) that does not block SR when infused into the dorsal hippocampus of ovariectomized female mice 15min prior to testing. We then determined whether this dose of LAT-A could prevent the enhancing effects of E2 (as in Phan et al., 2015) in a task where controls do not typically perform SR. These paradigms consist of habituation trails where two female conspecifics are presented and one test trial where one conspecific is novel and the other is familiar. The paradigms are completed within 40min of E2 administration, thus enabling investigation of rapid effects of estrogens. Actin polymerization was found to be necessary for E2 to rapidly facilitate SR, as a dose of 25ng/hemisphere of LAT-A blocked the rapid facilitation of SR by E2. This provides a cell structural mechanism through which estrogens rapidly facilitate SR.

PS1.0060 HYPOTHALAMIC TANYCYTES OF THE ARCUATE NUCLEUS CONTROL FEEDING CENTRES.

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Hypothalamic **tanycytes** are glial cells lining the third ventricle of mammalians' brain. Recently, we have demonstrated that these cells play an important role into the regulation of body weight by **sensing the cerebrospinal fluid (CSF) of nutrients** such as glucose and amino acids, using influx of extracellular Ca2+ into the cell. This mechanism relays on tanycytic **ATP release**. These **cell-sensors contact** the CSF of the third ventricle, and send processes into the **hypothalamic nuclei that control food intake and body weight**.

Here, we asked the question of whether these cells are part of the feeding centres with which

these cells are in contact? Are they part of the hypothalamic anorexigenic or the orexigenic pathway?

We used CatCh, a channelrhodopsin-2 variant more permeable for Ca2+, to remotely activate tanycytes and mime responses to nutrients both in vivo and in vitro.
And indeed, tanycytic optostimulation induced depolarisation of neurones of the arcuate nucleus of both pathways: **neuropeptide Y-expressing (NPY) and proopiomelanocortin-expressing (POMC)**.
We also demonstrated that ATP release by tanycytes is also the transmitter that allows these neuronal responses.

PS1.0061 CORTISOL RESPONSIVENESS, OBESITY AND THE ROLE OF MELANOCORTIN AND OXYTOCIN SYSTEMS.

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We previously identified sub-populations of ewes (approximately 10% from each extreme) that have either high (HR) or low (LR) cortisol responses to a low dose of ACTH (0.2μg/kg). When placed on a high-energy diet, HR become more obese and have impaired melanocortin signalling; HR animals are resistant to the satiety effect of α-melanocyte stimulating hormone (αMSH). Herein we aimed to characterise the genetic and neuroanatomical basis of melanocortin resistance in HR animals. A single blood sample was collected from 4 LR and 5 HR ewes, white blood cells were isolated and used to extract genomic DNA. DNA was used to sequence 1500 bp promoter region of the melanocortin 4 receptor (MC4R) gene. Although, we identified numerous genetic variants in the MC4R promoter region, these did not align with the LR or HR phenotype. Neuroanatomical characterisation focused on the melanocortin-oxytocin pathway. Hypothalami were collected and perfusion fixed for double *in situ hybridisation* (n=3 LR and n=5 HR). The total number of MC4R ($P<0.01$) and oxytocin ($P<0.05$) expressing cells was greater in LR than HR animals, as was the total number of cells co-localising MC4R and oxytocin ($P<0.05$). The % of oxytocin cells that expressed the MC4R, however, was similar in LR and HR groups. In summary, reduced expression of MC4R in HR did not coincide with any genetic variation in the MC4R promoter region. Irrespective of this, HR exhibit reduced expression and colocalisation of MC4R and oxytocin, which is consistent with the obesity-prone phenotype.

PS1.0062 PALMITATE TREATMENT INCREASES AUTOPHAGY IN MHYPOE-46 AND MHYPOA 2-29 HYPOTHALAMIC CELL LINES.

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Autophagy is a well-known process that regulates cellular homeostasis by degrading malformed organelles and dysfunctional proteins. Normal autophagy is crucial to maintain the functionality of hypothalamic neurons, which are important in regulating energy balance. Palmitate, a saturated fatty acid commonly associated with obesity development, is directly sensed by hypothalamic neurons. Therefore, we hypothesized that palmitate affects the autophagy process in *Npy*-expressing hypothalamic neurons. We used the clonal, embryonic male, mHypoE-46, and adult male, mHypoA2-29, cell lines to evaluate autophagy modulation in response to palmitate treatment. We also used an *ex vivo* treatment model to understand the role of palmitate in the whole hypothalamus. We show that 50 µM palmitate decreased ps6 and increased LC3B-II protein content in both cell lines in a time-dependent manner, suggesting an increase in autophagy modulation. p62 protein content was also increased in both cell lines. p62 levels are usually inversely correlated with autophagy modulation. However, this protein can be associated with other cellular degradation systems, such as the ubiquitin proteasome pathway. Using the *ex vivo* protocol, we did not find any differences in autophagy marker and p-JNK protein levels after palmitate treatment for 4 hours. We are currently repeating the *ex vivo* treatment with 16 and 24 hours of palmitate exposure. We are also interested in studying the relationship between increased autophagy markers and *Npy* expression and cell survival after palmitate exposure in these cell lines. Understanding how palmitate affects autophagy in neurons that control food intake can represent a promising therapeutic target against obesity.

PS1.0063 A PVN TO PANCREAS NEURONAL INPUT RAPIDLY MODIFIES GLUCOSE-STIMULATED INSULIN SECRETION

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Insulin secretion is critical for the maintenance of euglycemia and therefore plays an important role in the pathogenesis of diabetes. Regulation of insulin secretion by intra-islet autonomic nerve terminals has been well studied. However, little is known about the role of central preautonomic circuits that can modify insulin levels via the autonomic nervous system. Retrograde tracing studies have shown that the paraventricular hypothalamic nucleus (PVN) is a critical preautonomic node in the integration of central signals towards autonomic regions. In

this study, we describe the role of PVN neurons, and in particular oxytocin neurons (OXT), in the regulation of glucose-stimulated insulin secretion. We find that chemogenetic stimulation of the majority of PVN neurons, the Sim-1 neurons ($Sim1^{PVN}$), leads to an acute suppression of insulin levels upon glucose challenge. Further, stimulation of OXT^{PVN} neurons leads to an even more profound diminution in plasma insulin. Similar activation of OXT neurons in the supraoptic nucleus of the hypothalamus (SON) did not have any impact on insulin levels. We also propose a novel neuronal circuit that involves OXT^{PVN}-induced inhibition of parasympathetic preganglionic neurons in the dorsal motor nucleus (DMN). Overall, in this study we uncover a brain to pancreas circuit that strongly regulates insulin secretion.

PS1.0064 ROLE OF TANYCYTE NETWORK IN THE REGULATION OF ENERGY HOMEOSTASIS

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Tanycytes of the arcuate nucleus of the hypothalamus (ARH) are specialized glial cells found in the lateral walls of the third ventricle, with one side contacting the cerebrospinal fluid (CSF) and the other sending a single process towards the ARH, the brain feeding center. Given their strategic location at the interface between CSF, which contains glucose, and glucose-sensitive ARH neurons, we hypothesize that tanycytes might provide information about body energy status to the brain. Biocytin, a gap junction permeable tracer, injected *via* a patch pipette into a single ARH tanycyte in acute hypothalamic slices from mice diffused extensively between tanycytes, revealing gap junction coupling between these cells. Immunostaining indicated dense expression of the gap junction subunit connexin (Cx) 43 in ARH tanycyte cell bodies. Because Cx43-mediated gap junctions allow the passage of energy metabolites, we tested the possibility for glucose to traffic through the Cx43-mediated tanycyte network to reach ARH neurons. The fluorescent glucose analog, 2-NBDG, injected into a single ARH tanycyte *via* a patch pipette diffused in neighboring tanycytes, illuminating their cell body and process projecting to the ARH. Using the Cre-LoxP system in mice, we found that selective knockout of Cx43 in tanycytes abolished 2-NBDG diffusion between tanycytes and altered energy balance *in vivo* towards increased food intake and decreased energy expenditure. Together, our data indicate that ARH tanycytes form a network of interconnected cells allowing the trafficking of glucose towards the ARH, a process which may contribute to brain glucose sensing.

PS1.0065 PERMEABILITY OF THE BLOOD-HYPOTHALAMUS BARRIER CHANGES ALONG THE DAY.

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The suprachiasmatic nucleus (SCN), organizes the metabolism and hormonal secretion circadianally. The arcuate nucleus (ARC) is one of the main nuclei for energy and hormonal regulation and is tightly regulated by the SCN. To accomplish its function, ARC depends on the information that receives from the periphery through circulation; however, this is restricted by the blood-hypothalamic barrier (BHB). We investigated whether the access of circulating information to the ARC was also timed and if so whether the SCN was involved. Through permeability assays performed in Wistar rats, we compared the penetrability of the ARC at different time points (ZT2,ZT11,ZT14,ZT22). We found that the penetrability of ARC/ME fluctuates daily having its peak at Zeitgeber Time (ZT)2. Interestingly, the penetration to the ARC at ZT2 is prevented with a bilateral lesion of the SCN, while unilateral lesions of the SCN induces ipsilateral closing of the ARC/ME barrier. Also, we evaluate the speed by which the BHB can change and we found that BHB requires at least 30 min to change significantly its permeability. Our findings indicate that there is a daily rhythm in the permeability of BHB and it is neuronally controlled by the SCN. By changing the permeability, SCN might control the amount of circulating information that accesses the ARC and in consequence, participate in the regulation of hormonal secretion. By understanding the communication between the periphery and the hypothalamus; we can aim to better understand the consequences of a defective crosstalk between them, manifested in diseases as Obesity and T2-Diabetes Mellitus.

PS1.0066 CORTICOTROPIN-RELEASING FACTOR RECEPTOR SIGNALING IN THE BRAIN STIMULATES GHRELIN SECRETION IN MICE

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Ghrelin is a stomach-derived hormone, which is secreted during fasting and plays a role in maintaining the plasma glucose levels during severe calorie restriction. Previously, we found that ghrelin secretion was stimulated by norepinephrine through β-1 adrenergic receptors on the ghrelin cells. The injection of β-1 adrenergic receptor antagonist or reserpine into mice inhibited the fasting-induced elevation of ghrelin level, suggesting that ghrelin secretion is regulated by the sympathetic nervous system. In this study, to understand the central mechanism of sympathetic nervous system activation, we examined the effect of central urocortin-1 (UCN1) and urocortin-2 (UCN2) administration on circulating-ghrelin levels using conscious mice. Intracerebroventricular (i.c.v) injection of UCN1 increased the plasma ghrelin concentration in fed state but not in fasted state. Similarly, i.c.v administration of UCN2 increased the plasma ghrelin concentration. However, peripheral administration of UCN1 did

not alter the plasma ghrelin concentration in fed state and RT-PCR showed that corticotropin-releasing factor receptor (CRF-R) -1 and -2 mRNAs were not expressed in the stomach of mice and in the ghrelin-producing cell lines. Further, to understand the mechanism of urocortin-induced ghrelin level elevation, we centrally injected β-1 adrenergic receptor antagonists and found that UCN1-induced ghrelin elevation in fed state was blocked by the pre-treatment with β-1 adrenergic receptor antagonists. Moreover, we showed that the increase in fasting-induced ghrelin level was significantly inhibited by the pre-treatment of a nonselective CRF antagonist. These results suggest that CRF-R signaling in the brain is involved in the ghrelin secretion through the regulation of sympathetic nervous system.

PS1.0067 IMPORTANT ROLE OF NEUROPEPTIDE-FF SIGNALLING IN THE REGULATION OF GLUCOSE AND ENERGY HOMEOSTASIS

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Neuropeptide FF (NPFF) is an eight-amino acid peptide belonging to the RFamide peptide family characterized by an arginine and amidated phenylalanine C-terminus. Within the central nervous system NPFF is predominantly expressed in the subpostrema area in the brain stem in close proximity to the circumventricular organ suggesting a role of NPFF in monitoring peripheral energy status and regulating energy homeostasis. However, so far this has not been formally examined. Here we show that the expression of NPFF responds to changes in energy status, i.e. decreasing after 6 weeks of high-fat diet feeding, and increasing after a 24-hour fast. Under basal *ad libtum* condition, $NPFF^{-/-}$ mice do not differ from WT with regards to body weight, adiposity, food intake, energy metabolism or physical activity. Importantly, however, during the refeeding period after a 24-h fast, $NPFF^{-/-}$ mice exhibit significantly greater weight gain, which is associated with significantly decreased energy expenditure but unaltered food intake compared to WT mice. Moreover, in response to a challenge of an intraperitoneal glucose bolus, $NPFF^{-/-}$ mice exhibit significantly reduced glucose excursion compared to WT mice, demonstrating an improved glucose tolerance due to the lack of NPFF signalling. Furthermore, in response to an intraperitoneal insulin injection, blood glucose recovered significantly quicker in $NPFF^{-/-}$ than WT mice, suggesting NPFF signalling is critical in counterregualtory mechanisms that restore glucose homeostasis. Taken together, these data indicate an important role of NPFF signalling in the regulation of energy and glucose homeostasis particular when the system is facing a challenge.

PS1.0068 INVESTIGATING OBESITY-ASSOCIATED BRAIN INFLAMMATION USING QUANTITATIVE WATER CONTENT MAPPING

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There is growing evidence that obesity is associated with brain Inflammation contributing to the pathogenesis of obesity. In humans, it is challenging to detect brain inflammation *in vivo*. Recently, quantitative magnetic resonance imaging (qMRI) has emerged as a tool to characterize pathophysiological processes in the brain with reliable and reproducible measures. Proton density imaging provides quantitative assessment of the brain water content, which is affected in different pathologies including inflammation. We enrolled 60 normal weight, overweight and obese men and women (body mass index (BMI) range 20.1-34.4 kg/m^2, age range 20-71 years, 73.3% men) to acquire water content mapping *in vivo* using MRI at 3 Tesla. We investigated potential associations between anthropometric measures of obesity with brain water content. No global changes in water content were observed with measures of obesity. However, the limbic lobe, midbrain and pons showed higher water content values with increasing BMI independent of age ($p<0.005$). Moreover, hypothalamic water content values revealed a strong relationship with BMI, especially in older adults. We identified the highest hypothalamic water content values in individuals fulfilling the definition for metabolic syndrome ($p<0.005$, adjusted for age). Using qMRI, we were able to detect marked water content changes in young and older obese adults. This is most likely due to chronic low-grade inflammation. Whether brain inflammation is a cause or consequence of obesity, in humans, stills needs to be investigated using a longitudinal study design.

PS1.0069 NUTRITIONAL LIPIDS AND GLIAL REMODELING

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Energy balance is finely regulated by the central nervous system. The hypothalamus (HT) integrates peripheral signals reflecting the energy status of the organism and in turn adapts food intake and energy expenditure in order to maintain a stable weight throughout adult life.

Several studies show that obesity induced by a high fat diet (HFD) leads to neuroinflammation in the HT. Moreover, lipids contained in HFD might be directly responsible for the onset of the inflammatory response. At cellular level, this inflammation is in part characterized by an activation of microglia cells and astrocytes in the HT. In rodent, recent studies show that hypothalamic proliferation of microglia cells and astrocytes is observed in the first 24 hours of consumption of HFD, well before the development of obesity, and seems to be reversible. We therefore assume that early glial activation would be an adaptive mechanism involved in the physiological regulation of energy balance and that overexposure to nutritional lipids could deregulate this inflammatory response and lead to obesity. In our study we observed an increase in the expression of the astrocytes and microglial cells markers in the HT after 1 h of HFD consumption. Moreover we observed morphological modifications of microglial cells after 3h of HFD consumption. This remodeling is associated with differential activation of specific inflammatory markers and hypothalamic peptides involved in energy balance regulation. Our results suggest that inflammation induced by HFD consumption is a very early phenomenon which might be involved in the central regulation of energy balance.

PS1.007 ESTROGEN SIGNALLING IN ANXIODEPRESSIVE BEHAVIOUR INDUCED BY SATURATED FAT

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Obesity with metabolic dysfunction significantly increases the risk of developing depression. The nucleus accumbens (NAc) is a brain region involved in goal-oriented behavior, reward and hedonic impairments associated with mood disorders. Our group has recently demonstrated that a saturated, but no monounsaturated,high-fat diet (HFD) promotes anxiodepressive behaviour via NAc inflammation in male mice. As depression diagnosisis near double in women as compared to men, we set out to investigate the metabolic and behavioural outcomes of saturated and monounsaturated HFDs in female mice as well as to verify the relative contribution of NAc inflammation. Adult C57Bl6 female mice were fed either a low-fat diet (17%kcal lipids; soybean oil), a saturated HFD (50%; palm oil) or a monounsaturated HFD (50%; olive oil) for 24 weeks (n=12/diet). Anxiodepressive behaviour was assessed with the elevated-plus maze and forced swim test. NAc gene expression for inflammatory markers and estrogen receptors (ER) was determined by RT-qPCR. Plasma 17β-estradiol quantified by ELISA. Mice fed the saturated, but not monounsaturated, HFD displayed increased anxiodepressive behaviour compared to controls. In opposition to males, inflammatory levels in the NAc did not differ between both HFDs. In fact, increased NAc ERα gene expression, as well as heightened 17β-estradiol plasma concentrations, were associated withthe anxiodepressive phenotype of mice fed the saturated HFD. This data suggests saturated, but not monounsaturated, high-fat feeding elicits alterations in estrogen signaling that can contribute to anxiodepressive behaviour. Such

results highlight a sexual dimorphism in the mechanisms underlying depression comorbid to obesity

PS1.0070 INVESTIGATING THE EFFECTS OF PROTEIN DIGESTION ON GUT CONTENT AND SATIETY

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Protein is recognised as the most satiating macronutrient, and high-protein diets are associated with weight loss and maintenance. However, the protein-derived bioactive peptides and amino acids that mediate these appetite-regulating effects, and their underlying physiological mechanisms, remain unclear. Activation of nutrient-sensing mechanisms in the gut leads to release of gut hormones, such as glucagon-like peptide 1 (GLP-1), which are involved in appetite regulation pathways. We are conducting studies to explore acute effects of protein on satiety in healthy volunteers. Metabolomic and microbial profiling will identify metabolites and microbes from the ileum and colon obtained via enteral tube. Blood analysis will provide gut hormone concentrations, and visual analogue scales will assess subjective appetite. To establish causality of small and large intestinal effects on gut hormone release and satiety, we are concurrently screening candidate appetite-suppressing metabolites using a 3D intestinal stem cell-derived organoid culture system. These 'mini-guts' functionally recapitulate intestine physiology, providing a useful model to investigate nutrient sensing and incretin responses. Our hormone secretion assays in mouse ileum organoids revealed significantly higher GLP-1 release after 24 hours of treatment with the amino acid L-Phenylalanine, an effect significantly attenuated by addition of a calcium-sensing receptor (CaSR) antagonist. This suggests that L-Phenylalanine stimulates GLP-1 secretion, and the CaSR may mediate satiety in the gastrointestinal tract through detection of amino acid products from protein digestion. Understanding how the gut senses ingested protein to reduce food intake may aid development of foods that promote the feeling of fullness, which could be used to treat obesity.

PS1.0071 PATTERN DEPENDENT GENOME-WIDE DYSREGULATION OF GLUCOCORTICOID RECEPTOR ACTION IN LIVER

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Adrenal glucocorticoid (GC) secretion displays characteristic circadian and ultradian patterns, which are highly conserved across mammals. GCs such as corticosterone (cort) act on the glucocorticoid receptor (GR), a ligand-activated transcription factor that binds to specific DNA response elements modulating target gene transcription. Recent cell work has provided evidence that constant cort exposure prolongs GR activation and alters the temporal dynamics of RNA PolymeraseII (Pol2) recruitment. However, the effects of altering the GC ultradian rhythm *in vivo* are less well understood. Here, we reveal how altering the pattern of cort exposure results in differential modulation of transcriptional responses in the liver. Adrenalectomized male Sprague Dawley rats were intravenously administered a physiological dose of cort via hourly 20 min pulses or matched constant infusions over 3 hours. Liver samples were collected at timepoints corresponding to pulse peak (2hr20min) and nadir (3hr), chromatin immunoprecipitated with GR and Pol2 antibodies, and sequenced. We found that GR recruitment to genomic loci in the liver samples closely tracked the respective circulating cort profiles, with characteristic pulsatile dynamics in the case of the ultradian infusion and persistent GR binding in the case of the constant infusion. However the relationship between cort pattern and Pol2 activity was far more complex, with a clear dissociation between cort pattern and Pol2 activity in many key metabolic target genes. Our findings support a model where dysregulated cort exposure acts to drive aberrant gene expression of critical metabolic targets in the liver and may therefore contribute to the development of metabolic pathology.

PS1.0072 LEPTIN AND INSULIN DO NOT EXERT REDUNDANT CONTROL OF METABOLIC FUNCTION VIA DOPAMINE NEURONS

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Leptin and insulin's hunger-suppressing and activity-promoting actions in the hypothalamus are well characterized, yet the mechanisms by which they modulate the midbrain dopamine system to influence energy homeostasis remain less clear. A subset of midbrain dopamine neurons express receptors for leptin (Lepr) and insulin (Insr), and direct administration of either leptin or insulin to the midbrain reduces food intake. Leptin-dopamine signaling also reduces running reward and homecage activity. However, dopamine-specific deletion of Lepr does not affect body weight or food intake in mice. This could be explained by leptin indirectly modulating dopamine neurons to exert its effects. Alternatively, insulin-dopamine signaling could compensate for the absence of leptin-dopamine signaling. To investigate the degree to which insulin and leptin exert overlapping (i.e. redundant) versus discrete control over dopamine neurons, we generated transgenic male and female mice exhibiting dopamine-specific deletion of either Lepr (Lepr-KO), Insr (Insr-KO) or both Lepr and Insr (Dbl-KO) and assessed their feeding behavior, voluntary activity, and energy expenditure compared to control mice (CON). No

differences in body weight, daily food intake, energy expenditure or hyperphagic feeding of palatable chow were observed between Lepr-KO, Insr-KO or Dbl-KO mice and CON mice. However, consistent with previous findings, Lepr-KO (but not Insr-KO or Dbl-KO) male mice exhibited significantly increased running wheel activity compared to CONs (P=0.007). These data demonstrate that insulin and leptin do not exert redundant control of dopamine neuron-mediated regulation of energy homeostasis. Furthermore, neither plays a critical role in the regulation of dopamine neurons with regards to feeding behavior.

PS1.0073 EFFECTS OF THE SATURATED FATTY ACID PALMITATE ON NEURONAL MORPHOLOGY AND FUNCTION

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Diabetes and obesity are known to contribute to the development of Alzheimer's disease. A western diet, enriched in long-chain saturated fatty acids, is a risk factor for these conditions. Although the importance of polyunsaturated fatty acids for the maintenance of cognitive function is well known, possible implications of saturated fatty acids are less well understood. Here we investigated effects of palmitate, the most abundant saturated fatty acid in the western diet, on the morphology and function of hippocampal and cortical neurons. Furthermore, we analysed whether the unsaturated fatty acid docosahexaenoic acid (DHA) prevents potential palmitate-mediated cellular changes. Primary hippocampal and cortical rat neurons were treated with either 200µM palmitate, 200µM DHA or an equimolar combination of both. Dual label-immunocytochemistry for the Microtubules Associated Proteins MAP2 and Tau revealed that palmitate, but not DHA, leads to severe morphological changes in these neurons, including swelling of the cell body and blebbing in axons and dendrites compromising healthy cell function. These phenomena were due to a breakdown of the microtubules, as revealed by β-tubulin staining. A three-dimensional analysis of the synaptic input, visualised by Synapsin1 staining, furthermore exhibited a reduction in the number of synapses after palmitate treatment leading to reduced cell excitability. Interestingly, DHA was able to prevent all these changes, if applied simultaneously. It emerged that these lipids affect specific neuronal insulin signalling which may have direct functional implications for Alzheimer’s disease. We furthermore discovered that DHA enriched HFD prevents HFD diet-induced metabolic alterations and alters neuron morphology in mice.

PS1.0074 CCL5 PROTECTS AGAINST THE DEVELOPMENT OF OBESITY, DIABETES AND ASSOCIATED NEUROPATHIC PAIN.

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CCL5/RANTES is a chemoattractant cytokine well known for its role in cerebral and peripheral inflammation. Together with its receptors CCR1, CCR3 and CCR5 it also contributes to neural function and diseases such as obesity, type 2 diabetes (T2D) and neuropathic pain. Herein, we investigated the role of CCL5 in the development of diet-induced obesity (DIO), metabolic impairment and neuropathic pain. We tested the long-term effects of high-fat (HFD) or standard diet on the development of obesity in adult $CCL5^{-/-}$ mice and wild-type mice (WT) and discovered that $CCL5^{-/-}$ mice seem to be protected from weight gain and the associated impairment of glucose metabolism. To evaluate the implication of CCL5 in neuropathic pain associated with diabetes, thermal pain sensitivity of $CCL5^{-/-}$ mice was measured in both conditions. Remarkably, in HF condition, $CCL5^{-/-}$ displayed higher tolerance to heat pain compared to control mice. Furthermore, $CCL5^{-/-}$ mice show a different expression pattern of inflammatory markers and hypothalamic neuropeptides compared to control mice. Preliminary results of immunostaining of CCL5 and its receptors revealed expression of CCR3 and CCR5 in the hypothalamus in WT mice. Our results indicate that under a HF challenge the absence of CCL5 seems to have a protective effect on the development of obesity and associated metabolic impairment as well as an ameliorating effect on thermal pain sensitivity. CCL5 could be involved in the maintenance of overfeeding, possibly through an indirect action on neuronal hypothalamic systems, in the deregulated central control of energy balance found in obesity.

PS1.0075 BISPHENOL A INCREASES AGRP MRNA LEVELS IN HYPOTHALAMIC NEURONS THROUGH MAP KINASES, JNK AND ERK

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Bisphenol A (BPA), an environmentally ubiquitous compound found in plastics, can linings and receipts, is termed an 'obesogen' as it leads to adipogenesis, lipogenesis and weight gain in animal models. AgRP and NPY are orexigenic neuropeptides produced in the hypothalamus and a dysregulation of these peptides can lead to hyperphagia and obesity. Using quantitative PCR, we have previously shown 100 micromolar BPA upregulated AgRP and altered NPY mRNA levels in six NPY/AgRP-expressing cell lines across 24 hours. We hypothesized that these BPA-mediated changes occur through induction of neuroinflammation and endoplasmic reticulum stress or through activation of nuclear receptors. We found that BPA increased mRNA levels of inflammatory cytokines (IL6, TNFalpha, IL10), ER stress markers (CHOP, GRP78, Bax/Bcl2) and

nitric oxide synthases (iNOS, nNOS). These findings are currently being validated in hypothalamic primary culture. Western blot analysis showed BPA increased phosphorylation of the MAP kinases, JNK and ERK. Inhibition of JNK (SP600125) and MEK/ERK (PD0325901) partially abolished the BPA-mediated upregulation of AgRP, but not NPY, suggesting divergent mechanisms by which BPA regulates AgRP versus NPY. Furthermore, inhibition of NFkappaB activation (PS1145) and nuclear receptors, ERRgamma (GSK5182) and AhR (CH223191), did not have an effect on BPA-mediated AgRP or NPY induction. Future studies will focus on the nitric oxide pathway, using inhibitors to iNOS, nNOS and downstream effector, guanylyl cyclase. These studies will provide a potential mechanism by which BPA may act as an obesogen in the hypothalamus by increasing orexigenic neuropeptide expression, ultimately to increase food intake, often leading to obesity.

PS1.0076 RESCUING BPA-MEDIATED POMC GENE DYSREGULATION WITH ANTI-INFLAMMATORIES IN HYPOTHALAMIC NEURONS

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Endocrine disrupting chemicals, such as bisphenol A (BPA), have been linked to obesity. However, the effect of BPA on the hypothalamic pro-opiomelanocortin (POMC) neurons remains unexplored. POMC neurons produce the anorexigenic peptide αMSH and altering POMC expression disrupts energy homeostasis, leading to weight gain. We hypothesized that BPA alters *POMC* gene expression via induction of neuroinflammatory and nuclear receptor signaling, ultimately impairing feeding regulation. To examine this, POMC-expressing hypothalamus neuronal models, mHypoA-POMC/GFP-2 and mHypoE-43/5, were treated with 100 µM BPA. The transcription of *POMC,* circadian clock genes, pro-inflammatory genes, endoplasmic reticulum (ER) stress markers, estrogen and related receptor genes, and peroxisome proliferator-activated receptor gamma gene (PPAR*γ*) were examined using real-time quantitative PCR (RT-qPCR). BPA upregulated the mRNA levels of *POMC,* pro-inflammatory *NFkB, IL6*, *TNFα* and *IkBα*, *estrogen related receptor gamma (ERRγ), PPARγ*, and ER stress markers *CHOP, GRP78* and *Bax/Bcl2* at 4h, whereas BPA downregulated *GPR30* mRNA levels at 4h. The induction of *POMC* by BPA was abolished after pre-treatment with anti-inflammatory compounds GnRH, the IkB Kinase/NFkB inhibitor PS1145, a combination of metformin and melatonin, and the PPAR*γ* antagonist T0070907. In conclusion, our study is the first to explore the direct effects of BPA on POMC neuronal cell models, demonstrating altered expression of inflammatory and ER stress markers, steroid receptors and *POMC*genes. This study demonstrates that BPA can have direct effects on POMC neurons in the hypothalamus and that this region may be linked to the BPA-mediated alterations in energy homeostasis.

PS1.0077 LONG-TERM CONSEQUENCES OF REPRODUCTIVE EXPERIENCE ON METABOLIC HOMEOSTASIS IN MOTHERS

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During pregnancy and lactation, the maternal body undergoes many changes in the regulation of appetite, body weight and glucose homeostasis to deal with the metabolic demands of the growing fetus and subsequent demands of providing milk for offspring. The aim of this study was to investigate whether the adaptive changes during pregnancy and lactation have long-term effects on energy homeostasis in female mice following completion of lactation. Furthermore, reproductively experienced (one cycle of pregnancy and lactation) and control (virgin, aged matched) female mice were challenged with a high fat diet (HFD), to determine if having experienced a major challenge to energy homeostasis such as pregnancy and lactation leads to increased susceptibility to a second challenge, that of HFD. After weaning of pups, reproductively experienced (RE) mice maintained a higher body weight compared to age-matched control mice. While there was no significant difference in daily food intake, or the feeding response to exogenous leptin administration, RE mice were significantly less active than age-matched control mice as measured by average daily x + y beam breaks or average daily ambulatory distance. While both RE and control mice gained a similar amount of body weight on the high fat diet, only the RE mice had significantly impaired glucose tolerance when consuming the high fat diet, thus demonstrating an increased susceptibility to the negative consequences of high fat diet after pregnancy and lactation. Overall, these data indicate the experience of pregnancy and lactation have long-term consequences on energy homeostasis in mothers.

PS1.0078 NUTRITIONAL RECOVERY : IMPACT OF PHYSICAL ACTIVITY IN A CHRONIC FOOD RESTRICTION MOUSE MODEL

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The potential impact of physical activity on anorexia nervosa (AN) patients in remission is not well known, more specially its implication on the restoration of metabolic and neuroendocrine factors. It is then crucial to determine whether the inappropriate physical activity currently described in AN patients, might constitute a brake to a reliable recovery. To study the impact of physical activity, we first exposed young female mice to a chronic food restriction (FR) combined or not with access to a running wheel (W) for 9 weeks, followed by a 3-week's of

nutritional recovery. The mice (n=6/group) were compared to a group of mice fed *ad libitum* (AL). During nutritional recovery, FR and FRW mice exhibited a transient hyperphagia, associated with a rapid body weight recovery. FR and FRW mice restored their fat and lean mass, oestral cycle, but FR mice displayed the highest body weight gain. Acyl- and des-acyl ghrelin were differentially impacted by physical activity. Indeed, the kinetic of changes of these two isoforms was different across food restriction and refeeding periods. mRNA expression of orexigenic and anorexigenic hypothalamic peptides and their respective receptors was differentially altered in FR and FRW mice. In the recovery period, expression of neuropeptides was normalized and return to control values. However, alterations in the expression of the receptors persist especially in FR mice. In conclusion, physical activity seems to impact the recovery and can be an important factor to consider for a reliable remission in AN patients.

PS1.0079 MOLECULAR MECHANISMS OF LEPTIN TRANSPORT ACROSS THE TANYCYTES OF MEDIAN EMINENCE

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Leptin is a hormone secreted by adipose tissue that acts in the central nervous system to regulate appetite. This dialogue between the periphery and the brain is essential to maintain energy homeostasis but is impaired in obese people. We have recently hypothesised that this so-called leptin-resistance, could be the consequence of a defective leptin transport into the brain. The median eminence, a hypothalamic structure located below the third ventricule contains specialized ependymoglial cells called tanycytes. These cells line the floor of the third ventricle and contact 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 leptin-sensitive regions can be reach. However, the cellular and molecular mechanisms controlling leptin internalization, its transcytosis as well as its release from apical site of the tanycytes remain unknown. Using fluorescence and electron microscopy on primary cultures of rat tanycytes, we are deciphering the endocytic route taken by leptin. Moreover, in order to maintain the cell polarity in vitro, we are implementing tanycytes culture in basal membrane extract gels. Finally, leptin release from tanycytes was monitored by ELISA assay and we focused on the role of vesicule-associated membrane proteins (VAMPs), components of SNARE complex, which is a key component of the exocytotic machinery in glial cells. Altogether, these data constitute a promising start toward the understanding of the leptin journey in tanycytes.

PS1.008 CENTRAL PROLACTIN RECEPTOR DISTRIBUTION IN BREEDING AND NON-BREEDING ZEBRA FINCHES

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Parental care is a widespread phenomenon observed in many diverse taxa and is an important component of fitness. The hormone prolactin (PRL) has a well-established role in mediating mammalian maternal behavior through its actions on central prolactin receptors (PRLR). We have recently showed that PRL also plays a causal role in the onset of male and female parental care in zebra finches. However, there is a considerable lack of information on the distribution of PRLRs in the avian CNS to test the hypothesis that post-hatch parental care is mediated through central PRLRs. In order to advance the research on the role of central PRL in avian parental care, we developed a novel immunohistochemisty protocol to visualize the distribution of central PRLRs in the zebra finch brain. Additionally, we compared the central PRLR distribution in brains from breeding and non-breeding zebra finches. This is the first detailed description of PRLR in a songbird, and we provide the first evidence that PRLRs are upregulated in breeding birds in several brain regions relevant to parental care and other social behaviors. This work is an essential first step to facilitate future research that uses central PRL targets to manipulate parental behavior, and/or other physiological or behavioral functions of PRL.

PS1.0080 HYPOTHALAMIC REGION-SPECIFIC ASTROCYTE REGULATION OF GLUCOSE HOMEOSTASIS AND ENERGY BALANCE

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The hypothalamus is organized in defined neuronal nuclei that have key role in the control of energy balance. Synaptic transmission and activity of these neurons are intimately regulated by astrocytes whom role in the control of energy metabolism have only been recently addressed. By combining pharmacogenetic approaches (DREADDs) *in vivo* and intracellular Ca^{2+} imaging (GCamP) selectively in astrocytes, we explored if specific *in vivo* Ca^{2+} manipulation in glial fibrillary acidic protein or aldehyde dehydrogenase 1 family member L1 expressing astrocytes located in the ventromedial nucleus (VMH) or in the paraventricular nucleus (PVN) differentially participate in the control of glucose and energy homeostasis. E*x vivo* chemogenetic activation of Aldh1L1 expressing astrocytes by bath application of clozapine-n-oxide evoked a specific

DREADD-dependent increase in intracellular Ca^{2+} release. Additionally, *in vivo* specific manipulation of astrocyte in the VMH induced a change in peripheral substrate utilisation and an exacerbated response to neuroglucopenia by enhancing counter regulatory responses to 2 deoxy-glucose induced hypoglycemia. In contrast, manipulation of astrocyte in the PVN decreased glucose tolerance and energy expenditure. Finally, we provide evidence that astrocytic control of energy balance is partially mediated through adaptive change in the autonomic nervous system. In conclusion, we show that *in vivo* modulation of astrocyte populations located in discrete hypothalamic nuclei have distinct biological output onto glucose and energy homeostasis. In addition, our data support a concept in which obesity-associated diseases might be at least partially mediated through molecular and signaling change in hypothalamic astrocytes.

PS1.0081 GHRELIN: A KEY HORMONE IN METABOLIC AND NEUROENDOCRINE ADAPTATIONS TO UNDERNUTRITION

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Anorexia nervosa affects mostly young women and is characterized by a severe chronic food restriction, excessive physical activity and increased plasma ghrelin. Ghrelin, identified as the endogenous ligand of GHS-R, is a powerful GH secretagogue and orexigenic hormone. To investigate the role of ghrelin in neuroendocrine and metabolic adaptations to chronic undernutrition, GHS-R KO mice were submitted to chronic food restriction combined with voluntary activity (FRW) compared with *ad libitum* fed group with a free access to a running wheel (ALW). Behavioral (wheel activity), metabolic (body composition, blood glucose) and neuroendocrine (ghrelin, GH, IGF-1, insulin) parameters were monitored prior to food distribution. Under FRW conditions, *Ghs-r-/-* mice exhibited a more severe fat loss than *Ghs-r+/+* mice whereas a similar decrease in muscle mass was observed in both genotypes. Decreased blood glucose induced by food restriction alone was prevented by access to the running wheel in *Ghs-r+/+* mice but not in *Ghs-r-/-* mice. While plasma IGF-1, insulin and ghrelin levels did not differ between genotypes in AL conditions, low blood glucose in *Ghs-r-/-* mice under FRW conditions was associated with low plasma insulin and IGF-1 and high plasma ghrelin and GH concentrations. In *Ghs-r-/-* mice, metabolic and neuroendocrine alterations were accompanied with altered expression of hypothalamic NPY and GHRH, two main sensors of energy and glucose status. These data indicate that **ghrelin signaling in the presence of physical activity plays a key role in neuroendocrine adaptations to chronic undernutrition** by regulating the GH/IGF-1 axis in order to preserve glucose homeostasis.

PS1.0082 CHEMERIN RECEPTOR SIGNALLING PATHWAYS, TRAFFICKING AND REGULATION IN THE HYPOTHALAMUS

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Chemerin is a newly discovered chemoattractant adipokine, implicated in inflammation, adipogenesis and energy metabolism. Chemerin has recently been hypothesised as a possible link between obesity and the development of type II diabetes. In humans affected by obesity, chemerin gene expression and circulating levels are elevated, correlating well with increasing body mass index. In animal studies, chemerin has been shown to modulate hypothalamic neuropeptides that control feeding and energy homeostasis. We found expression of chemerin and its G protein-coupled receptor chemokine-like receptor 1 (CMKLR1) in tanycytes lining the third ventricle and neurones of the arcuate nucleus of the hypothalamus of Sprague Dawley rats, indicating a possible neuroendocrine role for chemerin. Using neuropeptide Y(NPY)/Agouti-related peptide (AgRP) hypothalamic mouse cell lines, we show that chemerin, through activation of CMKLR1, activates the extracellular signal-regulated kinases 1/2 (ERK1/2) and Akt pathways. Phosphorylation of both pathways peaks within 20 minutes of chemerin treatment indicating that chemerin may be involved in key cell regulating events, including cell growth response, differentiation and survival. We found that application of chemerin to NPY/AgRP hypothalamic cell lines triggers the endocytosis of CMKLR1. Chemerin seems to be involved in acute hypothalamic inflammation, as our data demonstrate that chemerin treatment results in the increased expression of pro-inflammatory markers IL-6 and TNF alpha. Furthermore, chemerin treatment upregulates appetite regulatory genes (NPY and AgRP), suggesting that it contributes to the neuroendocrine control of appetite. Our data demonstrate that the adipokine chemerin is a promising candidate for urgently needed pharmacological treatment strategies for obesity.

PS1.0083 EFFECTS OF HYPOGLYCEMIA ON GROWTH HORMONE RELEASING HORMONE NEURONS

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Maintenance of normal blood glucose levels is a complex process involving coordinated action of the central nervous system (CNS) and peripheral organs. In diabetes, recurrent hypoglycemia is a common complication that can cause cognitive and metabolic defects. While the brain monitors and adjusts blood glucose levels constantly, the neuronal adaptations to repeated hypoglycemic episodes are poorly understood. There are several counter-regulatory responses to hypoglycemia including increased growth hormone. In previous work, we found neurons

expressing growth hormone releasing hormone are highly responsive to low glucose. Therefore, this hypothalamic population of neurons provides a model to study how glucose-responsive neurons respond and adapt to hypoglycemia. *In vitro* studies using N38 mouse hypothalamic cells, which express GHRH, show changes in activity and gene expression in response to altered glucose. In vivo, hypoglycemia increases plasma growth hormone and expression of the early immediate gene c-fos in GHRH neurons in GHRH-GFP mice. In addition, GHRH neurons are synaptically connected to peripheral organs regulating glucose metabolism suggesting they may regulate peripheral glucose via mechanisms other than growth hormone. We also examined the effects of repeated hypoglycemia on the activity, gene expression and neuroanatomy of N38 cells in vitro and GHRH neurons in vivo. These studies suggest GHRH neurons may contribute to the counter-regulatory response to hypoglycemia and to the blunted counter-regulation with repeated hypoglycemia.

PS1.0084 THE VGF-DERIVED PEPTIDE TLQP-62 POTENTLY STIMULATES INSULIN SECRETION TO ENHANCE GLUCOSE CLEARANCE.

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The *vgf* gene is highly expressed in the hypothalamus and certain peripheral tissues, and the VGF polypeptide it encodes is processed to biologically active peptides in a tissue and cell type specific manner. VGF and VGF-derived peptides are stored in the islet of Langerhans, as well as in intramural fibres and ganglia of the pancreas. In addition, islet cultures and pancreatic β-cell lines were shown to secrete the VGF-derived peptides TLQP-21 and TLQP-62, suggesting a possible function in modulating insulin release, especially upon a glucose load. The aim of this study was to investigate the effects of TLQP-21 and TLQP-62 on glucose metabolism. After an overnight fast female mice (n=4/group) received an intraperitoneal (ip) infusion of either saline, TLQP-62 (5mg/kg) or TLQP-21 (5mg/kg) followed by either glucose (ip, 2g/kg), or saline 30 minutes later. Peripheral blood glucose and insulin were measured 15 min later. As expected glucose levels were significantly increased in saline+glucose treated mice (19.2±1.3mM), compared to saline+saline controls (9.5±0.5mM, $p<0.001$). Such change was significantly attenuated by pre-treatment with TLQP-62 (13.6±1.0mM, $p<0.05$), but not with TLQP-21 (19.7±1.8mM, p=ns.). Furthermore plasma insulin was significantly higher in TLQP-62 treated mice compared to all other groups (saline+saline: 2.9±0.4ng/ml; saline+glucose: 4.4±0.4ng/ml; TLQP-62/glucose: 8.4±1.1ng/ml, $P<0.001$). We also observed that pre-treatment with TLQP62 significantly improved glucose clearance in Siberian hamsters maintained in long photoperiod to generate an obese phenotype (saline+saline: 7.6±0.4mM; saline+glucose: 19.0±1.2mM; TLQP-62+glucose: 13.8±1.4mM, $P<0.001$). In conclusion, TLQP-62 may be a powerful insulinotropic peptide that can be targeted for innovative antidiabetic drug discovery programs.

PS1.0085 PROSTAGLANDIN E2 ACTIVATES MELANIN-CONCENTRATING HORMONE NEURONS THROUGH MULTIPLE MECHANISMS

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Prostaglandin E2 (PGE2) is an inflammatory mediator that plays a critical role in the hypothalamus to induce sickness syndrome during disease states, including anorexia and weight loss. Since melanin-concentrating hormone (MCH) is a hypothalamic neuropeptide known to promote food intake and weight gain, we hypothesized that PGE2 inhibits MCH neurons to induce sickness behavior. To test this, we performed whole-cell patch clamp to record MCH neuron activity in acute rat brain slices. Contrary to our hypothesis, PGE2 significantly depolarized the resting membrane potential (RMP) of MCH neurons, which was blocked by the PGE2 EP2 receptor antagonist, PF04418948. This led us to speculate that the PGE2-induced excitation of MCH neurons contributes to diet-induced obesity, a condition that accompanies low-grade hypothalamic inflammation. Indeed, we found that the RMP of MCH neurons from animals fed high-fat diet (HFD) for 4 weeks was depolarized. This effect was completely reversed by PF04418948, suggesting that endogenous PGE2 mediates HFD-induced activation of MCH neurons. HFD also hyperpolarized the spike threshold in MCH neurons, which was mimicked by PGE2 and blocked by PF04418948. Interestingly, after 11 weeks of HFD, the RMP remained depolarized while the spike threshold returned to control levels. Together, our results suggest that HFD increases PGE2 production in the hypothalamus, which in turn acts on the EP2 receptor on MCH neurons to increase neuronal excitability by multiple mechanisms. This provides a novel mechanism by which inflammation modulates the neuronal control of energy homeostasis to promote food intake and weight gain.

PS1.0086 LEPTIN TARGETS LH AND VTA GABA NEURONS TO REDUCE DOPAMINERGIC DRIVE TOWARDS FOOD REWARD

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Leptin reduces motivation to obtain food rewards when in negative energy balance. Although leptin directly reduces activity of ventral tegmental (VTA) dopamine neurons, most of these dopamine neurons do not project to the accumbens which is the dopamine projection implicated in driving food reward seeking. It has been proposed that leptin-receptor expressing GABA neurons in the lateral hypothalamus (LH) mediate leptin's effect on dopaminergic activity. Gaps in knowledge that we address here: 1) which leptin-receptor expressing neurons connect to the dopamine system, 2) how does leptin modulate their activity and 3) which of these neurons is implicated in regulating the motivation to obtain food reward. Using optogenetics-assisted circuit mapping in leptin receptor cre mice, we find that LH GABAergic neurons project to VTA GABA neurons. Leptin inhibits the activity of these neurons. Chemogenetic activation of these LH neurons increased the motivation to press lever to obtain

a sucrose reward likely by decreasing VTA GABAergic input onto VTA dopamine neurons and only when mice were ad libitum fed. We also find that VTA GABA neurons expressing leptin receptors project onto VTA dopamine neurons. Activation of leptin receptor expressing neurons in the VTA using chemogenetics reduced the motivation to press lever for a sucrose reward. Thus, in the VTA leptin receptor expressing GABA neurons are more important than leptin receptor expressing dopamine neurons in mediating the effect of leptin on reducing motivation for food reward. We conclude that leptin indirectly targets multiple inputs to the dopamine system to reduce food reward seeking.

PS1.0087 COLONIC L-PHENYLALANINE STIMULATES PANCREATIC HORMONE RELEASE

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High protein diets improve glucose tolerance and insulin sensitivity. In the gut, protein is broken-down into amino acids that can be detected by a series of nutrient sensors, mediating processes including gut hormone secretion. These sensors are present on cells in the small intestine, but also in the colon. The greater the protein load, the higher the concentrations of amino acids reaching the colon. Additionally, amino acids can enter the circulation and act on other endocrine organs such as the pancreas. Amino acid ingestion is linked with insulin and glucagon release to facilitate the movement of amino acids into tissues without suppressing blood glucose levels. However, the mechanisms mediating these effects are poorly characterised. L-Phenylalanine is an essential aromatic amino acid that orally suppresses food intake and stimulates the secretion of anorectic gut hormones. We examined the effect of colonically administered L-Phenylalanine on food intake, glucagon, insulin and glucose plasma levels, and on neuronal activation in mice. *In vivo,* colonic administration of 0.1ml of 10Mm L-Phenylalanine supressed food intake for two hours post administration, and increased plasma levels of insulin (control: 1.79ng/ml Vs. L-Phe: 2.85ng/ml) and glucagon (control: 45.04pg/ml Vs. L-Phe: 70.22pg/ml) thirty minutes post administration, without affecting plasma glucose. L-Phenylalanine administration caused a 1.7 fold increase in cells expressing the marker of neuronal activation c-fos in the brain stem, a region involved in energy homeostasis and glycaemic control. Identifying the pathways by which colonically administered L-Phenylalanine modulates glucagon and insulin secretion may reveal novel targets for improving glucose homeostasis.

PS1.0088 HYPOTHALAMIC VASCULATURE REMODELING UPON HYPERCALORIC FEEDING DEPENDS ON ASTROGLIAL HIF1A AND VEGF

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Obesity and diabetes exert deleterious impact on neurovascular beds, a phenomenon most prominently observable in the retina of the eye. We reported that high-fat high-sugar (HFHS) diet induces neovascularization within the hypothalamus, but not elsewhere in the brain, of both mice and humans. Interestingly, hypothalamic hypervascularity was found to be reversible upon weight loss in diet-induced obese mice. In order to identify which mechanisms are involved in the initiation of this pathologic vascularization during nutritional excess, we interrogated bioenergetic changes within the hypothalamus. Here we provide evidence that HFHS diet increased cellular mitochondrial respiration, which was associated with a lower local oxygen availability in the hypothalamus. We further showed that HFHS diet significantly increases hypoxia-inducible factor 1a (Hif1a) protein levels as well as its downstream mediator, vascular-endothelial growth factor (VEGF), which was mainly observed in glial-fibrillary acidic protein (GFAP)-positive astrocytes in the hypothalamus Given that astrocytes are an integral part of the neurovascular unit and VEGF production is regulated by hypoxia-inducible factor 1α (HIF1a), we generated an astrocyte-specific mouse model to postnatally ablate HIF1a in those glial cells by using a tamoxifen-dependent loss-of-function mouse model. Interestingly, mice lacking astrocytic HIF1a prevented the up-regulation of VEGF and hypothalamic angiogenic response upon a HFHS diet. Overall our findings indicate that diet-induced hypothalamic angiopathy is involving astrocytic HIF1a -VEGF signaling.

PS1.0089 FUNCTIONAL ROLE OF TANYCYTE PYROGLUTAMYL PEPTIDASE II FOR THYROID AXIS REGULATION

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Tanycytes, glial cells that surround the third ventricle of the medial basal hypothalamus, are components of the hypothalamus-pituitary-thyroid (HPT) axis. They express pyroglutamyl peptidase II (PPII), the thyrotropin releasing hormone (TRH) degrading ectoenzyme. Since PPII activity in the median eminence is regulated by TH levels and fasting, that β2-tanycyte end-feet contact hypophysiotropic TRH terminals, and that TRH controls PPII activity, tanycyte PPII may

control TRH bioavailability before entrance into portal vessels, and thus thyrotropin (TSH) secretion. This hypothesis is consistent with previous data showing that the ip injection of a PPII inhibitor can enhance serum TSH concentration, although this response could also be attributed to expression of PPII in other sites. To test the hypothesis that median eminence PPII is critical for the control of TSH secretion, we used serotype-1 adenoassociated virus (AAV1), which transduces median eminence tanycytes when injected into the third ventricle. Administration of rats with AAV1 expressing PPII increased median eminence PPII activity two or three weeks after virus administration. This change was associated with decreased serum TSH and T4 concentrations, with no effects over serum T3 concentration. The injection of AAV1 expressing a dominant negative truncated isoform of PPII had the opposite effect, with an increase of serum TSH and T4 levels two weeks after virus administration. AAV1 expressing PPII or the truncated isoform didn't change the activity of the circulating form of PPII. These observations support the hypothesis that β2-tanycyte PPII activity is critical for regulation of HPT axis activity.

PS1.009 RATS IN CAHOOTS: EXAMINING COOPERATION IN A 2X2 GAME

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We study the neurobiology of social behavior and cooperation. In our rat model of Prisoner's Dilemma, a classic 2x2 game (2 players, 2 responses), each player chooses to cooperate or defect without knowledge of their partner's choice. We compared rats' cooperative responses between two payoff matrices. Mutual cooperation [Reward, R] delivered 3 sugar pellets each. Mutual defection [Punishment, P] delivered 1 pellet. Unilateral cooperation [Sucker, S] earned no pellets; his partner was Temptation [T]. Rats were tested with iterated Prisoner's Dilemma (IPD, T=5 pellets) or Stag Hunt (SH, T=2 pellets). SH offered the highest payoff/trial for R, vs T for IPD. Therefore, we hypothesized rats would make more cooperative responses, earning more pellets in SH. In each pair, 1 rat was a stooge whose responses were computer-controlled using the Tit-For-Tat strategy: initially cooperative, then repeating the partner's response from the previous trial. Rats were more cooperative with SH (44.6%±9.5%) vs IPD (35.5%±9.2%), showed a similar likelihood to cooperate on the first trial (nice): 0.47±0.10 for SH, 0.43±0.14 for IPD, and earned similar numbers of pellets. The number of T and S trials was similar but rats had more R trials for SH (7.5±2.4/session vs 4.2±1.9 for IPD) and fewer P trials. Rats were more likely to cooperate after cooperation than to cooperate after defecting in the previous trial. These data suggest that rats alter cooperative responses according to the payoff matrix in a 2x2 game. This provides a model to investigate the neurobiology of cooperation.

PS1.0090 HIGH SALT INTAKE CAUSES CYTOSKELETON REORGANIZATION IN VASOPRESSIN NEURONS OF THE SUPRAOPTIC NUCLEUS

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High dietary salt (HDS) strongly correlates with cardiovascular diseases and is a major factor contributing to the pathogenesis of hypertension. Recent studies show that HDS leads to neurogenically-mediated increases in sympathetic activity, vascular resistance, and water and sodium retention, revealing a possible involvement of central sodium detection mechanisms in salt-sensitive hypertension. Changes in plasma sodium are detected by specialized osmosensory neurons located in hypothalamic *organum vasculosum lamina terminalis* (OVLT) and supraoptic nucleus (SON). Under normal physiological conditions, increased plasma sodium causes activation of these neurons and release of vasopressin (VP), antidiuretic hormone that mediates water retention by the kidney and vasoconstriction, to achieve body fluid homeostasis. Chronic exposure to HDS is associated with excessive activation of VP neurons, causing a VP-mediated increase in blood pressure. The molecular mechanisms underlying excessive secretion of VP in HDS are not fully understood. Osmosensory neurons harbor unique cytoskeleton networks comprised of a subcortical actin layer and somatic scaffold of interweaved microtubules, regulating the sensitivity of neuronal activation. Chronic exposure to HDS increases the density of actin and microtubules in osmosensory neurons. mDia1 is the major direct downstream effectors of RhoA, mediating its effects on actin and microtubule polymerization and stability. Our data suggest that mDia1 is elevated in the OVLT and SON following HDS. We hypothesize that chronic exposure to HDS causes an activation of the RhoA-mDia1 pathway to increase the cytoskeletal density in osmosensory OVLT and VP neurons, leading to excessive VP secretion, volume expansion, elevated blood pressure, and hypertension.

PS1.0091 ANATOMICAL ORGANIZATION OF THE MOUSE SUBFORNICAL ORGAN

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The subfornical organ (SFO) is one of the brain's sensory circumventricular organs (CVOs), which are highly vascularized midline structures lacking a complete blood-brain barrier (BBB). CVOs are characterized by the presence of tanycytes, specialized glia-like cells lining the ventricular floor of the CVOs and interacting with their extensive fenestrated vasculature, thereby contributing to the regulation of the BBB in these areas. Due to the lack of a complete BBB, SFO and other CVOs are unique sites where peripheral circulating factors can penetrate into the central nervous system, influencing neuronal activity and allowing brain cells to monitor blood-borne signals. This provides the brain with information from the periphery and contributes to the generation of centrally-mediated physiological responses to humoral

feedback and physiological stressors. Accordingly, SFO plays a key role in the regulation of cardiovascular status, hydromineral balance, energy homeostasis, and metabolism. SFO neurons express a variety of receptors for peripheral signals. Moreover, SFO neurons can be activated by numerous circulating molecules associated with fluid balance (e.g. angiotensin II, sodium, endothelin, vasopressin) and metabolism (e.g. leptin, ghrelin, glucose). While extensive studies have focused on the characterization of the SFO neurons and their roles in the regulation of cardiovascular and metabolic status, the contribution of non-neuronal cells to this regulation is unclear. In this study, we use histological techniques to characterize the spatial outline of the SFO and to examine the location of neurons, as well as non-neuronal cells, including tanycytes, astrocytes, ependymocytes, and endothelial cells within its confines.

PS1.0092 ATP IS RELEASED FROM GLP-1 SECRETING ENTEROENDOCRINE CELLS AND SIGNALS TO VAGAL AFFERENT NEURONES

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Objectives: To test the hypothesis that glucagon-like peptide-1 (GLP-1) secreting enteroendocrine cells release the small molecular neurotransmitter ATP alongside classical peptide hormones. ***Results:*** ATP was detected in distinct punctae in primary cultured GLP-1-producing L-cells and the GLP-1-secreting cell line GLUTag. In response to known L-cell stimuli, ATP release was detected using two methods: i) with a luminescence-based assay to measure ATP in culture supernatants (basal, 3.1±0.4 nM; 1µM angiotensin-II, 6.7±1.0 nM; 10µM forskolin+IBMX, 8.3±1.3 nM; *p<0.01*, N=15) and ii) using "sniffer patches" from HEK293 cells overexpressing $P2X_2$ channels, allowing detection of ATP currents: forksolin+IBMX and angiotensin-II triggered ATP currents when sniffer patches were placed adjacent to GLUTag cells (-64±27 pA, N=5) and primary colonic L-cells (-771±301 pA, N=7), respectively. To address whether ATP released from enteroendocrine cells participates in cross-talk to neurons, we co-cultured nodose ganglion neurons with GLUTag cells expressing Gq-DREADD, enabling cell-restricted stimulation of GLUTag cells by CNO. CNO triggered Ca^{2+} elevation in most GLUTag cells as predicted, and elevated Ca^{2+} in 30% of co-cultured neurons. The broad-spectrum purinergic receptor antagonist PPADs (100µM) reduced the CNO-induced Ca^{2+} rise in nodose ganglion neurons by 53%, without abolishing responses in GLUTag cells, supporting the idea of ATP-mediated signalling from GLUTag cells to neurones. ***Conclusions:*** ATP is released from GLP-1 secreting cells and can signal to vagal afferent neurons. Similar cross-talk to neurons has been reported for serotonin-secreting enterochromaffin-cells but our study suggests a more widespread importance of small molecular neurotransmitters in the gut-brain axis.

PS1.0093 NEURONAL ACTIVATION OF PROOPIOMELANOCORTIN AMELIORATES AGING ALTERED HEPATIC GLUCOSE METABOLISM

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Hypothalamic β-endorphin neurons have been shown to regulate glucose metabolism and immune function. Although these neurons do not directly innervate the lymphoid or pancreatic tissues, they have control over autonomic nervous system and it's influences on the peripheral tissues. Recently, we have established a method to prepare proopiomelanocortin (POMC) neurons from neuronal stem cells from fetal rats. Upon transplantation of these cells in the hypothalamus, we have found that these cells produce POMC at the site of transplants and stay viable for a long period of time. These cells are also capable of blocking components of inflammation, including proinflammatory cytokine production in various cancer models. In this study, we determined the effects of POMC neuronal transplantation on hepatic glucose homeostasis during aging. Male Sprague Dawley rats were eitherere transplanted with cortical cells (control transplants) in the PVN, other twere received POMC neuron transplants in the PVN (POMC transplants) or one wereas non- manipulated control group. Increased expressions of gluconeogenic and glycolytic enzymes, insulin receptor were observed in the liver of old animals with control transplants, whereas POMC neuronal implantation in old animals restored the expression of these gene levels towards normal level. POMC neuronal transplantation also has been was shown to normalize the level of pro-inflammatory cytokines in old animalseffect. A significant decrease in plasma insulin concentrations were observed in control old animals whereas POMC transplantation normalized the level towards control value. TAll these findings suggest that, POMC neuronal transplantation may regulates glucose homeostasis by increasing hepatic insulin sensitivity.

PS1.0094 MATERNAL HIGH-FAT DIET ALTERS LEPTIN SENSITIVITY AND AFFERENTS TO LATERAL HYPOTHALAMUS IN RAT PUPS.

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The early nutritional environment is critical for the development of neural circuits regulating energy homeostasis and food intake. The lateral hypothalamus (LH) links homeostatic hypothalamic nuclei to the mesocorticolimbic dopamine neurons that modulate food reward. The LH is sensitive to leptin and higher exposure to this hormone in neonates through maternal high-fat feeding might promote neuronal LH afferent projections growth and leptin sensitivity. Here we examined whether a maternal high-fat (HF) diet 1) modifies leptin signaling in identified LH neurons of neonates, and 2) increases the density of projections from the ventromedial (VMH) and dorsomedial (DMH) hypothalamus to LH orexin cells projecting to

dopamine neurons. Mothers received either a control (C) or HF diet (60% Kcal from fat) from gestation day 13-14 throughout lactation. The production of second messengers (pERK and pSTAT3) in pup LH neurons (GABA, CART, and orexin cells) was quantified by double immunofluorescence histochemistry after vehicle or leptin challenge (3mg/kg ip). Leptin increased pERK and pSTAT3 on PND16 in different neuronal populations, particularly in HF offspring. To evaluate diet-induced changes in the density LH afferents, fluorescent retrograde microbeads were injected stereotaxically into the LH orexin field on PND5. Preliminary results indicate that HF pups exhibit a greater density of projections from the DMH and VMH compared to C pups. Thus, a maternal HFD might increase both LH afferent fiber density and sensitivity to metabolic hormones in neonates, possibly until adulthood (Supported by CIHR grant #130323).

PS1.0095 HUDDLING MODULATES MONOAMINE NEUROTRANSMITTERS AND GUT MICROBIOTA TO ORCHESTRATE METABOLISM

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Huddling is highly evolved as a cooperative behavioral strategy for social mammals to maximize their fitness in harsh environments. The coevolution of the mammals with their microbial communities confers fitness benefits to both partners. We hypothesized that huddling behavior altered energetics and thermoregulation by shaping caecal microbiota in small herbivores. Brandt's voles (*Lasiopodomys brandtii*) were kept in huddling or separated condition and were exposed to warm (23 ± 1 °C) or cold (4 ± 1 °C). The voles had higher energy intake, resting metabolic rate and nonshivering thermogenesis in cold than those in warm, but huddling voles decreased all these parameters compared with separated voles in the cold. Huddling voles had higher concentrations of dopamine (DA) and 5-hydroxytryptamine (5-HT), but lower turnover of DA and 5-HT in hypothalamus, and higher concentrations of norepinephrine (NE) and DA and 5-HT turnover, but lower 5-HT concentration and DA turnover in amygdala than separated voles. Both cold and huddling induced a marked variation in caecal bacterial composition. Huddling voles increased α and β-diversity, the abundance of *Lachnospiraceae* and *Veillonellaceae*, but decreased the abundance of *Tenericutes*,
TM7, *Comamonadaceae* and *Sinobacteraceae* compared with separated voles. Transplantation of caecal microbiota from cold-separated voles but not from cold-huddling voles induced significant increases in energy intake and nonshivering thermogenesis than that from warm-separated voles. These findings demonstrate that huddling modulates monoamine neurotransmitters and gut microbiota to orchestrate metabolism and thermoregulation. It highlights the role of microbiota-gut-brain axis in regulating metabolism and thermogenesis by huddling in endotherms.

PS1.0096 PRESENCE OF RARE PATHOGENIC MUTATIONS ASSOCIATED WITH MENTAL HEALTH CONDITIONS IN EXTREME OBESITY

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Background: Obesity is associated with neuropsychiatric disease. Rare monogenic mutations associated with early childhood onset obesity and neuro-psychiatric phenotypes suggest a shared underlying etiology in some cases. We assessed the presence of rare (minor allele frequency <0.1%) pathogenic loss of function mutations in genes previously implicated in mental health disorders in a cohort of extremely obese adult patients. Methods: Extreme Obesity Study (EOS) is an ongoing study comprising adult patients with BMI >50 recruited from the Bariatric program at University Health Network. 65 patients have undergone detailed phenotyping. Whole exome sequencing has been undertaken in 51. Results: Incidence of mental health disease was: depression/dysthymia (32.3%) anxiety disorders (21.5%), intellectual disability (6.2%), psychosis (3.1%). Binge/emotional eating was seen in 21.5%. Previously unreported heterozygous protein truncating variants in genes implicated in autosomal dominant phenotypes (autism, schizophrenia and intellectual disability) were seen in 4 patients (7.8%) with a further 17 (33% of patients) missense variants predicted to be deleterious based on bioinformatics. These genes *(NRXN1, NRXN2, NRXN3, POGZ, EMC1, SHANK2, AUT2, GATAD2B, EPB41L1, NRIP1, UPF3B, ARHGEF6, NRG1, NTRK2, BDNF, DOCK8, DIP2B)* regulate synaptic function and neuronal function with high expression in the hypothalamus and limbic regions suggesting a plausible role in disordered food intake and obesity. Conclusions: The EOS cohort, which has an increased burden of mental health concerns, is enriched in rare variants in genes implicated in neuropsychiatric disease. These variants may also contribute to disordered eating and obesity.

PS1.0097 INTRANASAL DELIVERY OF A MICRORNA INHIBITOR TO MIR-1983 IMPROVES INSULIN SENSITIVITY IN MICE.

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Dysregulation of microRNA expression centrally in the hypothalamus has been shown to alter whole body energy homeostasis. Initial work in our laboratory sought to identify microRNAs upregulated in murine hypothalamic cell models during insulin resistance. We found that miR-1983 was increased by ~2 fold during insulin resistance in the embryonic male mHypoE-46 hypothalamic neuronal cell line with transfection of a miR-1983 mimic decreasing insulin receptor protein levels by 20% after 24 hours. Subsequent studies undertaken in male CD-1 mice placed on a 60% high fat diet (HFD) compared to a sucrose-matched control diet revealed

that miR-1983 was increased in the hypothalamus of mice with persistent hyperinsulinemia after 5 weeks. Based on these data, we hypothesized that administration of an inhibitor to miR-1983 would improve insulin sensitivity in mice fed HFD. Central administration of microRNA inhibitors has been traditionally conducted using intracerebroventricular administration, however, recent work suggests that intranasal delivery may be equally as effective. We placed CD-1 mice on a 60% HFD or control diet for two weeks, and under brief anaesthesia administered either a miR-1983 microRNA *in vivo*-ready inhibitor or control. Mice were continued on their respective diets, and insulin tolerance tests conducted one week post-administration revealed a significant improvement in insulin sensitivity for the inhibitor group. Two weeks post-administration, fasting blood glucose level was also improved in control animals. These results indicate that miR-1983 may serve as a biomarker of pre-diabetes and can be targeted at the level of the hypothalamus to alter whole body glucose homeostasis.

PS1.0098 COMPARISON OF ANTIPSYCHOTIC-INDUCED EFFECTS ON THE INSULIN AND MAPK PATHWAYS IN HYPOTHALAMIC NEURONS

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Antipsychotics are the gold-standard treatment for schizophrenia but cause serious metabolic side-effects. The hypothalamus is the primary brain region responsible for energy regulation, and disruptions in the hypothalamus are implicated in insulin resistance and obesity. Thus, dysregulation in hypothalamic signalling could be involved in antipsychotic-induced metabolic disturbances, yet direct effects of antipsychotics on the hypothalamus have yet to be examined. The hypothalamic cell lines, rHypoE-19 and mHypoE-46, were treated with olanzapine, clozapine, or aripiprazole. Western blotting was used to measure the energy sensing protein AMPK, components of the insulin signaling pathway (AKT, GSK3B), and components of the MAPK pathway (ERK1/2, JNK, p38), the latter linked to inflammation. In the rHypoE-19 cells, olanzapine and clozapine increased pERK1/2 and pJNK, while aripiprazole increased pJNK. Clozapine and aripiprazole increased pAMPK and inhibited insulin-induced pAKT. In the mHypoE-46 cells, olanzapine and aripiprazole increased pAMPK, while clozapine and aripiprazole again inhibited insulin-induced pAKT. Clozapine also increased pJNK and aripiprazole increased pERK1/2. Our findings suggest highly differential effects between antipsychotics on hypothalamic insulin and MAPK pathways. In the rHypoE-19 line, upregulation of MAPK proteins by all antipsychotics suggests potential upregulation of pro-inflammatory pathways. In the rHypoE-19 and mHypoE-46 lines, aripiprazole and clozapine inhibition of insulin-stimulated pAKT and induction of pAMPK suggests impaired insulin action. In addition, we also found marked variances between cell type, potentially due to species or receptor expression differences. Going forward, the mechanism of differences between antipsychotics in the hypothalamus will be explored using receptor antagonists and the anti-diabetic drug metformin.

PS1.0099 CLK2 IN NEURONS OF THE HYPOTHALAMUS BUT NOT IN THE AMYGDALA ALTERS THE ENERGY METABOLISM

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Cdc2-like Kinase 2 (Clk2) is ubiquitously expressed and is regulated by refeeding in the liver. Previously, we showed that hypothalamic Clk2 was important to maintain energy homeostasis through insulin and leptin signaling. However, it was not known in which neuron Clk2 was expressed. Thus, we aimed to investigate whether Clk2 was expressed in neurons and the phenotype of Clk2flox/flox mice injected with AAV-GFP expressing Cre recombinase in the mediobasal hypothalamus (MBH) or the central nucleus of the amygdala (CeA). AAV-GFP without Cre was a control. Clk2 was positive labeling in the MBH of POMC- and NPY-GFP mice. The deletion of Clk2 in MBH of adult mice resulted in an obese phenotype, explained by the increased body and fat mass, leptin resistance (unchanged food intake and reduced pSTAT3), hyperphagia and reduced Ucp-1, Pgc-1alpha and Prdm16 expression in the brown adipose tissue, suggesting reduced thermogenesis. They also presented enhanced fasting blood glucose and impaired ITT and GTT. Pepck, G6Pase, and Glucokinase genes were upregulated in the liver. Interestingly, we observed a robust Clk2 expression in the CeA, which was shown as an insulin-responsive nucleus and insulin resistant in obesity phenotypes. However, Clk2flox/flox mice injected with AAV-Cre-GFP in CeA had similar body weight over time, and same fat mass and food intake compared to Clk2flox/flox-AAV-GFP injected mice. Thus, our data suggest that neuronal Clk2 in mediobasal hypothalamus, but not in CeA has an essential role to maintain energy balance in mice. Clk2 in CeA may have a distinct role in animal physiology.

Poster Session 2 – Tuesday, July 17 to Wednesday, July 18, 2018

PS2.001 CASTRATION MODULATES ELECTROPHYSIOLOGICAL PROPERTIES OF HVC NEURONS IN ADULT MALE ZEBRA FINCHES

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The nucleus HVC (high vocal center, proper name) within the avian analog of mammal premotor cortex is the central part of two song control system pathways, which are VMP and AFP.HVC which produces stereotyped instructions through the motor pathway leading to precise, learned vocalization by songbirds, is the most important premotor nucleus critical for singing. Androgens such as testosterone play an important role in stabilizing birdsong. Castration can change levels of plasma testosterone. In this study, we investigated the effect of castration on electrophysiological properties of neurons in the HVC of adult male zebra finches. Adult male zebra finches were castrated and. We recorded the electrophysiological changes

from HVC using patch clamp recording. We found that membrane time constants, and input resistance of HVC projection neurons (HVC PNs, both HVC-RA and HVC-X) in the castration group were lower than those of the control group. Afterhyperpolarization AHP time to peak and amplitude of spontaneous action potential (AP) was prolonged after castration. These findings suggest that castration decreases song stereotypy and excitability of HVC PNs in male zebra finches.

PS2.0010 SPONTANEOUS BINGE-LIKE EATING IN MICE USING UNPREDICTABLE ONCE WEEKLY ACCESS TO PALATABLE DIETS

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Many pre-clinical models of binge-like eating involve predictable, scheduled, access to a palatable diet high in fat (HF), which may be preceded by anticipatory behaviour. To introduce spontaneity into the binge-like consumption of palatable diets, mice were allowed 2-hours access on a random day once per week and at a random time within 8-hours around the transition from dark to light phase. Despite normal intake of stock diet prior to unpredictable access, mice immediately initiated a substantial eating episode when presented with HF diet. Following this intake, compensatory hypophagia was observed relative to stock fed controls, and cumulative energy intakes converged. There were no effects of HF diet on body weight or body composition over 12-weeks. Binge-like consumption was also observed on unpredictable access to the complete liquid diet, chocolate Ensure, and responses to HF diet and Ensure were similar in male and female mice. The timing of unpredictable access relative to light phase transition affected the magnitude of the eating episode, and the pronounced diurnal pattern of energy intake on stock diet across the 8-hour window was attenuated on palatable diets. Analysis of leptin and insulin in terminal blood samples revealed no effect of diet or timing relative to HF diet access. Gene expression analysis in the hypothalamus and nucleus accumbens showed only limited effects of diet and timing relative to the access period; there was a trend for NPY gene expression in the arcuate nucleus to be lower in HF groups compared to those fed stock diet throughout.

PS2.00100 HOW DO LEAK CHANNELS CONTROL THE DYNAMIC ACTIVITY OF ENDOCRINE PITUITARY CELLS?

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The pituitary gland produces a variety of hormones that regulate other glands and organs throughout the body in order to control critical bodily functions including growth, metabolism and stress. Endocrine pituitary cells are also electrically excitable and can generate action potentials. In fact, hypothalamic factors can control hormone secretion in endocrine pituitary cells by regulating their electrical activity. Therefore, in order to understand how hormone secretion is controlled in the anterior pituitary cells, we need to know how changes in the ion channels properties affect the electrical activity in those cells. Here, we study the role of leak channels, which play an important role in maintaining the resting membrane potential near the threshold for generating electrical activity in all pituitary cells. Our hypothesis is that the leak channels (sodium and potassium leak channels) can be effective targets for hypothalamic factors to control the frequency of electrical activity in endocrine pituitary cells. Despite their important role, the molecular identity of these channels remains unknown, so it is difficult to study their role pharmacologically. Instead, we use a technique called “dynamic clamp”, using this technique we can virtually vary the conductance of the channels and see how this affects electrical activity. We found that increasing the sodium leak conductance increased the frequency of electrical activity and increasing the potassium leak conductance had inverse effect in a heterogeneous population of pituitary cells. This suggests that leak channels can affect hormone secretion in response to hypothalamic signals through a consistent mechanism across pituitary cells.

PS2.00101 MITOCHONDRIAL AND ENERGY REGULATION IN SKELETAL MUSCLE BY THE CRH-LIKE PEPTIDE, TCAP-1

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Teneurin C-terminal associated peptides (TCAPs 1-4) are CRH-like bioactive peptides located at the end of each of the teneurin proteins. Recently, it has been demonstrated that TCAP-1 enhances glucose metabolism *in vivo* as well as enhances skeletal muscle function during fatigue, however, the mechanism of action remains unknown. This work aims to investigate its mechanism of action in energy metabolism *in vitro* using the C2C12 murine skeletal muscle cell line. First, TCAP-1 induced a rapid biphasic peak of cytosolic calcium that corresponds in a similar timeline to TCAP-1-mediated increase in inositol triphosphate (IP3) levels. Further, when an inhibitor for IP3 receptor (IP3R) was applied, TCAP-1 was not able to induce any calcium response. These data suggest that TCAP-1 induces calcium release from the SR via activation of the IP3R. To investigate the roles of the TCAP-1-mediated calcium release, the mitochondria was next assessed. TCAP-1 induced a significant mitochondrial membrane hyperpolarization event in a similar timeline to TCAP-1-mediated calcium release, possibly suggesting calcium import into the mitochondria. Mitochondrial activation is upregulated by calcium influx as calcium has stimulatory roles in the TCA cycle and ETC. Thus, TCAP-1-mediated glucose uptake was measured as enhanced mitochondrial activation should in turn affect glucose regulation.

TCAP-1 increased radioactive ^{3}H-deoxyglucose uptake by 300%, with a concomitant increase in GLUT4 at the membrane after 30 minutes of treatment. Thus, this work for the first time demonstrates that TCAP-1 has a novel role in skeletal muscle energy metabolism that is likely mediated via calcium regulation and mitochondrial activation.

PS2.00102 MECHANISMS UNDERLYING THE EFFICACY OF VERTICAL SLEEVE GASTRECTOMY - A FOCUS ON ENERGY EXPENDITURE

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Vertical Sleeve Gastrectomy (VSG) is a bariatric surgery that is associated with improved metabolic outcomes, however, the contribution of reduced energy expenditure to VSG-induced weight loss, particularly that in brown adipose tissue (BAT), remains unclear. Diet-induced obese male Sprague-Dawley rats (n=35) were sham-operation or underwent VSG surgery. Animals were implanted with biotelemeters between the interscapular lobes of BAT to assess local changes in BAT temperature and metabolic parameters were assessed. To elucidate the contribution of BAT thermogenesis to VSG-induced weight loss, chow-fed rats (n=33) underwent excision of the interscapular BAT or chemical denervation (6-OHDA). In a separate cohort(n=20) elevated Fos protein in the nucleus of the solitary tract (NTS) was assessed in response to VSG following intragastric infusion of water or a mixed meal (Ensure). VSG caused significant reductions in food intake commensurate with reductions in both body weight($P<0.0001$) and fat mass($P<0.05$) and an increase in BAT thermogenesis, as demonstrated by an elevation in iBAT temperature ($P<0.05$) and UCP1 expression($P<0.01$). There was beiging of white fat, as indicated by elevated Cited1 mRNA($P<.01$) expression in subcutaneous (inguinal) fat. The positive effect of VSG on weight loss and fat mass was significantly reduced in animals with disrupted iBAT function. Infusion of Ensure resulted in twice as many Fos-labelled neurons in the NTS compared to that detected after the same volume of water following VSG. These data support a role for BAT thermogenesis in VSG-mediated weight loss and show that both stretch and nutrients are likely to contribute to recruitment of brainstem neural relays following VSG.

PS2.00103 TENEURIN C-TERMINAL ASSOCIATED PEPTIDE (TCAP-1) ANTAGONISES CRF SIGNALLING IN HYPOTHLAMIC NEURONS

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Teneurin C-terminal associated peptide (TCAP)-1 is a member of an evolutionarily ancient family of neuropeptides that regulates cellular energy production and protects against

organismal stress. Encoded in the terminal exon of teneurin-1, TCAP-1 can be expressed as part of the full-length teneurin-1 mRNA, or by a separate mRNA. TCAP-1 acts antagonistically to corticotrophin releasing factor (CRF) in both invertebrates and mammals; however, the intracellular signaling pathways and downstream targets of this interaction remained unresolved. Calcium signalling is integral to neuronal communication, and modulation of calcium cascades has important downstream effects of neurotransmission and energy metabolism. This suggests that the TCAP-mediated suppression of CRF actions in neurons may result from modulation of CRF-induced calcium signaling. Therefore, the aim of this study was to assess if the anxiolytic effects of TCAP-1 are due to the suppression of CRF-activated calcium signalling. Using an in vitro approach with immortalized hypothalamic neurons, in combination with live-cell fluorescent imaging, we show that CRF increases cytosolic calcium and depolarizes mitochondrial membrane potential. Pre-treatment with TCAP-1 prevents these CRF-induced changes. Application of TCAP-1 alone decreases cytosolic calcium in neurons and hyperpolarises mitochondrial membrane potential, indicating that a TCAP-mediated calcium signal stimulates mitochondrial energetics. Together, these data indicate that the anxiolytic effects of TCAP-1 result from inhibition of CRF-induced increases in calcium and may also stimulate mitochondrial energy metabolism.

PS2.00104 SIRT1-MEDIATED EPIGENETIC CONTROL OF KISS1 EXPRESSION IN RESPONSE TO NUTRITIONAL UNBALANCE

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The onset of mammalian puberty is highly sensitive to metabolic cues. Energy balance is strongly influenced by nutritional challenges throughout early development. Although the premature initiation of puberty is prevented by an epigenetic mechanism of transcriptional repression, the epigenetic pathways that mediate the effects of nutrition and obesity on pubertal timing are unknown. Here, we identify Sirtuin 1 (SIRT1), a fuel-sensing NAD+-dependent deacetylase with an essential role in metabolic homeostasis, as a molecule that acts within the neuroendocrine brain to restrain female puberty via epigenetic repression of the puberty-activating gene, *Kiss1*. Sirt1 is expressed in hypothalamic *Kiss1* mRNA-containing KNDy neurons and operates as a central epigenetic link between energy status and *Kiss1* expression in KNDy neurons by changing the histone landscape of the *Kiss1* promoter. Moreover, SIRT1 recruits the Polycomb silencing complex member, EED, to the *Kiss1* promoter, enhancing the repressive activity of SIRT1 on *Kiss1* transcription. Eviction of SIRT1 from the *Kiss1* promoter during puberty is associated with increased abundance of activating histone post-translational modifications (H3K9ac, H4K16ac, H3K4me3), and a decrease in the repressive histone mark H3K27me3. This molecular switch from repressive to permissive chromatin configuration is seemingly dictated by the nutritional influence on SIRT1 activity at the *Kiss1* promoter: Early-onset overnutrition accelerate these changes and causes precocious puberty. Contrastingly,

undernutrition results in protracted repression and pubertal delay, which is mimicked by pharmacological activation of SIRT1. Our findings identify SIRT1-mediated inhibition of *Kiss1* transcription as a key epigenetic mechanism by which nutritional cues and obesity influence mammalian puberty.

PS2.00105 GHRELIN INHIBITION OF THE M-CURRENT IN KNDY NEURONS AND THE IMPACT OF 17 BETA-ESTRADIOL

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Obesity and anorexia both result in dysregulation of the hypothalamic-pituitary-gonadal axis, which negatively impacts reproduction. The gut peptide, ghrelin, secreted from the stomach, potentially mediates negative energy states and the neuroendocrine control of reproduction by acting through its receptor, growth hormone secretagogue receptor (GHSR). GHSR is expressed in hypothalamic arcuate (ARC) Kisspeptin/Neurokinin B (*Tac2*)/Dynorphin (KNDy) neurons. Ghrelin signaling is known inhibit the M-current produced by KCNQ channels in other ARC neurons. In addition, we have shown 17-beta-estradiol (E2) to increase *Ghsr* expression in KNDy neurons by 6-fold and increase the M-current in NPY neurons. We hypothesize that E2 increases GHSR expression in KNDy neurons to increase ghrelin sensitivity during negative energy states. Furthermore, we suspect ghrelin targets the M-current in KNDy neurons to control reproduction and energy homeostasis. We utilized ovariectomized (OVX) Tac2-GFP adult female mice, pre-treated with estradiol benzoate (EB) or oil and performed whole-cell-patch-clamp recordings to elicit the M-current in KNDy neurons using standard activation protocols in voltage-clamp. Using the selective KCNQ channel blocker XE991 (40 µM) to target the M-current, oil-treated and EB-treated mice, showed a decrease in the maximum peak current by 63.08±31.54 (n=8) and 57.65±28.82 (*n*=10), respectively. To determine the actions of ghrelin on the M-current, ghrelin perfusion (100 nM) in oil-treated and EB-treated mice suppressed the maximum peak current by 42.60±21.30 (n=7) and 57.11±28.56 (*n*=8), respectively. KNDy neurons appeared more sensitive to ghrelin when pre-treated with EB. This reveals ARC KNDy neurons are more sensitive to ghrelin during states of high E2.

PS2.00106 GABAB RECEPTOR DELETION IN KISS1 NEURONS/CELLS ALTERS BODY WEIGHT AND ANOGENITAL INDEX

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Female, global GABAB1KO mice show reproductive alterations with disrupted estrous cycles and compromised fertility. GABAB1KO mice co-express GABAB receptors (GABABRs) in Kiss1 neurons and show a dramatic increase in Kiss1 expression in male and female extrahypothalamic areas (amygdala, BNST); its expression did not differ in AVPV-PeN/ARC. To establish the impact of GABABRs on kisspeptin physiology we developed a strain of mice with specific deletion of GABABRs in Kiss1 cells/neurons and characterized them from reproductive and metabolic perspectives. Kiss-Cre mice (Jackson's Lab) were crossed with GABAB-floxed mice (donated by Dr. Bettler) to obtain Kiss1-GABAB1KO (KO) mice. Body weight (BW) was evaluated from postnatal day (PND) 7 to PND84. Sexual differentiation was evaluated by ano-genital distance (AGD) on PND7, PND14 and PND21 and relativized to BW (AG-index). KO females had increased BW [BW (g) PND84: F-KO=29.5±1.0 (n=6) vs F-WT=25.4±1.2 (n=8), $p<0.001$]; no differences were observed in males. In KO males AGD/BW was significantly increased at all ages, being more marked on PND7 [AGD/BW (cm/g)= M-KO=0.067±0.005 (n=5) vs M-WT=0.055±0.003 (n=9), $p<0.05$]. In KO females AGD/BW was decreased at all time points, being more marked on PND21 [AGD/BW (cm/g)= F-KO=0.033±0.003 (n=6) vs F-WT=0.043±0.003 (n=8), $p<0.04$]. Fertility was not compromised in young KO mice. First results from Kiss1-GABAB1KO mice demonstrate a significant increase in BW in females, compatible with kisspeptin's metabolic effects and alterations in sexual differentiation, where KO males are masculinized while KO females are feminized. Further evaluation of this colony will increase our understanding of GABAB control of Kiss1 cells/neurons. Funding: CONICET-UBA-ANPCYT-Fund.Williams-Fund.R.Barón.

PS2.00107 IGSF1 DOES NOT REGULATE FSH SYNTHESIS OR SECRETION IN VIVO OR IN VITRO

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Loss of function mutations in the X-linked immunoglobulin superfamily, member 1 (*IGSF1*) gene result in central hypothyroidism, often associated with macroorchidism. *Igsf1* knockout mice are also centrally hypothyroid, due to impaired TRH receptor expression and TRH action in the pituitary. The mechanisms underlying testicular enlargement are unclear and disputed. IGSF1 was originally characterized as an inhibin co-receptor. As inhibins negatively regulate FSH secretion, it was hypothesized loss of IGSF1 would lead to impaired inhibin action, elevated FSH, and, as a result, enhanced Sertoli cell proliferation during post-natal development. However, IGSF1 does not associate with inhibin A or B in heterologous binding assays. More recently, IGSF1 was proposed to inhibit signaling by the activin type IB receptor (ALK4). As activins stimulate FSH, the loss of this inhibition should lead to enhanced FSH levels. However, neither humans nor mice with IGSF1-deficiency have elevated FSH. Moreover, the methods used to demonstrate IGSF1 regulation of human FSHβ (*FSHβ*) promoter-reporter were conducted in a heterologous assay system in which such reporters lack activin/ALK4-dependent activity. Here, we further demonstrate that, when over-expressed in a homologous cell system

(LβT2 cells), IGSF1 does not impair induction of murine or human *Fshb/FSHβ* promoter-reporters by activin A or a constitutively active form of ALK4. Preliminary data further indicate that *Fshb* mRNA expression is similarly antagonized by inhibin A in primary cultures of pituitaries from wild-type and *Igsf1*-deficient mice. Collectively, the available data fail to support a role for IGSF1 in FSH regulation by activins, inhibins, or otherwise.

PS2.00108 A ROLE FOR GFAP-EXPRESSING CELLS IN REGULATING GONADOTROPIN-RELEASING HORMONE (GNRH) NEURON ACTIVITY

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GnRH neurons regulate fertility via episodic hormone release that suggests coordination among these cells, but how this coordination arises is unknown. GnRH neurons are surrounded by astrocytes, which can modulate neuronal activity and communicate over long distances. The gliotransmitter prostaglandin E2 (PGE2) increases GnRH activity and LH levels in rodents. We hypothesized astrocytes play a role regulating GnRH neuron activity. To begin to test this, we crossed GFAP-cre and floxed hM3Dq (Dq) mice. Dq is a DREADD (designer receptor exclusively activated by designer drugs) that activates Gq signaling in the presence of clozapine n-oxide (CNO). Most GFAP-positive cells in the brain are astrocytes. Extracellular recordings were used to monitor firing activity of GFP-identified GnRH neurons in brain slices from GFAP-cre Dq males and GFAP-cre littermate controls for a 10-min control period, then 1μM CNO was bath applied. CNO increased firing rate ≥ 50% in 6 of 8 GnRH neurons (baseline 0.2±0.07Hz, CNO 0.4±0.1Hz, $p<0.006$). No change was observed in cells from GFAP-cre controls (baseline 0.1±0.03Hz, CNO 0.07±0.02Hz). Pretreatment with a broad-spectrum prostaglandin E receptor antagonist failed to block this effect, suggesting factors other than PGE2 are involved in GnRH firing induced by activation of astrocyte Dq-signaling. Preliminary data indicate intraperitoneal injection of CNO induced a dramatic and rapid increase in LH in the GFAP-cre-Dq mouse and decreased LH in GFAP-Di mice, in which a DREADD activating Gi signaling was targeted. These data provide preliminary evidence that altering signaling in astrocytes changes GnRH neuron firing activity and LH release. NIH-R01HD34860

PS2.00109 FLUOXETINE INCREASED IL-1BETA IN THE MATERNAL HIPPOCAMPUS BUT NOT MOOD

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Postpartum depression affects approximately up to 10-15% of women. Fluoxetine is a common selective serotonin re-uptake inhibitor (SSRI) prescribed to treat postpartum depression. The

pleiotropic cytokine interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) are elevated in patients with depression compared to healthy controls. Here, we used a rodent model of postpartum depression to determine how maternal stress hormone exposure and SSRI treatment affect the cytokine levels within the maternal brain. We hypothesized that maternal corticosterone and SSRI treatment will have a more inflammatory cytokine profile than non-treated control dams. Dams were given corticosterone (40mg/kg) to model postpartum depression and fluoxetine (10mg/kg) for 21 days. Dams were tested for depressive-like behaviour using the Forced-swim test (FST) at the end of their treatment period. Then dams were sacrificed and brain tissues were used for cytokine analysis. Preliminary cytokine data showed a decrease in IL-6 and TNF-α within the hippocampus in corticosterone treated dams. Fluoxetine treatment increased the levels of interleukin-1beta (IL-1β) within the dam hippocampus. Further analyses will look at the cytokine profile within the prefrontal cortex of the dams to determine any possible effects by brain region. With more understanding of how antidepressant and maternal stress hormone exposure can affect the cytokine signatures within the brain, it can provide insights as to whether pregnant women or new mothers will seek antidepressants or alternative treatments for depression.

PS2.0011 ALTERED ALZHEIMER'S RELATED BRAIN GENES, COGNITION AND LOCOMOTION IN KISSPEPTIN RECEPTOR KO MICE

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Gonadal sex steroids have been implicated in the processing of beta-amyloid precursor protein, which generates beta-amyloid, an important factor for Alzheimer's disease (AD). Kisspeptin signalling controls the hypothalamic-pituitary-gonadal axis responsible for regulation of sex steroid levels, however kisspeptin is also neuroprotective against beta-amyloid toxicity, independent of its receptor, through direct binding to the beta-amyloid peptide. We used a hypogonadal kisspeptin receptor (Kiss1r) knockout (KO) mouse model to investigate the relationship between kisspeptin signalling/sex steroid deficiency and AD-like neuropathology. Brain regions were dissected and analysed for gene expression of AD- and neurosteroidogenic-associated markers. Behavioural testing was also performed in the male cohort. Object-recognition and object-in-place deficiencies in 6-month-old Kiss1r KO males were paired with increased expression of the beta-amyloid-associated gene presenilin 1 (PSEN) within the hippocampus. Gene expression analysis of brain regions in male and female mice showed an increase in expression of beta-amyloid-associated genes beta-amyloid cleaving enzyme (BACE), PSEN, and apolipoprotein E with age in males only. Females tended to exhibit higher levels of expression of these markers than males. We also used immunohistochemistry to examine forkhead box protein P2 (Foxp2) expression in the cortex, a transcription factor thought to regulate several hundred genes in the nervous system and regulate neurogenesis, neuroplasticity, synaptic function and signalling – these studies are ongoing. Kiss1r KO males also displayed reduced locomotion but no significant change in immuno-positive dopamine

neuron number was observed. The results of these experiments suggest that kisspeptin may act as a regulator of cognition and possibly beta-amyloid and AD-like behaviours.

PS2.00110 CORTISOL ADVANTAGE TO NEIGHBORING THE OPPOSITE SEX IN-UTERO

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Population sex ratios naturally fluctuate around equality. It is argued that the production of an equal number of male and female offspring by individual parents should be favored by selection. Theoretically, an even-sex ratio should yield the highest probability for a fetus to be adjacent to a fetus of the opposite sex in utero. This may cause developmental costs or benefits. We examined the physiological and developmental parameters associated with in-utero sex ratios in the nutria (*Myocastor coypus*), an invasive wildlife species with a strong reproductive output. Using hair-testing, we found that litters with even-sex ratios had the highest average cortisol levels. Fetuses neighboring an opposite sex embryo exhibited longer trunks than those neighboring the same sex, which might imply better lung development. In addition, although male fetuses had higher testosterone levels than female fetuses, females neighboring a female fetus had higher testosterone levels than those neighboring males. Our results introduce novel ideas about steroid dynamics in-utero, and the advantages to neighboring a fetus of the opposite sex. They also suggest that fetal cortisol may be a mechanism by which even-sex ratios are maintained.

PS2.00111 ANDROGEN RECEPTOR SIGNALLING IS A NOVEL REGULATOR OF PITUITARY PROLACTIN PRODUCTION

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The classical paradigm of lactotroph prolactin production and release is based around tonic inhibition by hypothalamic dopamine and stimulation by estrogen. We have recently shown that conditional ablation of pituitary androgen receptor (Foxg1-Cre ARKO) increases circulating prolactin in male mice, highlighting androgen signalling as a novel negative regulator of prolactin production. We aimed to refine our knowledge of the site and mechanism of action of this control by performing further genetic, pharmacological and surgical experiments to ablate the production and/or action of prolactin and/or androgens. Male mice with a genetic ablation of AR in neurons (Nestin-Cre ARKO) have no increase in circulating prolactin, confirming that the increase in prolactin seen in the Foxg1-Cre ARKO mouse is not due to loss of hypothalamic

AR, but specifically pituitary AR. However, Foxg1-Cre ARKO mice treated with the dopamine agonist bromocriptine show a decrease in circulating prolactin. Male mice with postnatal AR ablation in ~50% lactotrophs (Prolactin-Cre ARKO) have no increase in circulating prolactin, suggesting that either the mechanism is not lactotroph-specific or that ablation of AR needs to occur earlier or in a greater number of lactotrophs to result in an increase in circulating prolactin. Mice castrated in adulthood have no increase in circulating prolactin, suggesting that the control mechanism is not an acute response to changes in androgens. Further experiments are currently being undertaken to ablate AR in other cell populations and at earlier time points with the aim of pinpointing the mechanism of control of prolactin production by androgen receptor signalling.

PS2.00112 FROM UNZIPPING SINGLE MOLECULES OF CHROMATIN TO UNDERSTANDING GONADOTROPIN GENE EXPRESSION

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We have shown previously the differential organization of nucleosomes on the promoters of the two luteinizing hormone (LH) subunit genes, *Cga* and *Lhb*. The *Cga* promoter is largely nucleosome-free, while the *Lhb* promoter, encompassing binding sites of the transcription factors, Sf-1, Egr1 and Pitx1, is packaged into nucleosomes. Also the histone variant H2A.Z is located at different locations: at the TSS of *Lhb*, but at the +1 nucleosome of *Cga*. Using a single-molecule optical trap for high resolution force-distance measurements, incorporation of H2A.Z was seen to reduce stability and increase mobility of the nucleosome at these sites. We hypothesized that these differences in underlying chromatin organization determine not only the distinct expression levels but also diverse mechanisms of gene activation. RNAPII accumulates at the *Cga* TSS indicating promoter stalling which would be reduced by H2A.Z incorporation and also by the GnRH-induced histone modifications we observe at this +1 nucleosome. At the *Lhb* promoter, binding of GnRH-activated Egr-1 is increased by the presence of H2A.Z, and the associated histones are also modified by GnRH, contributing further to the remodeling of this nucleosome that exposes the transcription factor binding sites. Sf1 likely comprises the pioneer factor at the *Lhb* gene, binding one of its two sites to induce initial nucleosomal instability and co-operative binding of the other factors. We have generated a Sf1 stable knockdown cell model in which *Sf1* and *Lhb* mRNA levels are dramatically reduced, and are now pursuing more extensive studies on its role in the *Lhb* chromatin landscape.

PS2.00113 ROLE OF INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) IN MATERNAL ADAPTATION OF THE CENTRAL NERVOUS SYSTEM

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Adaptation to motherhood includes maternal behaviour and lactation with major organizing centres located in hypothalamic medial preoptic area (MPOA) and arcuate nucleus, respectively. We performed proteomics in hypothalamic synaptosomes comparing lactating and pup-deprived rat dams, which suggested that IGF- regulates several maternally changing synaptic proteins. Then, we re-evaluated our previous microarray study of the preoptic area and performed its validation with RT-PCR and in situ hybridization histochemistry, which in turn demonstrated that IGF binding protein-3 (IGFBP-3) has higher expression in MPOA of lactating mothers. Prolonged intracerebroventricular (icv.) administration of IGF-1 and an IGFBP-3 ligand inhibitor (NBI-31772) lengthened the pup-retrieval time. Furthermore, IGF-1 administration decreased suckling-induced prolactin release and consequently the weight gain of pups. The induction of IGFBP-3 expression occurred in tuberoinfundibular (TIDA) neurons of dorsomedial arcuate nucleus, which control prolactin secretion. IGF-1 elevated the expression of tyrosine-hydroxylase (TH) in TIDA neurons *in vivo* and induced not only expression but also activation of TH by phosphorylation *in vitro*. We also demonstrated suckling-induced IGF-1 release, which correlates with prolactin, reaches its maximum 30 minutes after the start of suckling and is diminished by prolonged icv. IGF-treatment. In conclusion, we propose a model that IGF-1 serum level is increased during suckling, which would inhibit maternal adaptation in the central nervous system. However, IGFBP-3 induced in MPOA and arcuate nucleus may be able to counteract this action of IGF-1 by possibly sequestering it from the extracellular space.

PS2.00114 REGULATION OF THE PHOENIXIN GENE BY BISPHENOL A, PALMITATE AND OLEATE IN HYPOTHALAMIC NEURONS

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Phoenixin (PNX) is a highly conserved, amidated 20 amino acid peptide expressed most highly in the hypothalamus. In hypothalamic neurons, PNX upregulates gonadotropin-releasing hormone and kisspeptin expression and is therefore a positive regulator of the hypothalamic-pituitary-gonadal axis. However, how PNX and its receptor, GPR173, are regulated is unknown and will be important for determining possible additional functions, such as a role in energy homeostasis. We hypothesize that expression of the PNX gene, *Smim20,* and PNX receptor gene, *Gpr173*, will be affected by the sex hormone, 17β-estradiol (E2); the endocrine disrupting

chemical, bisphenol A (BPA); the fatty acids, palmitate, oleate, docosahexaenoic acid (DHA) and palmitoleate; and by the activation of individual signaling pathways. Gene expression was measured from 2 to 24 hours with quantitative PCR in hypothalamic cell lines. E2, DHA and palmitoleate had no effect on PNX; however, BPA significantly decreased *Smim20* and *Gpr173* mRNA levels by 16 hours. Palmitate and oleate significantly upregulated *Smim20* mRNA, while palmitate downregulated *Gpr173* expression. Activation of protein kinase C (12-*O*-tetradecanoylphorbol-13-acetate), elevated levels of cAMP (forskolin), the nitric oxide donor sodium nitroprusside (SNP), and induction of neuroinflammatory signaling (lipopolysaccharide) had no effect on *Smim20* gene expression over 24 hours, while SNP slightly increased *Gpr173* at 24 hours. We will continue to investigate the effects of these compounds using enzyme immunoassays to determine PNX peptide levels. An understanding of how PNX is regulated will aid in determining its physiologic function at the hypothalamic level, and its potential for therapeutic applications.

PS2.00115 A GABAB ANTAGONIST INCREASES OVARIAN CYP19A1 AND ESR1/2 MRNA IN PND6 MICE AND DELAYS PUBERTY ONSET

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GABAB antagonist (CGP55845) administration to neonatal BALB/C mice significantly decreased ARC *Kiss1* expression in both sexes. Neither *Cyp19A1*, *Esr1/2*, *Pgr* hypothalamic expression were not affected by this treatment. Nevertheless, ovarian and testicular E2 contents were significantly increased in CGP-treated mice. Here we analyzed *Cyp19A1*, *Esr1/2*, *Kiss1* and *Kiss1r* in the ovaries of control and CGP-treated neonates and determined body weight (BW), anogenital index (AGI=AGD/BW) and puberty onset. Female Balb/c mice were injected with CGP55845 (1 mg/kg, sc) or saline from postnatal day 2 (PND2) to PND6±1, three times/day (8AM, 1PM, 6PM). One set of mice was sacrificed at 3PM (after two injections on the last day). One ovary was collected to determine *Cyp19A1*, *Esr1/2*, *Kiss1* and *Kiss1r*expression by qPCR; the other ovary was used to analyze ovarian morphology. In the second set of animals BW and AGI were evaluated on PND7, PND14 and PND21 and puberty onset was determined by vaginal opening (VO). CGP55845 increased *Cyp19A1, Esr1 and Esr2* expression in neonatal ovaries (*Cyp19A1*: CTRL:0.97±0.32 vs CGP:2.5±0.55: p<0,04; *Esr1*:CTRL:0.85±0.30 vs CGP:3.80±0.54: p<0,001; *Esr2:*CTRL:1.11±0.39 vs CGP:6.99±1.65: p<0,003). Neither Kiss1 nor Kiss1r ovarian expression was altered by CGP55845 (ns). CGP55845 decreased PND7 BW, increased PND7 and PND21 AGI and delayed puberty onset (VO/BW). Our data clearly show that lack of GABAB signaling stimulates the ovarian estrogenic system. CGP55845 impacts BW, sexual differentiation and puberty onset. Whether these are peripheral and/or CNS-dependent effects of CGP55845 remains to be determined. (Supported by CONICET, ANPCYT, UBA, Fundación René Barón, Fundación Williams).

PS2.00116 ESTRADIOL ENABLES STRESS-LEVELS OF CORTICOSTERONE TO INHIBIT LH PULSES IN FEMALE MICE.

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Two common responses to stress include elevated circulating levels of glucocorticoids and impaired LH pulsatility. We have shown that a stress-level of corticosterone (CORT) can prevent the estradiol-induced LH surge and impair ovarian cyclicity in mice; whether CORT disrupts LH pulses has not been investigated in this species. Here, we tested whether CORT can inhibit pulsatile LH secretion in female mice and if estradiol is necessary for this inhibition. Our approach was to measure LH pulses prior to and following administration of CORT or cholesterol (CHOL, control) in ovariectomized C57bl6 mice pretreated with one of three estradiol doses: 21.5 ng (low), 43 ng (med), 107.5 ng (high). Approximately one week after ovariectomy and estradiol pretreatment, tail-tip blood was collected every 6 minutes for 90 minutes to determine a baseline LH profile (PRE sampling). After sampling, animals were implanted with CORT or CHOL and sampled 2 days later (POST sampling). Regardless of estradiol dose, LH did not differ between PRE and POST sampling in CHOL-treated animals. In contrast, CORT significantly suppressed mean LH (60% lower) and basal LH (65% lower) in the POST sampling *only* in animals pretreated with high estradiol. Interestingly, only the high estradiol treatment was sufficient to slow LH pulse frequency during the PRE sampling, compared to an ovariectomized frequency, suggesting a sub-threshold level of the lower two estradiol treatments. These data support the interpretation that CORT can impair LH pulsatile secretion in the female mouse, but the response to CORT is dependent on circulating levels of estradiol.

PS2.00117 CAN GLUCOCORTICOIDS ACT DIRECTLY IN KISSPEPTIN NEURONS TO SUPPRESS LH SECRETION?

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Stress inhibits reproduction at least in part by suppressing LH secretion. However, the specific neural and/or endocrine mechanisms are unclear. Glucocorticoids (CORT) are released in response to a variety of stressors and have been demonstrated to suppress LH secretion in sheep, though conflicting or contrary data are available in other species. We previously demonstrated that administration of a subcutaneous CORT-containing implant (to achieve stress-like levels of CORT) caused a persistent state of diestrus, thereby inhibiting estrous cyclicity in mice. Here, we tested the hypothesis that CORT acts directly in kisspeptin neurons to suppress LH. First, we used immunohistochemistry to determine that 78% of ARC Kiss1 neurons and 82% of AVPV Kiss1 neurons contain glucocorticoid receptor (GR). Next, we treated GR flox/flox Kiss1-Cre+ (Kiss1-specific knock-out) or Cre- mice with cholesterol or CORT implants (n = 3 per group) and monitored estrous cycles. All cholesterol-treated animals had normal

estrous cycles (5.6 days) that did not differ by genotype. As expected in Cre- females, CORT induced a persistent state of diestrus that lasted ~14.5 days; disrupted cyclicity was also observed in Cre+ females. We sought to confirm a reduction of GR protein in Kiss1 neurons but detected no difference in the percentage of Kiss1 neurons that contain GR in the ARC or AVPV. Though examination of the necessity of GR signaling in Kiss1 neurons was hindered by an incomplete reduction of GR protein in Kiss1 neurons, we have demonstrated that a majority of both ARC and AVPV Kiss1 neurons contain GR in mice.

PS2.00118 SUPRACHIASMATIC VASOPRESSIN NEURONS PROJECT TO PREOPTIC KISSPEPTIN NEURONS

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The activity of kisspeptin neurons in the rostral periventricular region of the third ventricle (RP3V) drives the surge release of gonadotropin-releasing hormone and the downstream gonadotropins that triggers ovulation. In female rodents, this preovulatory hormonal surge is dependent on inputs from the suprachiasmatic nucleus (SCN). One potentially important input is that from SCN vasopressin (AVP)-expressing neurons to RP3V kisspeptin neurons. Here, we used either transgenic or viral-mediated expression of a red fluorescent reporter to reveal the axonal projections of SCN AVP neurons to the RP3V, in mice expressing cre-recombinase enzyme in AVP-expressing neurons. Reporter-positive fibres, indicating AVP neuronal projections, and kisspeptin neurons were stained using immunohistochemistry. We quantified the extent of RP3V innervation by SCN AVP neurons by measuring the area of the RP3V taken up by reporter-positive fibres. This revealed that reporter-positive fibres occupy 5.00 ± 1.64% of the RP3V (n = 4). In contrast, in animals in which the supraoptic nucleus and the paraventricular nucleus – but not the SCN – were transfected, reporter-positive fibres occupy only 0.16 ± 0.10% (n = 4) and 0.12 ± 0.03% (n = 4) of the RP3V, respectively. Furthermore, our preliminary observations suggest that SCN-derived reporter-positive fibres make close appositions with 80% of RP3V kisspeptin neuron somata (1.33 ± 0.21 appositions per soma). These results suggest an extensive projection from SCN AVP neurons to RP3V kisspeptin neurons, potentially providing a circadian input to drive the preovulatory hormone surge.

PS2.00119 ELUCIDATING MKRN3'S MECHANISM OF ACTION USING HIPSCS-DERIVED HYPOTHALAMIC NEURONS.

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Makorin ring finger protein 3 (MKRN3) was identified as a regulator of puberty initiation with the report of loss-of-function mutations in association with central precocious puberty (CPP). In juvenile mice, *Mkrn3* is highly expressed in the arcuate nucleus, with a reduction in expression before puberty initiation. These findings suggest that MKRN3 is acting at the hypothalamic level to inhibit puberty initiation. MKRN3 is an E3 ubiquitin ligase but, to date, its exact mechanisms of action are still unknown; we aimed to generate a human cell model to identify the hypothalamic targets of MKRN3's inhibitory effects. Using a directed differentiation protocol, we generated hypothalamic neurons from wild-type (WT) and CRISPR/Cas9-generated MKRN3-deficient isogenic human induced pluripotent stem cells (hiPSCs). RT-PCR analysis showed that *MKRN3* mRNA was not detected in MKRN3-deficient cells, but was expressed in WT neuronal progenitors and hypothalamic neurons. RT-qPCR analyses showed that *OCT4*-expressing WT and MKRN3-deficient hiPSCs differentiated with high efficiency into *NKX2.1*-expressing neuro-hypothalamic progenitors and then into *MAP2*-expressing neurons. Furthermore, *POMC*, *KISS1*and *TAC3* were expressed in both WT and MKRN3-deficient hypothalamic neurons. Our results indicate that *MKRN3* expression is highly upregulated during differentiation of hypothalamic neurons, yet not essential for this process. Rather, we hypothesize that, consistent with the clinical features, MKRN3 may be more important as a postnatal regulator of hypothalamic function. These WT and MKRN3-deficient hypothalamic neurons will serve as valuable models for transcriptome and proteome comparison to identify hypothalamic targets of MRKN3 action and thus reveal MKRN3 mechanisms of action within the human hypothalamus.

PS2.0012 THE OXYTOCIN SYSTEM AS A POTENTIAL TARGET FOR THE NEUROPSYCHIATRIC COMPONENT IN HUNTINGTON'S DISEASE

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Although oxytocin is associated with a number of social behaviors including maternal care, social bonding and attachment that are essential for social interaction, it has also been implicated in a number of psychiatric disorders as well as Huntington's disease (HD). This is a genetic and fatal neurodegenerative disease characterized by motor symptoms primarily associated with basal ganglia pathology as well as early metabolic dysfunction and psychiatric disturbances such as depression and anxiety. Dysfunction of the oxytocin system originating in the hypothalamus occurs before motor onset; we have previously established a loss of oxytocin neurons in human post-mortem samples with Vonsattel grades 2-4 (HD stages with prevalent striatal pathology) and reductions in protein and mRNA levels of oxytocin in HD mice. More recently, we reported a similar selective loss of oxytocin neurons in an HD case with Vonsattel grade 0 (absence of striatal atrophy) and medical records revealed that the patient suffered

non-motor symptoms including anxiety and sleep disturbances. Our work now focuses on understanding whether dysfunction of the oxytocin system contributes to the early neuropsychiatric component in HD and if it can be targeted as a novel therapeutic intervention using experimental mouse models of HD.

PS2.00120 THE IMPACT OF CHRONIC ACTIVATION OF ARCUATE GABA NEURONS ON FERTILITY

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Gonadotropin-releasing hormone neurons (GnRH-N) are heavily innervated by arcuate nucleus (ARN) GABA neurons (GABA-N). In a mouse model of polycystic ovary syndrome, the most common infertility disorder worldwide, an increase of ARN GABA-N inputs onto GnRH-N is observed. However, it is unclear how selective chronic activation of ARN GABA-N directly impacts GnRH-N activity and fertility. To address this question, we are using chemogenetic tools coupled with a Cre/lox approach in mice. We expressed the designer receptor hM3Dq specifically in ARN GABA-N via stereotaxic injection into the ARN of vesicular GABA transporter (VGAT-Cre) mice. The delivery of the designer drug (CNO) to activate hM3Dq was coupled with serial tail-tip blood sampling to detect luteinizing hormone (LH) secretion as a readout of GnRH secretion. *In vivo*, we have been able to accurately target hM3Dq to ARN GABA-N and observed cFos expression specifically in ARN GABA-N after a single peripheral injection of 1.5mg/kg of CNO. However, LH secretion was not affected in gonadally intact male (n=12) and female (n=9) mice or ovariectomised females (n=4). Interestingly, a single injection of CNO directly onto the rostral preoptic nucleus of bilaterally transfected unconscious male increased LH release (n=2). To investigate longer term chronic activation of ARN GABA-N, CNO was delivered in the water for 2 weeks. This disrupted estrous cyclicity in bilaterally transfected intact females (n=4). These results suggest that the specific modulation of the ARN GABA-N can stimulate GnRH/LH secretion. However, a chronic activation of this circuit can cause dysregulation of estrous cyclicity.

PS2.00121 PERIVENTRICULAR KISSPEPTIN NEURONS SEND PROJECTIONS TO THE PARAVENTRICULAR NUCLEUS IN THE MOUSE

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Magnocellular oxytocin neurons are found in the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei. Their cell bodies synthesize oxytocin, which is released into the circulation from the posterior pituitary gland in response to action potential firing. Oxytocin promotes uterine contractions during parturition and milk let-down during lactation. In late-pregnant rats (days 18 – 21 of gestation), central kisspeptin administration increases oxytocin neuron activity, but not in non-pregnant rats. We have unpublished data from late pregnant mice that show increased kisspeptin projections to the PVN and SON. To identify the origin of these kisspeptin fibres, we injected a retrograde tracer into the PVN of pregnant mice. The fluorescent retrobeads are taken up by axon terminals in the PVN and retrogradely transported along the axon to the cell body. Four days after PVN injection, mice were perfused with 4% paraformaldehyde and brains were processed for immunohistochemistry to identify kisspeptin cell bodies and co-localization of green fluorescent retrobeads. The periventricular nucleus of the hypothalamus showed co-expression of green retrobeads in kisspeptin cell bodies. Other kisspeptin neuron populations in the anteroventral periventricular nucleus and arcuate populations did not contain retrobeads. Taken together, these results show that periventricular kisspeptin neurons increase their fibre innervation in the PVN and SON at the end of pregnancy in the mouse. As yet the functional significance of the increase in kisspeptin fibres during pregnancy is unknown but it appears likely to increase the excitability of oxytocin neurons for birth.

PS2.00122 USING GENETIC MANIPULATIONS TO UNDERSTAND THE ROLE OF RFRP-3 IN THE REGULATION OF REPRODUCTION

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In 2000, gonadotrophin-inhibitory hormone was discovered in birds and shown to inhibit gonadotrophin secretion. The mammalian ortholog was concurrently discovered in humans and rats and termed RFamide-related peptide-3 (RFRP-3). We have recently shown, by administering RFRP-3 centrally, that the effects of RFRP-3 on gonadotrophin secretion are sex- and cycle stage-dependent in mice and hamsters. In order to further our understanding of the ways in which RFRP neurons modulate GnRH function, we have developed two novel transgenic mouse lines. Using a Cre-loxP conditional transgenic method, we knocked the receptor for RFRP-3 (GPR147) out of GnRH neurons and analysed puberty onset in these mice. While the absence of GPR147 on GnRH neurons had no effect on puberty onset in males, it resulted in a 3.8 day delay in female puberty ($P<0.05$ vs GPR147-intact littermates). However, no deficits in reproductive cycles or male and female adult fertility were noted. In parallel, we have been using a chemogenetic approach to elucidate the effect of activation of RFRP neurons on LH secretion. In mice expressing the hM3Dq DREADD in RFRP neurons, 1 mg/kg clozapine-N-oxide (CNO, the DREADD ligand) had no effect on circulating LH levels in either sex. In males, an additional dose of 10mg/kg of CNO had no effect on LH secretion. Further studies will aim at

characterising the effect of CNO administration on preovulatory surge LH secretion in females. Taken together, these new tools provide us with the possibility to advance our understanding of the functions of the RFRP neuronal system in mice.

PS2.00123 ELECTRICAL CHARACTERISTICS OF THE RFRP NEURONS IN MICE

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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 gonadotropin hormones, which in turn control the gonadal activity. A hypothalamic neuropeptide, RF (Arg-Phe) amide-related peptide (RFRP), has been shown to act upstream of the GnRH neurons. The effect of the RFRP peptide on the gonadotropic axis however is complex and may depend on species, sex or environmental conditions. The aim of this study is to use RFRP-CRE mice to explore the role of the endogenous RFRP on the gonadotropic axis of adult male and female mice, as well as to investigate what drives the RFRP neuronal activity. In order to establish the electrical firing characteristics of the RFRP neurons, we performed cell-attached electrical recordings on coronal brain slices. Our 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. Moreover, preliminary data point towards a small-scale response of the RFRP neurons to vasopressin, indicating a potential circadian control of the RFRP system. Finally, using channel rhodospin expressing virus, we have performed optogenetic activation of the RFRP neurons in vitro in order to move forward to investigate the effect of in vivo optogenetic activation of RFRP neurons on gonadotropin secretion.

PS2.00124 DETERMINING THE EFFECTS OF DIETARY PHYTOSTEROIDS ON RED COLOBUS MONKEY REPRODUCTION

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Conservation strategies benefit from species-specific knowledge of reproduction gained through observation and hormone monitoring; however, basic reproductive information for many endangered species is lacking. Further, understanding patterns of reproductive physiology and behavior is obscured by effects of dietary chemistry on the endocrine system.

To examine interactions between reproduction, dietary chemistry, and hormones, we studied one group of red colobus monkeys (*Procolobus rufomitratus*) in Kibale National Park, Uganda, and determined patterns of phytosteroid consumption, fecal hormone levels, and reproductive behavior. Differences in average fecal estradiol for cycling (102.3 ± 108.6 ng/g), lactating (96.4 ± 217.5 ng/g) and pregnant females (561.9 ± 555.8 ng/g) were detectable ($F_{2, 138} = 32.5$, $P < 0.0001$, n = 14 females); however, on an individual level estradiol data yielded mixed results. Regularly cycling females did not follow an expected estrous pattern as spacing between peaks from baseline were often irregular, but females that became pregnant and then lactated tended to follow an expected estradiol profile. Differences in average fecal progesterone levels were not detectable among reproductive states. One possibility for discrepancies between fecal hormones and observed reproductive state was the consumption of phytosteroids, as some red colobus plant foods were estrogenic and others bound to progesterone antibodies. Consumption of estrogenic young leaves was positively related to fecal estradiol and cortisol levels in adult males and their mating patterns. We are currently examining if a similar effect is seen in females and suggest that field endocrinologists consider the effects of endocrine-active plants on their study species.

PS2.00125 THE CENTRAL EFFECT OF THE INJECTED B-NGF IN THE DROMEDARY CAMEL FEMALES (CAMELUS DROMEDARIUS).

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The dromedary camel (*Camelus dromedarius*), a well-adapted desert mammal, is sexually active during short photoperiod. Females display a provoked ovulation induced by the nerve growth factor beta (β-NGF) present in the seminal plasma of males. The mechanisms by which β-NGF triggers the ovulation are still unknown. The aim of this study is to identify the central targets of β-NGF when injected to dromedary camel females during the breeding season. Females were injected intramuscularly with 0 (control saline), 250, 500, 1000µg of β-NGF and two hours later, brains were sampled and prepared for immunohistochemistry analysis of c-Fos and reproductive peptides (GnRH, kisspeptin, RFRP3). Only the injection of 1000µg β-NGF induced c-Fos expression which was observed in various hypothalamic area: the preoptic area (POA) and the paraventricular (PVN), dorsomedial hypothalamus (DMH) and arcuate nuclei (Arc). Immunostaining with GnRH, kisspeptine or RFRP3 antibodies showed a clear expression of these peptides in neurons in the POA and Arc for GnRH and Kisspeptin and PVN, DMH, ventromedial hypothalamus nuclei for RFRP. However, none of these neurons co-expressed β-NGF-induced c-FOS. These results indicate that the ovulatory effect of β-NGF in dromedary

camel may not be directly mediated by GnRH, Kp or RFRP3 neurons and may act on still to be identified neurons where c-Fos expression was observed.

PS2.00126 PROLACTIN-INDUCED AND NEURONAL ACTIVATION IN THE BRAIN OF MOTHER MICE

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The brain regions neuronally activated by suckling generally overlap with those containing prolactin activated neurons. The precise comparison between these cell populations has not been performed, even though they are both responsible for a variety of different brain adaptations to motherhood. To separate the actions of these different mechanisms, neurons directly activated by prolactin were visualized by pSTAT5 immunohistochemistry in relation to Fos-expressing neurons in suckled mother mice. We first mapped pSTAT5-ir neurons in the maternal mouse brain 2-h after reuniting the dams with their litter following a 22-h separation. Suckling also induced Fos expression in all of those brain regions. Then, double labeling of pSTAT5 and Fos was performed with and without pup-exposure, and the colocalization in brain regions containing pSTAT5 was quantitatively analyzed. In addition, both pSTAT5 and Fos were also double labeled with estrogen receptor alpha (ERα), which revealed a very high degree of co-localization between pSTAT5 and ERα in several brain regions with much less potential interaction between Fos- and ERα-containing neurons. The results suggest that most neurons responding to suckling in mothers, and likely to be involved in maternal responsiveness, are driven either by prolactin or direct neuronal input from the pups while some neurons are affected by both types of inputs. In addition, the ratio of neurons directly influenced by both routes varies in different brain region, and estrogen-sensitive neurons are more likely to be affected by prolactin than by direct neuronal activation. Support: NKFIH-4300-1/2017-NKP_17, NKFIH-2920-1/2016-VEKOP-2.3.-15, NKFIH-6785-1/2016-VEKOP-2.3.3-15, OTKA K116538.

PS2.00127 TRPV1 CHANNELS REGULATE THERMOREGULATORY RESPONSES OF ESTRADIOL-TREATED OVARIECTOMIZED RATS

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The core body temperature is maintained by mechanisms of loss and heat production. Vasodilatation of skin tail is one of the main heat loss effectors. Estradiol participates in the neural pathways responsible to activate heat loss by a mechanism that is not complete elucidated. TRPV1 channels regulate core body temperature. Therefore we tested whether these channels participate in this mechanism. Ovariectomized rats were treated for 14 days with pellets of 17β-estradiol (180 µg/mL, E2-OVX) or of corn oil (control group). The tail blood flow measured by *Doppler* perfusion was higher in the E2-OVX rats compared to control. E2-OVX rats were treated with resiniferatoxin (RTX) in a sufficient dose (20 µg/Kg) to promote peripheral TRPV1 channel desensitization. This treatment reduced tail skin blood flow. TRPV1 channels and estradiol therefore regulates the tail skin vasomotor. Thus, we tested the role of these channels in the skin tail heat loss after a thermal challenge. Another set of E2-OVX rats injected with RTX was exposed to temperature of 34 ºC (above of the thermoneutral zone). This procedure increased core body and skin tail temperature. RTX blunted the tail heat dissipation, increased the heat loss threshold, and decreased heat loss sensitivity. Thus, TRPV1 channels participate in the maintenance of tail vasomotor tonus, which facilitates heat loss essential to keep core body temperature in the E2-OVX rats. These results may contribute to our understanding of neural pathways compromised by the withdrawn of estradiol like observed in the menopause. FAPEMIG APQ-01173-17, CAPES, CNPq

PS2.00128 HIGH DEGREE OF PLASTICITY IN LUTEINIZING HORMONE (LH) CELL POPULATION IN MEDAKA (ORYZIAS LATIPES)

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Pituitary gonadotropins are key players in pubertal development and reproduction across all vertebrates. However, very few studies have been performed on gonadotrope cell development after the embryonic stages. Using a transgenic line of the modelfish medaka, where Gfp expression is under the control of the endogenous *lhb* promotor, we studied different aspects of the Lh cell population comparing juveniles and adults in both sex. Confocal imaging and 3D reconstruction did not reveal any major difference between sexes and stages in Lh cell distribution, but an increased number and an increased size of Lh cells in adults compared to juveniles, in both males and females. BrdU incubation experiments in combination with PCNA staining showed that Lh cells are able to divide themselves. Time-lapse recordings of primary pituitary cell cultures further showed that non-Lh-cells have the possibility to become Lh-positive. Thus, the increased number of Lh-cells in adult vs juvenile pituitaries seem to arrive both from Lh-cell proliferation, and from phenotypic plasticity. Finally, when treating the fish with 17β-estradiol we observed an increased cell proliferation in the adult pituitary, in particular in Lh cells. RNA-seq of FACS sorted Lh cells, as well as *in situ* hybridization revealed that Lh cells express two estrogen receptors, suggesting a direct effect of 17β-estradiol on Lh

cell proliferation. These results reveal a high degree of plasticity of the Lh population in the medaka pituitary during puberty, presumably due to both estrogen-dependent cell proliferation and changing of phenotype from other cell types.

PS2.00129 ANALYSIS OF THE DIRECT ACTION OF MELANOCORTINS ON KISS1 NEURONS IN THE CONTROL OF REPRODUCTION

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Reproduction, controlled by the excitatory action of the neuropeptide kisspeptin, is an energy costly function for the organism and, therefore, tightly regulated by metabolic factors. Among those, hypothalamic melanocortins are essential in the control of metabolism. Inactivating mutations in the proopiomelanocortin (*Pomc*) gene or melanocortin receptor 4 (MC4R) cause severe obesity and subfertility in humans and mice. However, whether the reproductive impairment is a direct consequence of the lack of melanocortin signaling, or indirect from the obesity phenotype, remains unknown. Alpha-MSH (derived from *Pomc*) stimulates LH release by acting on or above Kiss1 neurons. Moreover, POMC neurons project to Kiss1 neurons, which express MC4R. To determine whether the alpha-MSH regulation of reproduction is mediated by MC4R on Kiss1 neurons, we ablated MC4R specifically from Kiss1 neurons by generating a specific Kiss1-MC4R.KO (*Kiss1*-cre^{+}/MC4R.lox$^{+/+}$) mouse line. These mice exhibit similar body weight as controls (MC4R.lox$^{+/+}$); however, females display disrupted estrous cycles with prolonged diestrous phases, suggesting a direct action of melanocortins on Kiss1 neurons. Our ongoing studies are aimed at 1) identifying the specific population of Kiss1 neurons that mediates this effect, 2) characterize the contribution of the melanocortin-kisspeptin pathway to the metabolic regulation of fertility in these mice and 3) delineate the neuronal circuits that connect POMC and Kiss1 neurons. Understanding the neuroendocrine mechanism underlying the metabolic regulation of reproduction will allow us to improve fertility rates in patients affected by metabolic disturbances.

PS2.0013 REORGANIZATION OF PERINEURONAL NETS IN THE RAT'S MEDIAL PREOPTIC AREA DURING PREGNANCY AND LACTATION

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Perineuronal nets (PNNs) are aggregations of extracellular matrix associated to specific neuronal populations in the CNS, suggested to play key roles in neural development, synaptogenesis, neuroprotection and experience-dependent synaptic plasticity. Pregnancy and lactation are characterized by a remarkable increase in neuroplasticity, however it is unknown how PNNs associated to maternal circuits are regulated during these periods. We analyzed the structure of PNNs in a key nucleus of the maternal circuit, the medial preoptic area (mPOA), in female rats during pregnancy (Gestation Day (GD) 10, GD14, GD21) and postpartum period (Postpartum Day (PPD) 2, PPD7 and PPD 22) using the glycosaminoglycan label obtained with the lectin of *Wisteria floribunda* (WFA).To assess whether gonadal steroids associated to gestation modulate PNNs we tested ovariectomized females treated with a hormone-simulated gestation protocol: daily injections of Estradiol (days 1-16), Estradiol+Progesterone, (days 17 to 21), and cycling females. We found that PNNs start to ensemble on GD14 and are highly organized on GD21, fading from PPD2 on. Cycling, as well as vehicle-treated females, did not exhibit any label of WFA in the mPOA, while the hormone-simulated treatment mimicked the PNNs ensemble described above. In conclusion, the different organization of PNNs is associated with changes in the plasticity of the mPOA, most likely underlying the dynamics of maternal behavior and physiological adaptations to lactation. Moreover, this appears to be highly dependent on gonadal hormonal levels characteristic of gestation. How PNNs are involved in expression and maintenance of maternal behavior remains to be explored. Support: ANII-SNB/SNI, PEDECIBA

PS2.00130 EXPRESSION OF EPIGENETIC ENZYMES IN THE REPRODUCTIVE NEUROENDOCRINE AXIS OF THE SIBERIAN HAMSTER

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Seasonal species such as the Siberian hamster use external stimuli such as day length to time reproductive biology. Annual variation in day length is coded in mammalian species via the nocturnal production of melatonin by the pineal gland. In hamsters, long summer days (LD) induce an active reproductive phenotype and short-winter days (SD) trigger reproductive involution. Recent work has indicated that annual changes in DNA methylation regulate seasonal reproductive biology. Specifically, reduced methylation of the iodothyronine deiodinase 3 (dio3) gene in the ependymal layer of the 3rd ventricle initiates a cascade of molecular neuroendocrine events that leads to gonadal involution. Using the female Siberian hamster, we investigated the role of estrogen for the regulation of DNA methyltransferase 1 and 3a enzymes (dnmt1 and dnmt3a) across the annual reproductive and estrous cycle. We propose that the variation in epigenetic enzyme expression is tissue-dependent (i.e. hypothalamic and uterine) and

driven by estrogen. The findings indicate that DNMT1 immunoreactivity is predominantly expressed along the ependymal layer; SDs reduced signal intensity in the ventral region. DNMT3a showed a robust switch in the endometrial layer of the uterus with greater expression in the regressed SD phenotype. Ovariectomized females administered a single bolus injection of estrogen and progesterone was found to express significantly lower levels of uterine dnmt3a and dnmt3b. The data presented here indicates that epigenetic modifications exhibit robust plasticity in key tissues and provides a novel mechanism that govern female reproductive biology.

PS2.00131 GENE NETWORKS INVOLVED IN THE METABOLIC CONTROL OF FEMALE PUBERTY.

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Energy balance is widely affected by nutritional challenges during early development, i.e. during late gestation in humans and during early post-natal life in rodents. Alterations in the developmental programming affect the female neuroendocrine reproductive development. While increased nutritional availability advances the timing of puberty, nutritional deficiency results in puberty protracted repression. Here, we performed RNA-Seq analysis of the female rat arcuate nucleus (ARC) throughout peripubertal development under different nutritional conditions. RNA-Seq reads were aligned to the rat reference genome using Bowtie2/Tophat2. Differential expression between time points was analyzed using the generalized linear modeling approach implemented in edgeR. Genes with ± 1.5 fold expression change compared to animals grown under conditions of normal nutrition were selected for Gene Ontology pathway analysis using the Database for Annotation, Visualization, and Integrated Discovery. During pubertal development, 517 genes were down-regulated in the ARC. Biological processes significantly ($p<0.05$) enriched in this group included: cell division, cell proliferation, cell adhesion, Notch signaling, and response to estradiol. Moreover, 566 genes were up-regulated showing enrichment in: neuropeptide signaling, immune response, apoptosis and retinoid metabolism. Female rats grown under conditions of nutritional deficiency showed pubertal delay as well as increased enrichment in down-regulated biological processes. Contrarily, nutritional excess advanced pubertal development and increased enrichment in up-regulated biological processes. Our study provides new evidence that alterations in the developmental programming of energy balance affect the timing of female puberty by rewiring gene networks involved in the fine-tuning of the developing ARC. **Funding**: NIH grants 1R01HD084542 to A.L; F32-HD-86904 to C.A.T.

PS2.00132 QUANTIFYING UNCONJUGATED URINARY STEROIDS IN THE BIG BROWN BAT ACROSS THE REPRODUCTIVE CYCLE

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Research shows that sex steroids can be excreted by an individual into the environment and directly impact a conspecifics behaviour and physiology. Studies in mice demonstrate that the failure of blastocyst implantation after insemination, precocious puberty, and changes in sexual behaviour can all be induced by exposing a female to a novel male. Application of 17beta-estradiol to the mucus membranes of females can cause the aforementioned effects. Furthermore, enzyme linked immunosorbent assays (ELISA's) show the presence of bioactive unconjugated 17beta-estradiol in the urine of male mice, and simply applying male urine to the female can cause behavioural and physiological changes, demonstrating the ability of sex steroids to act as a pheromone. Using radioactive tracers, our lab has shown the transfer of 17beta-estradiol from male to female big brown bats during the mating season, as well as the transfer of progesterone between female conspecifics, however the mechanism of steroid transfer in remains unknown. With urine as a likely vector, the current study uses ELISA's to quantify unconjugated urinary steroid levels across the annual reproductive season of the big brown bat. Urine was collected over an 18-month period from adults and juveniles. Male urine was analyzed for 17beta-estradiol, previously shown as a vector in male mice and to transfer from male to female bats, as well as 17beta-estradiol and progesterone in the urine of female bats. We are the first to show the presence of unconjugated steroids in the urine of bats, and will discuss the observed changes across the reproductive cycle.

PS2.00133 REGULATION OF THE PRIMATE MENSTRUAL CYCLE BY TWO DISTINCT GNRH NEURONAL POPULATIONS.

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Gonadotropin-releasing hormone (GnRH) neurons represent the primary neuroendocrine link between the brain and the rest of the reproductive system, and traditionally it has been assumed that a single population of GnRH neurons controls both pusatile LH release as well as the preovulatory LH surge. This view has profoundly influenced our strategies for contraception and for the treatment of infertility in women. Recent data from our laboratory, however, questions the validity of this fundamental assumption. Using the female rhesus monkey as a translational animal model, we found that: 1) Primates express two distinct molecular forms of GnRH, both of which are highly effective at stimulating LH release; 2) GnRH-I and GnRH-II, are encoded on different chromosomes, and the neurons that secrete them have completely distinct locations in the hypothalamus; 3) GnRH-I neurons respond to estrogen exclusively in a negative manner, while GnRH-II neurons respond to estrogen exclusively in a positive manner.

Taken together, these data suggest that different aspects of reproductive function in primates are orchestrated by two distinct populations of GnRH neurons, with GnRH-II neurons playing the primary role in mediating the estrogen–induced preovulatory LH surge. Moreover, these findings suggest that it may be possible to selectively silence this subpopulation of neurons in humans, using pharmacological agents - thereby blocking ovulation while leaving the rest of the reproductive axis relatively unperturbed.

PS2.00134 DIFFERENTIAL GENE EXPRESSION IN RESPONSE TO ESTRADIOL WITHDRAWAL IN PERIMENOPAUSAL DEPRESSION

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The risk of depression increases 2-3 fold for women during the menopause transition compared to premenopausal women. Additionally, peri/postmenopausal women with even minor depression are at an increased risk of cardiovascular mortality. Clinical studies show both the therapeutic benefits of estradiol (E2) in perimenopausal depression (PMD) (Schmidt et al, 2000, Soares et al, 2001) and the symptom-provoking effects of E2-withdrawal (E2WD) in women with past PMD, which are not experienced by those without past PMD (Schmidt et al, 2015). Thus, it has been posited that heightened sensitivity to changes in ovarian steroids such as E2 may contribute to the onset of PMD. We hypothesized that the differential affective/behavioral responsivity to E2WD in PMD could be observed on a cellular level. In this study, we used lymphoblastoid cell lines (LCLs) derived from women with past PMD, or from asymptomatic controls (AC). LCLs were maintained in 100nM E2 for 72 hours, and then withdrawn and collected 24 hours after the final E2 exposure. Cells were then examined for changes in gene expression levels using whole-transcriptome RNA sequencing. Preliminary analysis (n=7 AC, n=6 PMD) revealed significant differences in RNA expression between women with PMD and AC, as well as several molecular pathways that appear to be differentially altered in women with PMD. In particular, the gene CXCL10, which has been previously linked to cardiovascular disease, is significantly upregulated in women with PMD. Future studies with these data may help to establish a cellular basis for the differential behavioral response to E2WD in PMD.

PS2.00135 PUTATIVE ROLE OF HYPOTHALAMIC NEUROGENESIS IN NEUROENDOCRINE CONTROL OF PUBERTAL SUPPRESSION.

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Pubertal maturation is associated with changes in release of gonadotropin-releasing hormone from the medial pre-optic area (mPOA), which is in turn regulated by neuropeptidergic cell populations in the arcuate nucleus (Arc). Eusocial naked mole-rats provide an intriguing opportunity to study the neuroendocrine mechanisms associated with pubertal suppression and subsequent maturation: they live in large colonies consisting of a single reproductive female,1-3 breeding males and dozens of socially subordinate, reproductively suppressed adults. Reproductive suppression is more rigid in females and subordinates of both sexes undergo puberty when removed from the colony's suppressive cues. Here, we begin to explore a role for hypothalamic neurogenesis in the socially-mediated pubertal transition of the naked mole-rat. We injected a sex-balanced group of adult subordinate naked mole-rats daily with BrdU (proliferative cell marker) for seven days and collected them at 1 day, 1 week, 3 weeks, 5 months and 12 months post-injection; a separate group was collected 2 hours after a single injection. The number of BrdU immunoreactive cells was quantified for each collection time in the mPOA and Arc. Patterns of hypothalamic neurogenesis show a larger number of cells present after 2 hours in the Arc as compared to the mPOA, with both regions showing a peak in BrdU immunoreactive cells at 3 weeks. A significant sex difference, favoring females, was found in the mPOA at 1 and 3 weeks. These results are consistent with the hypothesis that plasticity in hypothalamic circuits accompanies reproductive transitions in adult naked mole-rats.

PS2.00136 EFFECTS OF OXYTOCIN RECEPTOR KNOCKDOWN IN THE MIDBRAIN DORSAL RAPHE ON POSTPARTUM BEHAVIORS

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Oxytocin (OT) is well-known for influencing mammalian maternal caregiving. OT acts in many brain sites to affect postpartum behaviors, but midbrain sites sensitive to OT, such as the dorsal raphe (DR; source of most forebrain serotonergic innervation) are rarely studied. Our lab previously found a ~ 60% increase in oxytocin receptor (OTR) autoradiographic binding and ~ 60% higher OT-immunoreactive fiber density in the postpartum DR compared to diestrus. Additionally, we demonstrated that ~ 40% of serotonergic neurons in the female rat DR express OTR immunoreactivity. These postpartum increases in DR OT measures may be functionally relevant, as manipulating the serotonergic system modifies many postpartum behaviors. Here we hypothesized that elevated OT signaling specifically in the DR influences the display of mothers' socioemotional behaviors. To test this hypothesis, we created an adeno-associated virus (AAV) expressing a short hairpin RNA (shRNA) targeted to OTR mRNA (AAV-OTRKO) or a scrambled shRNA control. We predict that mothers treated with the AAV-OTRKO in the DR during pregnancy will later show impairments in aspects of their caregiving behaviors,

increased anxiety-like behavior, and decreased maternal aggression compared to scrambled shRNA treated control dams. Preliminary results using these vectors support our predictions. These results would demonstrate that OTR signaling in the DR is necessary for the display of numerous postpartum behaviors in laboratory rats and that OTR-5HT interactions are an understudied mechanism underlying successful motherhood.

PS2.00137 DAILY CHANGES IN GNRH SYSTEM SENSITIVITY TO RFRP-3 INHIBITION

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In spontaneously ovulating species, the timing of the LH surge is controlled by the master circadian pacemaker in the suprachiasmatic nucleus (SCN). We have previously shown that the SCN initiates the LH surge through the coordinated control of two, opposing neuropeptidergic systems that lie upstream of the GnRH system, kisspeptin and RFamide-related peptide-3 (RFRP-3; also known as gonadotropin-inhibitory hormone (GnIH)). Additionally, we found that the GnRH system exhibits time-dependent sensitivity to kisspeptin stimulation, further contributing to the precise timing of the LH surge. Whether this time-dependent sensitivity is unique to kisspeptin or also occurs in response to negative regulation is unknown. To answer this question, we explored daily changes in sensitivity of GnRH neurons to RFRP-3 inhibition. Female hamsters were ovariectomized to eliminate estradiol negative feedback and injected with RFRP-3 or saline (i.c.v.) in the morning (prior to the LH surge) or late afternoon (around the time of the LH surge). We hypothesized that inhibition by RFRP-3 would reduce LH in the morning, as reproductive axis inhibition is essential prior to ovulation. In contrast to expectation, LH concentrations did not differ between RFRP-3- and saline-injected animals in the morning, but were markedly suppressed by RFRP-3 in the afternoon. *LH beta* mRNA expression was suppressed in the afternoon, however no difference in *Gnrh* or *Gpr147* mRNA expression was found in the mPOA. Together, these results underscore the necessity of circadian-controlled RFRP-3 system inhibition during the LH surge as we have previously demonstrated.

PS2.00138 EFFECTS OF PREGNANCY STRESS ON MATERNAL CAREGIVING, ANXIETY, AND MIDBRAIN SEROTONIN 2C RECEPTORS

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Mammalian mothers show a unique suite of behavioral responses beginning around the time of parturition that include increased offspring caregiving, high maternal aggression, and low anxiety. The neurotransmitter, serotonin (5-HT), is known to influence many socioemotional behaviors, and much of it is synthesized by cells in the midbrain dorsal raphe nucleus (DR). Our lab has shown that the DR undergoes structural and neurochemical changes during the peripartum period, and some recent findings also revealed a decrease in serotonin 2C receptor (5-HT2C) mRNA in the DR at parturition and early lactation. This finding is interesting because others have found that activation of 5-HT2C during early lactation disrupts maternal behaviors, and that central 5-HT2C receptors modulate the behavioral effects of chronic stress. The aim of the current study is to determine whether the normal expression of 5-HT2C in the DR across female reproduction is altered by pregnancy stress, which impairs later postpartum caregiving and increases anxiety. Female rats receiving repeated mild variable stress during pregnancy will be assessed for caregiving, anxiety-like, and depression-like behaviors after parturition. RT-qPCR is being used to analyze 5-HT2C in the DR of stressed and unstressed dams. We predict that pregnancy stress will prevent or even reverse the normal peripartum reduction in DR 5-HT2C mRNA expression, which will be associated with less caregiving and increased anxiety. This work could reveal that disruptions in the normative expression of serotonin receptors in the DR across reproduction may contribute to the stress-induced maladaptions in postpartum socioemotional behaviors.

PS2.00139 NERVE GROWTH FACTOR (NGF) RECEPTORS IN THE HYPOTHALAMUS OF AN INDUCED AND A SPONTANEOUS OVULATOR

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Nerve growth factor (NGF) is abundant in seminal plasma and is responsible for inducing ovulation in some species of induced ovulators. To examine the mechanism of action, we compared the distribution of NGF receptors in the hypothalamus of female llamas (induced ovulator) and sheep (spontaneous ovulator). The hypothalamus of llamas (n=5) and sheep (n=4 in the breeding season and n=5 in the non-breeding season) were fixed, and serially cryo-sectioned at 50 um intervals. Immunohistochemistry was performed against P75 and trkA (low- and high -affinity receptors for NGF, respectively), and double immunofluorescence was performed against P75 and vimentin (marker of tanycytes). Cell counts were compared by two-way ANOVA using hypothalamic area and species/season as factors. For both species, trkA immuno-reactivity was found in the diagonal band of Broca, septum and lateral preoptic area, but llamas also displayed immunoreactivity in the periventricular area and the supraoptic nuclei whereas sheep did not. For both species, a population of P75 immuno-reactive cells was detected in the diagonal band of Broca and septum. The number of immunoreactive cells for both P75 and trkA was influenced by an interaction between hypothalamic area and species/season ($P<0.05$). Double immuno-labelling of P75 and vimentin revealed co-localization in the epithelium of the third ventricle and in the organum vasculosum of llamas, but not in

sheep. Results suggest a differential distribution of NGF receptors between species representative of induced vs spontaneous ovulators, and support the hypothesis that NGF acts at the level of the hypothalamus to induce ovulation in llamas.

PS2.0014 UNACYLATED GHRELIN REDUCES HIPPOCAMPAL NEUROGENESIS & IS ALTERED IN PARKINSON'S DEMENTIA

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New neurones are formed from neural stem/progenitor cells (NSPCs) in the adult mammalian dentate gyrus (DG) throughout life. This process termed, adult hippocampal neurogenesis (AHN), is important for learning and memory, however, it is not fully understood. Calorie restriction (CR) has been shown to modulate the DG and improve cognitive function, albeit via unknown mechanisms. Previously, we showed that acyl-ghrelin (AG), which is elevated during CR, increases AHN in the DG and enhances pattern-separation memory. Ghrelin-receptor (GHSR) was expressed on mature DG neurones but wasn't expressed in DG NSPCs of GHSR-eGFP mice, suggesting a non-cell autonomous mechanism of action. We also show that CR enhances AHN in WT but not in GHSR-ko mice, demonstrating that CR induces AHN in a GHSR-dependant manner. Here, to determine whether unacylated-ghrelin (UAG), a so-called inactive form of ghrelin, regulates AHN, WT and GOAT-ko mice - which lack circulating AG - were treated with vehicle or UAG for 7-days (48ug/day i.v). Surprisingly, UAG-treated WT mice had reduced proliferating $Ki67^+$ cells ($p<0.01$), DCX^+ neurones ($p<0.05$) and newborn ($BrdU^+/DCX^+$) neurones ($p<0.01$). GOAT-ko mice had similarly reduced AHN and impairments in hippocampal-dependent memory that were restored by AG-treatment. Finally, we show that circulating levels of AG:UAG in Parkinson's disease dementia (n=8) was significantly reduced compared to both age-matched healthy controls (n=20)($p<0.05$) and a cognitively normal PD group (n=20)($p<0.05$). These data identify a novel role for UAG in regulating hippocampal plasticity and memory, and suggest that AG:UAG may be a biomarker of dementia in humans.

PS2.00140 EFFECTS OF ACUTE PSYCHOSOCIAL STRESS ON LH PULSES, KISS1, AND RFRP-3 IN MALE MICE

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Multiple types of stress, including psychosocial stress, disturb reproductive hormone secretion, but it is not fully understood how or where in the brain this reproductive suppression occurs. Our previous work in ovariectomized female mice demonstrated that short-term restraint stress significantly and rapidly reduces LH pulsatility and *Kiss1* neuronal activation, while increasing RFRP-3 neuron activation. To determine if such restraint stress has sex specific effects on any of these measures, we exposed awake castrated male mice to either 90 min of restraint stress or no stress (controls), during which time we took repeated serial blood samples to measure pulsatile LH secretion. LH pulse frequency, mean LH, and basal LH levels were all significantly decreased in stressed males compared to controls. Next, to determine the effects of acute restraint stress on reproductive neuropeptides known to regulate GnRH neurons, we examined kisspeptin/NKB and RFRP-3 neurons, positive and negative regulators of the reproductive axis, respectively. Castrated male mice were exposed to either 45, 90, or 180 min restraint stress or not stressed (controls). *Kiss1 (*arcuate nucleus) and *Rfrp* (dorsal medial nucleus) expression did not differ between control and stress groups. Current experiments are examining arcuate *Tac2* (NKB) expression as well as *Kiss1* and *Rfrp* neuronal activation. These data indicate that acute restraint stress rapidly disturbs LH pulsatility in both male and female mice. Current experiments will elucidate if the stress-induced suppression of the reproductive axis in male mice is due, at least in in part, to regulation of *Kiss1/NKB* and/or *Rfrp* neuron activation.

PS2.00141 CHRONIC PROLACTIN ADMINISTRATION DOES NOT AFFECT KISSPEPTIN OR OXYTOCIN EXPRESSION IN VIRGIN MICE

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Oxytocin is secreted from the posterior pituitary gland by hypothalamic neurons in the supraoptic and paraventricular nuclei (SON and PVN) and is required for normal parturition. Kisspeptin fibre density surrounding oxytocin neurones increases during pregnancy and we have previously demonstrated that kisspeptin excites oxytocin neurones only in late pregnancy. Kisspeptin and oxytocin neurons express prolactin receptors. Placental lactogen, which acts on prolactin receptors, is elevated in late pregnancy. Thus, we hypothesised that prolactin receptor activation might increase kisspeptin fibre expression to excite oxytocin neurones in late pregnancy. Here, we determined the effect of prolonged prolactin infusion on kisspeptin and oxytocin neurones in virgin mice. Following subcutaneous infusion of ovine prolactin (1500 µg/day at 1µl/hr for seven days) or vehicle (0.01M $NaHCO_3$), kisspeptin and oxytocin immunohistochemistry (IHC) was carried out. There was no significant difference in the mean number of kisspeptin-labelled cells in the hypothalamic periventricular nucleus (58.6 ± 23.7 vs 49.1 ± 11.7, P = 0.20) or in oxytocin-labelled cells in the PVN (139.9 ± 22.8 vs 138.1 ± 19.6, P = 0.47) or SON (48.8 ± 8.9 vs 52.1 ± 2.5, P=0.21). To determine whether there is a change in the kisspeptin fibre density surrounding the PVN and SON following prolactin infusion, confocal images of brain sections double labelled for oxytocin and kisspeptin are being analysed.

PS2.00142 RNASEQ REVEALS TRANSCRIPTOME CHANGES IN MPOA OF WISTAR-KYOTO RAT MODEL OF POSTPARTUM DEPRESSION

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Postpartum depression (PPD) is a severe mental illness that affects millions of mothers and their babies worldwide. However, the molecular mechanisms of postpartum depression are currently unclear. The present study used the Wistar-Kyoto (WKY) genetic rat model of depression, which demonstrates cognitive, motivational, and parenting dysfunctions that are representative of PPD symptomatology, for identification of altered molecular mechanisms involved in the disease phenotype. Following behavioral phenotyping of WKY and control Sprague-Dawley (SD) mother rats, RNAseq transcriptomic analysis was used to identify differentially expressed genes (DEGs) in the medial preoptic area (mPOA), a region that plays a major role in orchestrating cognitive and motivational aspects of parenting. RNAseq revealed 584 DEGs in the postpartum mPOA that had at least a 2-fold-change in expression between WKY and SD mothers, including oxytocin, mitogen-activated protein kinase, the monoamine signaling genes vesicular monoamine transporter 2 (Vmat2) and tyrosine hydroxylase (Th), and the immediate early genes Fos, FosB, and Egr1. Gene Ontology (GO) and enrichment analyses on DEGs identified signaling pathways associated with cellular metabolic and biological processes, including chromatin organization, synaptic plasticity, and response to stress and hormones. An additional ongoing study is examining the impact of gestational stress (a known risk factor for postpartum depression) on depressive phenotype severity and mPOA transcriptional profile of WKY and SD mothers. Together, these results provide additional insight into pathophysiology of depression and its impact on parenting.

PS2.00143 MATERNAL VERSUS PATERNAL CARE BEHAVIORS, FROM GENOME TO PHENOME

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Becoming a parent often requires major changes in an organism's physiology and behavior to promote offspring survival. While research continues to uncover crucial endocrine players responsible for these changes, we know relatively less about the underlying genomic activity driving them. In species in which both parents care for offspring, are the genetic mechanisms facilitating similar maternal and paternal behaviors the same in both sexes, or do they differ? Conversely, do sex-specific behaviors originate from the activity of the same or different genes? To answer these questions, our team characterized how gene activity changes in mothers versus fathers over the course of parental care using the socially monogamous and bi-parental species of the rock dove (*Columba livia*). At eight different time points, we assessed levels of gene transcription in tissues vital for reproduction in vertebrates: the hypothalamus and lateral septum in the brain, the pituitary gland, and the testes and ovaries. We found both similar and

sex-biased changes in gene expression at various time points in genes identified *a priori* for their known role in facilitating parental care. In addition, we uncovered novel targets for further investigations. The results of this large-scale study offer significant insight into the mechanisms driving maternal versus paternal care behaviors, from genome to phenome.

PS2.00144 GLUTAMATERGIC REGULATION OF ESTROGEN SYNTHESIS AND CELL CYCLE GENES IN RADIAL GLIAL CELLS

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Neuroestrogens regulate neurogenesis and sexual behaviour among other critical functions. Currently, little is known about the regulation of neuroestrogen synthesis by neurotransmitters. Teleost fish represent amenable models to elucidate the direct control of neuroestrogen production. Radial glial cells (RGCs) in fish are abundant neural progenitors and are the exclusive site of the expression of *cyp19a1b,* which encodes for aromatase B that synthesizes estradiol (E2) from testosterone. This contrasts mammals, where both neurons and astroglia produce estrogens. Characterization of the goldfish RGC transcriptome revealed that they express only one group of metabotropic glutamate receptors (mGluRs), group III mGluRs, which canonically inhibit protein kinase A (PKA) signalling. Using qPCR, expression of *cyp19a1b* and cyclin D1 *(ccdn1)*, a critical regulator of cell proliferation and differentiation, were measured in cultured female goldfish RGCs following 48 hour *in vitro* pharmacological treatments to determine the involvement of glutamate in regulating RGC function. L-AP4, a group III mGluR agonist, down-regulated *cyp19a1b* and *ccdn1* mRNA levels. Forskolin, a PKA signal inducer, reversed the inhibitory effect of L-AP4. Pre-treatment with CPPG, a group III mGluR antagonist, blocked the inhibitory effects of L-AP4 on *cyp19a1b* and *ccdn1*, indicating a receptor-mediated mechanism. Given that the expression of both *cyp19a1b* and *ccdn1* ceases as RGCs transition to neurons, the inhibitory effect of group III mGluRs activation suggests that glutamate regulates critical RGC functions. The origin of glutamatergic inputs and the context in which they control RGCs remains to be determined. (Funded by NSERC and uOttawa).

PS2.00145 INCREASED LH PULSATILITY AND ARCUATE KISSPEPTIN GENE EXPRESSION IN A MOUSE MODEL OF PCOS

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Polycystic ovary syndrome (PCOS) is characterized by hyperandrogenemia, chronic anovulation, and polycystic ovaries but the pathophysiology of this condition is not completely understood.

We recently reported a new mouse model of PCOS using letrozole (LET), an aromatase inhibitor, which recapitulates multiple phenotypes of PCOS, including polycystic ovaries and elevated levels of LH and testosterone. However, that study did not assess *in vivo* LH pulsatile secretion (which is elevated in PCOS women), relying instead on single "one-off" measures of LH. Here we examined LH pulses of LET female mice and compared them to placebo-treated mice as well as chronically-ovariectomized (OVX) mice. In addition, brains were examined for kisspeptin gene (*Kiss1*) expression in the arcuate nucleus. We found that LET mice have dramatically increased LH pulsatility, with more frequent pulses and higher basal levels than placebo mice. Interestingly, while LH pulse frequency and basal LH levels are similarly elevated between LET and OVX females, LH pulse amplitude is notably smaller in LET females. Brain analysis determined that LET females have markedly elevated arcuate *Kiss1* levels compared to placebo females but not as high as *Kiss1* in OVX females. Lastly, we used sensitive LCMS to measure for the first time E2 levels in LET mice and found lower E2 in LET than placebo mice. Collectively, these findings demonstrate that, like PCOS women, LET female mice have elevated LH pulsatility, which may help drive the elevated androgens in these conditions. Additionally, increased neural kisspeptin synthesis may be a key contributor to the elevated pulsatile LH secretion in the LET PCOS-like condition.

PS2.00146 OPTOGENETIC ACTIVATION OF POMC TERMINALS IN THE MEDIAL PREOPTIC NUCLEUS REGULATES LORDOSIS BEHAVIOR

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Lordosis, the stereotypical behavior of female sexual receptivity, is elicited by estrogen and progesterone signaling in a limbic-hypothalamic circuit, which integrates sensory and hormonal information. Estradiol acts through membrane receptors to rapidly activate a part of this circuit involving the arcuate (ARH), medial preoptic (MPN), and ventromedial nuclei of the hypothalamus. Activation of this sub-circuit is necessary for full lordosis behavior. Previous work has indicated that estradiol-dependent beta-endorphin release in the MPN from ARH pro-opiomelanocortic (POMC) neurons is required for full lordosis behavior. Beta-endorphin release activates mu opioid receptors (MOR), resulting in a transient, but necessary, inhibition of lordosis behavior. To functionally dissect this circuit, we tested the effect of activating POMC axon terminals in the MPN in sexually receptive mice. Female POMC-Cre mice were ovariectomized and channel rhodopsin 2 (ChR2) was delivered via an adeno-associated vector (AAV) bilaterally to the ARC, followed by ferrule fiber implantation into the MPN. Mice were primed with estrogen and progesterone prior to behavioral testing for sexual receptivity, which was assessed by measuring the lordosis quotient (number of times a female exhibits lordosis when mounted by a male, divided by total number of mounts). Following an initial trial for sexual receptivity, blue light was delivered for the duration of the lordosis test. Immunohistochemistry was run to evaluate MOR internalization in the MPN. Our results show

that optogenetic activation of POMC terminals in the MPN attenuates lordosis behavior, thus indicating that endogenous opioid inhibition of sexual receptivity requires the ARH POMC input to the MPN.

PS2.00147 MAPPING NEURONAL INPUTS INTO KISS1 NEURONS IN THE ARCUATE.

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Kisspeptin neuropeptides, encoded by the *Kiss1* gene, are key regulators of the mammalian reproductive axis and are required for both puberty and ovulation by stimulating GnRH release. *Kiss1* neurons are located in two main areas of the hypothalamus: the arcuate (ARC) region, which regulates basal GnRH pulsatility and the anteroventral periventricular (AVPV) region, which controls the preovulatory LH surge One of the fundamental steps in understanding how the reproductive axis is co-ordinated with other physiological processes is an accurate description of the neuronal circuitry communicating with *Kiss1* neurons. We have used a Kiss-CRE mouse line to undertake conditional viral tracing with genetically modified pseudorabies viruses to define afferent neuronal inputs to *Kiss1* neurons. Several of these neuronal populations have been implicated as physiologically relevant in controlling the reproductive axis. These include the suprachiasmatic nucleus, which communicates information about day length; the subfornical organ, which provides information about peripheral metabolic status; the amygdala, which responds to pheromone signals and POMC and NPY neurons in the ARC, which regulate feeding behaviour. We are currently studying these connections to determine whether they are primary or secondary inputs to *Kiss1* neurons and are defining the functional relevance of these.

PS2.00148 HORMONAL REGULATION OF BMPS (BONE MORPHOGENETIC PROTEINS) GENES TRANSCRIPTION IN RAT PITUITARY

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Bone morphogenetic proteins, a group of signaling molecules that belongs to TGFβ superfamily, are involved in pituitary gonadotropic activity We tested how central and peripheral stimulation might affect selected BMPs transcription *in vivo*. Ovariectomized 4 month old rats received intracerebroventricular (1 pulse /h/over 5h) microinjections of 1.5 nM GnRH; 2 nM GnRHR antagonist + 1.5 nM GnRH; 0.9% NaCl (controls) or, for three consecutive days, subcutaneous injections of 17β-estradiol (20 µg/0.2 ml DMSO), PPT (ESR1 agonist; 0.5 mg/0.2 ml DMSO), DPN

(ESR2 agonist; 0.5 mg/0.2 ml DMSO, and in controls, 0.2 ml DMSO. qRT PCR analysis was applied to determine BMP2, BMP4, BMP6, BMP7, BMP15, BMPRIA, BMPRIB, BMPR2, Smad1, Smad5, Smad8 mRNA expression in anterior pituitary gland. As a positive control for central/peripheral inputs, FSHβ, and GnRHR transcription levels were also evaluated. GnRH up-regulated BMP2 (by 60%), BMP-15 (by 35%) and BMPR-IA (by 41%) mRNA expression and increased BMPs signaling pathway transcription factors mRNA levels: Smad1 by 31% whereas Smad5 by 25%., respectively. In contrast, estrogenic stimulation down-regulated BMPs system activity.17β-estradiol, acting via ESR1, decreased BMP2 and BMP-6 mRNA level (by 56% and 26%, respectively) whereas down-regulation of BMP-15 transcription down-regulation (by 35%) required ESR1and ESR2 activation.17β-estradiol reduced also BMPR-1A (by 32%) and BMPR2 (by 27%) mRNA expression, both in ESR1-dependent manner. ESR1 and ESR2 were involved in estrogen-induced decrease of Smad5 transcription. In conclusion, obtained data suggest that BMPs signaling system activity depends on central and peripheral inputs exerted at the BMPs network transcriptional level in a gene-specific manner.

PS2.00149 CONTEXTUAL FEAR LEARNING AND MEMORY IN ALTERNATIVE STRESS COPING STYLES

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Animals frequently must overcome stressors and the ability to encode and recall these salient experiences is essential to an individual's survival. Across many taxa, studies have documented two alternative stress coping styles (proactive and reactive) that differ in behavior, cognition, stress physiology, and underlying neuromolecular mechanisms. The role of stress in cognitive traits (e.g. learning and memory) has been well documented, however, the influence of an animal's stress coping style on learning and memory capabilities is only beginning to be understood. Here, we developed a contextual fear learning paradigm to characterize learning and memory differences between proactive and reactive stress coping styles. Specifically, we trained zebrafish to associate an antipredatory response with a conditioned context (CS) using alarm substance as an unconditioned stimulus (US). Zebrafish with the reactive stress coping style acquired the fear memory at a significantly faster rate than proactive fish over four CS-US pairings. While both stress coping styles showed equal memory recall one day post-training with no significant difference in freezing time, reactive zebrafish showed significantly higher levels of freezing relative to proactive fish four days post-training. Altogether we find that an animal's stress coping style is closely linked to learning and memory capabilities. Specifically, how individuals cope with stress may also promote differences in information processing, decision making, and how salient experiences are encoded and recalled (e.g. cognitive biases).

PS2.0015 IS BREEDING-STATUS DEPENDENT NEURAL DIFFERENTIATION IN DAMARALAND MOLE-RATS CONTEXT-DEPENDENT?

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Damaraland mole-rats (DMR; *Fukomys damarensis*) are one of two mammalian species that are eusocial. This social system consists of overlapping generations of adults, and only a single breeding pair within the colony, the remaining members are non-reproductive, but support reproduction of the breeders. Unlike eusocial insects, subordinate DMR retain the capacity to become breeders throughout their lifetime if they are paired with an opposite-sex, unfamiliar individual. As compared to other mammals, sexual differentiation of the central nervous system (CNS) is greatly attenuated. Interestingly, DMR do exhibit status-dependent differentiation of the CNS. Thus, the brains of breeders, both male and female, differ from non-breeders. The brain nuclei that differ based on social/reproductive status, include the principal nucleus of the bed nucleus of the stria terminalis (BNST) and the paraventricular nucleus of the hypothalamus (PVN), which are among the same nuclei that are subject to sexual differentiation by gonadal steroids in other rodents. Steroid-dependent sexual differentiation occurs during a critical phase of sensitivity and is permanent. It remains unknown whether the social/reproductive status-dependent brain differentiation in DMR is permanent or reversible. We addressed this hypothesis by altering the social/breeding status of individual DMR, some were paired with opposite-sex individuals permanently for 3 months, while others were paired for 3 months but then returned to their natal colony for an additional 3 months. We then analyzed the volume of the BNST and PVN in each group. The results will indicate whether the neural changes are plastic or permanent.

PS2.00150 NEUROMOLECULAR MECHANISMS OF ALTERNATIVE STRESS COPING STYLES

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Animals experience stress in many contexts and often successfully cope. Many studies have documented two alternative stress coping styles (proactive and reactive) across a diverse range of taxa. Suites of correlated behavioral and physiological responses uniquely characterize each coping style. With evidence that these associated responses are heritable and have distinct genetic architectures, a fundamental goal is to understand how neural and molecular mechanisms interact to facilitate the display of proactive and reactive stress coping styles. Using RNA-sequencing, qPCR, and bioinformatic analyses we characterize the basal neurotranscriptome profiles of zebrafish lines selectively bred to exhibit the proactive and reactive stress coping styles. We show that a core set of genes linked to differences between

stress coping styles are associated with (1) neurometabolic activity and synaptic plasticity and (2) the magnitude of the behavioral response. To identify underlying neural mechanisms, we compared the expression of an immediate early gene and candidate genes in several brain regions between individuals coping with stress and controls. Our results expand the molecular mechanisms of stress coping from classic neurotransmitter systems to include a potentially significant role of neurometabolic activity. We also posit how neural activity across and within brain regions may lead to expression of a stress coping style. Altogether our studies begin to elucidate neuromolecular mechanisms underlying how animals cope with stress.

PS2.00151 METYRAPONE UNEXPECTEDLY ACTIVATES FKBP5 GENE TRANSCRIPTION IN THE HIPPOCAMPUS

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During the acute stress response, corticosterone (cort) is secreted from the rat adrenal gland and binds to two distinct receptor subtypes in the hippocampus; the mineralocorticoid receptor (MR) and glucocorticoid receptor (GR). Activated MRs and GRs bind to glucocorticoid response elements (GREs) on DNA, thereby inducing transcriptional changes in glucocorticoid target genes (Mifsud & Reul (2016) PNAS). Metyrapone inhibits 11-beta-hydroxylase, thereby inhibiting synthesis of cort from deoxycorticosterone in the adrenal gland. As such, it has been used to induce a chemical form of adrenalectomy (ADX). We investigated the effects of metyrapone administration on the stress-induced plasma cort response, MR-/GR- binding to GREs within the glucocorticoid target gene FK506-binding protein 5 (*Fkbp5*), and heteronuclear *Fkbp5* transcriptional (hnRNA) changes in the hippocampus of intact rats. We also assessed similar parameters in ADX rats. In intact rats, exposure to forced swimming (FS) resulted in significantly elevated plasma cort levels, and increased hippocampal MR-/GR-binding at *Fkbp5* GREs and *Fkbp5* gene transcription. Administration of metyrapone reduced the FS-induced cort response and inhibited FS-induced MR-/GR- binding to *Fkbp5* GREs but, surprisingly, significantly enhanced *Fkbp5* gene transcription. Furthermore, metyrapone significantly increased *Fkbp5* transcription under baseline conditions. ADX abolished FS-induced cort preventing both the binding of MRs and GRs to *Fkbp5* GREs and the surge in *Fkbp5* hnRNA. In view of these unexpected results, metyrapone is not a good substitute for surgical ADX due to off target effects on glucocorticoid responsive genes.

PS2.00152 ACUTE STRESS-INDUCED FKBP5 GENE ACTIVATION IN THE BRAIN-PITUITARY-ADRENAL STRESS AXIS

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Activation of the hypothalamic-pituitary adrenal (HPA) axis after acute stress triggers the secretion of glucocorticoid (GC) hormones which bind to mineralocorticoid (MRs) and glucocorticoid receptors (GRs) in the rat hippocampus. MRs and GRs initiate changes in gene transcription of FK506-binding protein 5 (*Fkbp5*) by binding to GC response elements (GREs) within *Fkbp5*. At present, it is unclear how the*Fkbp5* gene in the brain, pituitary and adrenals is regulated by MRs/GRs after acute stress. Understanding the regulation of this gene is important as it is involved in maintaining MR/GR activity within healthy limits. Genomic variations in the gene have been linked to increased risk of developing stress-related disorders. In this study, we examined MR and GR binding to *Fkbp5* GREs as well as *Fkbp5* mRNA expression in brain (hippocampus, amygdala, hypothalamus, prefrontal cortex and neocortex), pituitary and adrenal tissues under baseline and acute stress conditions in male rats. Forced swimming (FS) resulted in increased binding of both MRs and GRs at an *Fkbp5* GRE within intron 5 throughout the brain, peaking at 30 min after FS. This was accompanied by brain-wide elevation of *Fkbp5* mRNA levels, which peaked at 2h after FS. Experiments on hypothalamic, pituitary and adrenal MR/GR binding to *Fkbp5* GRE and *Fkbp5* mRNA are in progress. In various brain regions, enhanced binding of MR/GR was also found at the circadian peak in GC levels. This data demonstrates that acute stress results in enhanced MR-/GR-binding to *Fkbp5* GRE triggering increased *Fkbp5* transcription throughout the brain-pituitary-adrenal stress axis.

PS2.00153 CHRONIC VARIABLE STRESS AFFECTS RESPONSE OF HYPOTHALAMUS-PITUITARY-AXIS TO COLD EXPOSURE

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The hypothalamus-pituitary-thyroid (HPT) axis is involved in energy homeostasis. It is activated by a drop in external temperature and inhibited by stress; a previous stress exposure or corticosterone injection blunts cold-induced activation of the HPT axis. Certain types of chronic stress inhibit also the HPT axis, but their effects on the axis response to acute energy demanding stimuli are unknown (1,2). The aim of this work is to evaluate if chronic variable stress exposure interferes with the response of the HPT axis to cold. Adult Wistar male rats were subjected to 15 days of Variable Chronic Stress (VCS) at 75 days of age. On day 16, at 10:00 am, animals were exposed for 1h to 4ºC or 22ºC. Compared to naïve animals, VCS decreased food intake; thus, controls were pair-fed. VCS increased serum corticosterone

concentration up to the final day, with no habituation. Cold exposure increased paraventricular hypothalamic nucleus thyrotrophin-releasing hormone and corticotrophin-releasing hormone receptor-1 expression in controls but not in VCS. Serum concentration of triiodothyronine (T3) diminished in VCS rats exposed to RT or cold; cold did not stimulate T3 release. Only in controls, cold tended to increase thyrotrophin (p=0.056). The thermogenic organ, brown adipose tissue (BAT), showed increased expression of uncoupling protein-1 in cold-exposed controls but not in VCS. These results corroborate hypothalamus-pituitary-adrenal hyperactivity by VCS, and support the inhibitory effect of stress on HPT response to cold stimulation. Dysfunction of the HPT axis response may contribute to altered energy homeostasis. Funding: DGAPA IN204316. ACC,CONACyT-scholarship

PS2.00154 ACUTE SWIM STRESS ALTERS STEROID SYNTHESIS IN THE RAT BRAIN IN A REGION AND SEX-DEPENDENT MANNER

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A liquid chromatography-mass spectrometry method was developed to quantify a panel of 8 neurosteroids in the brain: corticosterone, pregnenolone, deoxycorticosterone (DOC), dihydrodeoxycorticosterone (DHDOC), progesterone, dihydroprogesterone (DHP), allopregnanolone and testosterone. The method showed good linearity, sensitivity and precision, and was used to determine steroid concentrations in plasma and 5 brain subregions (frontal cortex, hypothalamus, hippocampus, amygdala and brainstem) from male and female rats killed under basal conditions or 30 min after acute stress exposure (2 min forced swimming). Corticosterone, DOC and progesterone concentrations were significantly greater in the plasma and brain of both sexes following stress; however the response in plasma was exaggerated in females compared to males. This sex difference was also observed in the majority of brain regions for DOC and progesterone, but not corticosterone. Despite observing no changes in plasma concentrations of pregnenolone and the 5-alpha reduced steroids, DHDOC and DHP after stress, concentrations were greater in the brain, and this effect was more pronounced in females compared to males. Basal plasma and brain concentrations of allopregnanolone were significantly higher in females, compared to males. Moreover, following stress a significant increase in allopregnanolone was observed only in the females. Testosterone was undetectable in females, and stress had no significant effect on circulating or brain concentrations in either sex. Together these data indicate local regulation of neurosteroids in the brain following acute stress, especially for 5alpha- and 3alpha-hydroxysteroid dehydrogenase-reduced steroids. Furthermore, the greater neurosteroid response to stress in females, suggests sex-specific expression of steroidogenic enzymes in the brain.

PS2.00155 INHIBITORY EFFECTS OF AN EGFR INHIBITOR ON ACTH PRODUCTION AND PROLIFERATION OF ATT-20 CELLS

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Cushing's disease is primarily caused by hypersecretion of adrenocorticotropic hormone (ACTH) from a pituitary adenoma. Mutations in the deubiquitinase gene *USP8* have been found in human ACTH-producing pituitary adenoma cells. The mutational hotspot hyper-activates USP8, enabling it to rescue epidermal growth factor receptor (EGFR) from lysosomal degradation and ensure its stimulatory signaling in Cushing's disease. An EGFR inhibitor would be effective for anti-tumor in the EGFR-related tumors. Pituitary tumor-transforming gene 1 (PTTG1), a hallmark of pituitary tumors, stimulates pituitary cell proliferation. The stress response growth arrest and DNA damage-inducible 45b (GADD45β) and Cdk5 and ABL enzyme substrate 1 (CABLES1) are novel pituitary suppressors whose expression blocks proliferation, survival, and tumorigenesis. In the present study, we determined the effect of a potent EGFR inhibitor, lapatinib, on ACTH production and cellular proliferation in mouse AtT-20 corticotroph tumor cells. Lapatinib decreased proopiomelanocortin mRNA levels in AtT-20 cells and reduced ACTH levels in the culture medium of these cells. Drug treatment also decreased AtT-20 cell proliferation, and induced apoptosis. Lapatinib decreased PTTG1 mRNA levels, and increased both GADD45β and CABLES1 mRNA levels. GADD45β or CABLES1 knockdown partially inhibited the lapatinib-induced decrease in cell proliferation. GADD45β and CABLES1 would be involved in lapatinib-induced decreases in cellular proliferation.

PS2.00156 REGULATION OF CORTICOTROPIN-RELEASING FACTOR GENE EXPRESSION BY RELAXIN-3 IN RAT HYPOTHALAMIC CELLS

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Relaxin-3, the ancestral member of relaxin/insulin superfamily, is an important regulator of food intake, memory, and gonadotropin-releasing factor. Relaxin-3 has a high binding affinity to its cognate receptor of relaxin-family peptide receptor 3 (RXFP3), and it also binds to RXFP1. RXFP1 and RXFP3 are strongly expressed in the paraventricular hypothalamic nucleus (PVN), which is a main center of stress response. In the PVN, corticotropin-releasing factor (CRF), which plays a central role in regulating the stress response and is produced in response to stress, stimulates the release of adrenocorticotropic hormone from the anterior pituitary. Therefore, relaxin-3 has been suggested to implicate in a stress response. We hypothesized that relaxin-3 regulates CRF gene expression directly in the hypothalamus, and thus examined the direct effect of relaxin-3 on the promoter activity and mRNA levels of CRF in hypothalamic cells. To examine these pathways, we used hypothalamic 4B cells, a homologous PVN neuronal cell

line. Both RXFP1 and RXFP3 mRNA, and their proteins were expressed in the hypothalamic cells. Relaxin-3 stimulated CRF mRNA levels and CRF promoter activity directly in 4B cells following their transfection with the CRF promoter. Protein kinase A and protein kinase C inhibitors suppressed the relaxin-3-induced increases in CRF promoter activity. Further studies are required to elucidate the involvement of relaxin-3 on stress mechanism.

PS2.00157 S-ACYLATION CONTROLS CRH-INDUCED CALCIUM SIGNALLING IN ANTERIOR PITUITARY CORTICOTROPHS

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Anterior pituitary corticotroph cells, the central hub of the hypothalamic-pituitary-adrenal (HPA) axis, are essential for the neuroendocrine response to stress. S-acylation is a dynamic post-translational lipid modification that controls the properties and function of many proteins including receptors and ion channels that control corticotroph excitability. S-acylation is enzymatically controlled by the zDHHC family of S-acyl-transferases (zDHHC family) that are differentially expressed in corticotrophs. In this project, we have tested the hypothesis that S-acylation mediated by zDHHC23, which is highly expressed in corticotrophs, is important in regulation of corticotroph Ca^{2+} signalling. To address this, isolated mouse anterior pituitary cells were transduced with lentivirus to express the genetically-encoded calcium indicator GCaMP6s under the control of a minimal POMC reporter, which allowed specific labelling of mouse corticotrophs in vitro for calcium imaging experiments. We investigated CRH- and AVP- induced Ca^{2+} signalling in both male and female corticotrophs from wild-type mice and mice with a genetic deletion of zDHHC23. In isolated male cortciotrophs, CRH (200pM) and AVP (2nM) evoked robust calcium responses in wild-type corticotrophs. However, genetic deletion of zDHHC23 (zDHHC23-KO) had no significant effect on either CRH- or AVP-and induced calcium signals. In contrast, in female cortciotrophs, CRH-, but not AVP-, evoked calcium signals were significantly attenuated in corticotrophs from zDHHC23-KO mice compared to the wild-type. This reveals a sexually dimorphic role for zDHHC23 in corticotroph physiology through the regulation of CRH-, but not AVP-signalling pathway in females. The molecular mechanisms for zDHHC23 mediated control of CRH-induced calcium signalling remain to be determined.

PS2.00158 HYPOTHALAMIC NMDA RECEPTOR CONTRIBUTES TO HYPERACTIVITY OF HPA AXIS IN CHRONIC STRESS

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Chronic stress stimulates corticotrophin-releasing hormone (CRH)-expressing neurons in the paraventricular nucleus (PVN) of the hypothalamus and leads to hypothalamus-pituitary-adrenal (HPA) axis hyperactivity, but the mechanisms underlying this action are unknown. Because chronic stress enhances *N*-methyl-D-aspartate receptor (NMDAR) activity in various brain regions, we hypothesized that augmented NMDAR activity contributes to the hyperactivity of PVN-CRH neurons and the HPA axis in chronic stress. We performed whole-cell patch-clamp recordings on PVN-CRH neurons expressing CRH promoter-driven enhanced green fluorescent protein in brain slices from rats exposed to chronic unpredictable mild stress (CUMS) and unstressed rats. CUMS rats had significantly higher expression levels of the NMDAR subunits GluN1 in the PVN than unstressed rats. Furthermore, puff NMDA-elicited currents, evoked NMDAR currents, and the baseline frequency of the miniature excitatory postsynaptic currents (mess) in PVN-CRH neurons were significantly larger in CUMS rats than in unstressed rats. The NMDAR-specific antagonist AP5 significantly decreased the frequency of mEPSCs of PVN-CRH neurons in CUMS rats but did not change the frequency or amplitude of mEPSCs in unstressed rats. Bath application of AP5 normalized the elevated firing activity of PVN-CRH neurons in CUMS rats but not in unstressed rats. In addition, microinjection of the NMDAR antagonist memantine into the PVN normalized the elevated corticosterone levels in CUMS rats to the levels in unstressed rats, but did not alter corticosterone levels in unstressed rats. Our findings suggest that synaptic NMDAR activity is enhanced in CUMS rats and contributes to the hyperactivity of PVN-CRN neurons and the HPA axis.

PS2.00159 CORTICOSTEROID RECEPTOR BINDING TO GLUCOCORTICOID TARGET GENES IN THE RAT HIPPOCAMPUS AFTER STRESS

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Glucocorticoids (GCs), secreted after stress or during the circadian rise, act on the brain through binding to mineralocorticoid (MRs) and glucocorticoid receptors (GRs). MRs and GRs are then thought to bind to GC response elements (GREs) within GC target genes (e.g. FK506-binding protein 5 (*Fkbp5*), serum/GC-regulated kinase 1 (*Sgk1*), Period 1 (*Per1*)) to evoke changes in gene transcription. Until recently, this had not been studied under physiological conditions *in vivo*. Male Wistar rats were killed under baseline conditions or at various time points after exposure to stress and hippocampus tissue collected for chromatin immuno-precipitation (ChIP) to assess MR and GR binding to GREs within target genes or for RNA analysis by qPCR. In a separate experiment, rats were subjected to adrenalectomy (ADX) to remove endogenous corticosterone and the effect of ADX, with or without corticosterone replacement, on MR and GR binding to target genes assessed up to 12 weeks later. Different stressors resulted in similar levels of receptor binding to GREs, indicating that above a certain GC threshold these responses are independent of GC levels. MR and GR to GRE binding, as well as their binding as homo- versus heterodimers, was found to be very GRE- and gene-dependent and resulted in

associated changes in gene transcription. Long-term ADX changed the binding pattern of both MR and GR binding to target genes in a gene-specific manner. These findings highlight the complexity of GC receptor action, revealing new layers of regulatory control and opportunities for future investigation. Supported by BBSRC, UK

PS2.0016 SEX & COUPLES: BIPARENTAL CARE DOESN´T ALTER THE SEXUAL BEHAVIOR IN THE MONOGAMOUS PRAIRIE VOLE

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Most modern societies are increasing the engagement of the father in the raise of the children resulting in behavioral, emotional and cognitive benefits for the progeny. However, the neurobiological effects of biparental care are not well understood. *Microtus ochrogaster*, the prairie vole, is a good animal model for studying biparental care (BP) because they build a strong pair bond and share the care of the offspring. Voles raised only by their mother (monoparental, MP) need more time as adults to build a pair bond. We evaluated if the delay in pair bond formation is related with alterations in olfaction and/or sexual behavior. Our results show that BP pups were more frequently licked ($p<0.05$, $F_{1,15}= 4.778$) and displayed their ears earlier ($p<0.05$; Postnatal Day 3) than MP voles. When adult, both groups of male and female voles were able to discriminate between different odors ($p<0.001$) but MP females didn´t show preference for male-soiled bedding ($F_{2,18}=1.779$, $p>0.05$) as biparental females did ($F_{2,18} =14.333$, $p<0.001$). Our data showed no differences in the anogenital sniffing or the lordosis quotient between groups, but BP females received less ejaculations during the test ($F_{1,8}=9,224$, $p<0.05$). No differences were found between male groups in their preference for female-soiled bedding or their sexual behavior with receptive females. Our experiments demonstrate that monoparental raising decreases the preference for male odors in the females but doesn´t impair mating in male or female voles. ACKNOWLEDGMENTS: CONACYT252756,253631; FRONTERAS374; IN203615,210215; INPER212503230-21216-05-15; NIHP51OD11132

PS2.00160 EFFECTS OF SALT LOADING ON OSMOTIC DETECTION BY RAT MAGNOCELLULAR VASOPRESSIN NEURONS

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High dietary salt intake (HDSI) is a major risk factor for hypertension and is strongly correlated with the incidence of cardiovascular diseases and stroke. Increases in osmotic pressure due to increased plasma sodium levels are detected by osmosensitive neurons in the hypothalamus, called osmoreceptors. Osmoreceptors in the organum vasculosum laminae terminalis (OVLT) send an excitatory projection to the supraoptic nucleus (SON) and activate specialized magnocellular neurosecretory cells (MNCs), which are also intrinsically osmosensitive. These MNCs project to the neurohypophysis to release vasopressin (VP) into the circulation. Recent studies have demonstrated that exposure of rats to HDSI results in excessive activation of MNCs, leading to VP-mediated increases in blood pressure. Although this effect is associated with a reduction in the efficacy of inhibitory synaptic signaling by baroreceptors, it remains possible that a facilitation of osmoreceptor signaling and/or intrinsic osmoresponsiveness also contribute to this process. In this study, VP-eGFP Wistar rats were subjected to a 7-day salt-loading (SL) period in which their drinking water was replaced with 2% NaCl. Whole cell patch-clamp recordings of SON neurons were performed in both horizontal acute slice and in isolated cell preparations. Current clamp analysis revealed that the excitatory response of VP MNCs to a hypertonic stimulus was enhanced following HDSI. while voltage clamp analyses revealed no sensitization of afferent inputs. Ongoing analyses are investigating changes in intrinsic osmoresponsiveness of VP MNCs to mechanical stimulation following SL.

PS2.00161 REMODELLING OF THE ANTERIOR PITUITARY ION CHANNEL LANDSCAPE IN CHRONIC STRESS

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Chronic stress (CS) represents a huge burden on health and society. In the UK, stress-related illness accounts for 40% of work-related illness and costs the economy an estimated £6.5 billion per year. Following acute stress, glucocorticoids are beneficial. However, CS is associated with both desensitization of the HPA axis to glucocorticoid negative feedback, as well as hypersensitivity to novel stressors. Corticotrophs of the anterior pituitary are electrically excitable and stimulation with hypothalamic neuropeptides CRH and AVP results in a characteristic increase in excitability that can be suppressed by acute glucocorticoid treatment. For example, CRH induces a transition from spiking to bursting activity which raises intracellular calcium and is proposed to drive secretagogue-evoked ACTH secretion. To test the effect of CS, mice were subjected to a 14 day CS paradigm using a daily restraint stress. We reveal that both CRH- and CRH/AVP-evoked secretion is enhanced in isolated corticotrophs from chronically stressed male mice compared to their non-stressed controls. The effects are independent of ACTH content suggesting that hypersensitivity is at the level of secretagogue-evoked stimulus-secretion coupling. CS induces significant changes in anterior pituitary mRNA expression encoding ion channel pore- and regulatory-subunits. CS induced significant up- or down-regulation of mRNAs for a variety of pore-forming and regulatory subunits that encode for

voltage-gated calcium channels, voltage-gated and calcium-activated potassium channels, as well as other potassium-selective and non-selective cation conductances. Taken together, we predict that hypersensitivity is due to enhanced corticotroph excitability, which could explain elevated ACTH secretion in CS mice.

PS2.00162 METHYLATION IN ER GENES, ASSAY VALIDATION AND FINDINGS FROM THE WOMEN HEALTHY AGING STUDY

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Levels of estrogen receptors (ERs), mediating the estrogen signaling, have been repeatedly positively associated with attenuated anxiety- and depression- like behavior and are therefore likely to play a role in estrogen related psychopathology. Several studies indicate that methylation on various CpGs within the estrogen receptor genes (*ESR1*, *ESR2* and *GPER*) is associated with ERs gene expression and/or psychopathology, while aging, estradiol (E2) deprivation and early life adversities (ELA) have been shown to modulate methylation on a number of these CpGs. So far, only few of these CpGs were assessable with the commercially available Illumina BeadChips and a targeted bisulfite sequencing assay considering all these CpGs was missing. Therefore, the present study aimed at establishing a cost-effective targeted bisulfite next generation sequencing (NGS) approach to undertake a comprehensive analysis of all the above mentioned CpGs, using dried blood spots (DBS) DNA. This assay accurately assessed the 129 target CpGs with high sequencing depth and showed consistency with methylation values from fresh whole blood DNA (GSE 40279), validating our application of the DBS technology. The assay as well as findings from the women 40+ aging study (N=120), associating ERs genes methylation with E2 levels, age and ELA will be presented at the conference.

PS2.00163 EFFECT OF L-ARGININE AND D-RIBOSE SUPPLEMENTATION ON HORMONES IN PERIMENOPAUSAL RATS AFTER STRESS

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Perimenopause involves dynamic neuroendocrine reactions that promote exacerbation of stress responses. Female 28-day old rats were divided into 3 groups; Control injected subcutaneously with Corn oil (2.5 µl/g BW); VCD injected subcutaneously with 4-

vinylcyclohexene diepoxide (160 mg/kg BW) diluted in Corn oil (2.5 µl/g BW) both for 15 days; and Aged group was left to age naturally till 150 days. Seven weeks after VCD/corn oil administration, and 150 days in Aged group, rats were further divided into 3 sub-groups: L-Arginine (100mg/kg BW), D-ribose (200mg/kg BW) and a third group received neither for additional 30 days. At 130 days of age in Control and VCD groups., 180 days of age in Aged group on diestrus morning, animals were subjected to acute restraint stress for 30 minutes. Blood samples were drawn before and after stress from the retro-orbital sinus for measurement of serum prolactin, LH, FSH, progesterone, and corticosterone using radioimmunoassay technique. In unstressed and stressed animals, L-Arginine and D-ribose significantly increased ($p < 0.05$) progesterone and corticosterone concentrations in Aged and VCD groups compared to Control group. D-ribose significantly increased ($p < 0.05$) FSH, LH, and Prolactin concentrations in Aged and VCD groups compared to Control group. L-Arginine significantly decreased ($p < 0.05$) FSH and LH concentrations in Aged and VCD groups compared to Control group. L-Arginine significantly increased ($p < 0.05$) prolactin concentrations in unstressed animals and also significantly decreased ($p < 0.05$) it in stressed animals in the Aged and VCD groups compared to Control group.

PS2.00164 ANTI-OXIDANTS AMELIORATE NORADRENALINE AND SEROTONIN CONTENTS IN PERIMENOPAUSAL RATS AFTER STRESS

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The decreased antioxidant defense characteristic of perimenopause leads to a decrease in stress resilience upon exposure to external stressors. Female 28-day old rats were divided into 3 groups; Control (injected, Corn oil 2.5 µl/g BW); VCD (injected, 4-vinylcyclohexene diepoxide 160 mg/kg BW diluted in Corn oil) both for 15 days; and Aged group allowed to age naturally till 150 days. Seven weeks after VCD/corn oil administration, and 150 days in Aged group, rats were further divided into 3 sub-groups: L-Arginine (100mg/kg BW), D-ribose (200mg/kg BW) and a third group received neither for additional 30 days. At 130 days in Control and VCD groups., and 180 days in Aged group on diestrus morning, animals were subjected to acute restraint stress for 30 minutes. Blood samples were drawn before and after stress from the retro-orbital sinus for measurement of serum noradrenaline and serotonin concentrations using HPLC technique. Ovaries were processed through HPLC for assessment of noradrenaline and serotonin contents. In unstressed and stressed animals, D-ribose significantly decreased ($p < 0.05$) serum and ovarian noradrenaline, serum serotonin in Aged and VCD groups compared to Control group. It also significantly increased ($p < 0.05$) ovarian serotonin in Aged and VCD groups compared to Control group. In unstressed animals, L-Arginine significantly decreased ($p < 0.05$) serum noradrenaline, serum and ovarian serotonin in Aged and VCD groups compared to Control group. In stressed animals, L-Arginine significantly increased ($p < 0.05$) serum noradrenaline and ovarian serotonin in Aged and VCD groups compared to Control group.

PS2.00165 SALT LOADING PROMOTES SYNCHRONIZATION OF PHASICALLY FIRING NEURONS OF THE SUPRAOPTIC NUCLEUS.

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The antidiuretic hormone vasopressin (VP) is synthesized by magnocellular neurosecretory cells located in the hypothalamic paraventricular and supraoptic nuclei (SON) which project axons to the neurohypophysis. VP neurons increase their action potential firing rate in proportion with extracellular fluid osmolality to progressively increase VP secretion into the bloodstream. Under pronounced hyperosmotic conditions the electrical activity of these neurons changes to a type of bursting pattern called "phasic" to facilitate VP release. Phasic activity is defined by groups of spikes interspersed by silent pauses (each lasting >1 s) (Poulain & Wakerley, 1982). In acute hypertonic conditions *in vivo*, spontaneous phasic activity is asynchronous among VP cells. However, it remains unknown whether phasic firing remains asynchronous following chronic hyperosmotic stimulation. To examine this question, we performed paired extracellular recordings in superfused hypothalamic explants prepared from either euhydrated or salt loaded rats (6 days of 2% NaCl drinking solution). We found that spontaneous phasic firing is asynchronous in euhydrated animals. However, recordings from SON neurons in explants prepared from salt loaded rats revealed a high degree of synchronization between bursts recorded from adjacent cells. The basis for this effect remains to be determined.

PS2.00166 EFFECTS OF STRESS EXPOSURE ACROSS LIFE-HISTORY STAGES ON CELL DEATH AND SURVIVAL IN THE AGEING BRAIN

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Developmental stress often has negative long-term effects on the ageing brain. However, it may also be adaptive by preparing individuals to better cope with stressors later in life. As neural apoptosis and neurogenesis are two processes that are implicated in accelerated age-related cognitive decline, we set out to determine whether stress exposure throughout life can affect both neural apoptosis and neurogenesis within the brain. Here, we exposed male and female Japanese quail (*Coturnix japonica*) to a combination of pre-natal, post-natal and/or adult stress treatments. Using immunofluorescence, we determined both apoptotic (Caspase3) and neurotrophic (BDNF) activity across several brain regions in birds at peak reproductive (9-months) and senescent ages (24-months). We found that stress exposure during post-natal or adult stages led to increased Caspase3 expression in the hippocampus and hypothalamus in 9-months old birds while BDNF expression was reduced in the amygdala and hippocampus of 24-months old birds. We also found cumulative effects of pre and post-natal stress, resulting in reduced hypothalamic Caspase3 expression in adulthood, relative to single stress exposure at

either pre or post-natal stages. These data show that developmental stress has long-lasting effects on apoptosis and neurogenesis in the ageing brain, which may ultimately affect subsequent cellular responses to stress and impact on the health of the ageing brain.

PS2.00167 MATERNAL ANTI-OXIDANT TREATMENT PREVENTS SOME OF THE ADVERSE EFFECTS OF PRENATAL STRESS IN RATS

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Exposure to prenatal stress (PNS) programmes persistent neural and behavioural changes in the offspring. In rats, exposure to social stress during pregnancy results in greater neuroendocrine responses to stress and heightened anxiety behaviour in the adult offspring. The mechanisms involved in transmitting the effects of maternal stress to the fetuses are unclear; hence we investigated a role for oxidative stress in mediating the adverse impacts of PNS on the offspring. Pregnant rats were administered the antioxidant, mitoquinone attached to nanoparticles (MitoQ-NP; hence does not cross the placenta) or vehicle on gestational day (GD) 16 and were undisturbed or subjected to 10min/day social stress for 5d. Rats were either killed on GD20 or allowed to give birth so the offspring could be studied. PNS increased plasma corticosterone concentrations in the maternal, but not the fetal circulation. PNS also induced a significant increase in oxidative stress (reactive oxygen species; ROS) in the maternal brain, liver, placenta and fetal liver; which was prevented by maternal MitoQ-NP treatment. In contrast, the fetal brain showed no changes in ROS, regardless of treatment. PNS resulted in heightened anxiety-like behaviour, increased corticotropin-releasing hormone mRNA expression and decreased $GABA_{A\alpha 2}$ immunoreactivity in the amygdala of the male, but not female offspring; and these changes were prevented by maternal MitoQ-NP treatment. Maternal mitoQ-NP did not alter the corticosterone secretory response to acute stress in either control or PNS offspring. In conclusion, oxidative stress seemingly mediates the impact of PNS on anxiety-like behaviour, but not hypothalamo-pituitary-adrenal axis dysregulation in the offspring.

PS2.00168 MOLECULAR MECHANISMS OF STRESS HORMONES RELEASE IN HEALTHY VERSUS TUMORAL ADRENAL MEDULLA CELLS

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Chromaffin cells from the adrenal-medulla gland release catecholamines (dopamine, noradrenaline and adrenaline), the chemical messengers involved in the sympathetic stress-induced "Figth or Fligth" response. Nevertheless, catecholamine release at a basal level is also very important for the "rest and digest" response controlled by the parasympathetic nervous system allowing accurate homeostatic regulations. Today, the molecular mechanisms controlling the proper amount of catecholamines released according to the physiological demand remain poorly understood. Yet this is of primary interest since uncontrolled release of catecholamines causes serious illness and complications. This is the case for example for patients with pheochromocytoma, a neuroendocrine tumors arising from chromaffin cells of the adrenal medulla, and which is characterized by an excess of catecholamine secretion leading to hypertension, cardiomyopathy and high risk of stroke. In order to investigate the mechanisms leading to aberrant secretion of catecholamines we have compared, between healthy chromaffin cells and pheochromocytoma cells, both the exocytic activity and the expression level of proteins involved in the exocytic machinery. To do so, we have combined the highly sensitive carbon fiber amperometry technique on single cells with quantitative proteomic of tissue resection. We have demonstrated that hypersecretion is a direct consequence of a deregulation of the catecholamines secretion and we have identified several candidates involved in the changes leading to uncontrolled secretion. *This work has been supported by a Ligue Contre le Cancer (CCIR-GE), by a USIAS (University of Strasbourg Institute for Advanced Study) and by the ANR "SecretoNET" research grants to SG.*

PS2.00169 ACUTE STRESS RESPONSES OF RAMS REMAIN ALTERED AFTER DELAYING PUBERTAL ONSET WITH CHRONIC GNRHA

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Reproductive activity can be reversibly blocked with chronic gonadotrophin-releasing hormone agonist (cGnRHa) treatment. Use of cGnRHa-treatment in management of central precocious puberty is associated with lower resting heart rates and increased emotional reactivity. Limited information is available re effects of peripubertal cGnRHa-treatment in adulthood. This study investigated the effects of peripubertal cGnRHa-treatment on acute stress responses during puberty and adulthood. An all-male ovine model was used, Control (*n*=50), cGnRHa (*n*=50, goserelinacetate 4-weekly doses, 8-48 weeks of age) and cGnRHa+T (*n*=25, cGnRHa-treated as

above, testosterone replaced via 2-weekly i.m. injections of testosterone cypionate from 10-48 weeks of age). Puberty was expected at 10 weeks of age in Controls, but would be delayed in cGnRHa-treated rams (>48 weeks of age). A 15-min social isolation stress test was performed at 8, 28, 48, 87 and 101 weeks of age during which heart-rate-variability (HRV) was monitored and plasma cortisol quantified. HRV analyses indicated that GnRHa reduced (P<0.05) total autonomic cardiac control, whereas testosterone (exogenous and endogenous) was associated with parasympathetic dominance, at 28 and 48 weeks of age). Some of these effects persisted after a delayed pubertal transition (87, 101 weeks of age). Plasma cortisol responses where lower (P<0.05) for cGnRHa+T (vs Control and cGnRHa) at 28 (1.4-fold) and 48 (1.8-fold) weeks of age. Following delayed puberty, cortisol responses were lower in cGnRHa (vs Control) rams at 87 (1.3-fold), but not 101, weeks of age. Thus peripubertal cGnRHa-treatment may reduce the ability to cope with challenging situations. Funded by the BBSRC (BB/K002821/1).

PS2.0017 VAPORIZED CANNABIS DIFFERENTIALLY AFFECTS SEXUAL BEHAVIOR OF FEMALE RATS ACCORDING TO THE DOSE

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It is not clear how endocannabinoids modulate sexual behavior in females. In women, smoked cannabis has been associated with an increase in sexual desire; however, the effect of inhaled cannabis on rat's sexual behavior is unknown. Therefore, we hypothesized that vaporized cannabis augments sexually motivated behaviors in female rats. To test this hypothesis we compared the sexual behavior of late-proestrous females in a bilevel chamber after 10 min of confinement into a hermetic box where doses of 0 (control, n=8), 200 (n=9) or 400 (n=7) mg of cannabis (18% of delta-9-THC and undetectable levels of cannabidiol) were vaporized. Both doses of cannabis tended to increase the duration of the lordosis, but only the highest dose reduced lordosis quotient of females. While the low dose of cannabis augmented the display of hops and darts without altering the expression of solicitations of the females, the high dose did not affect the expression of hops and darts but reduced the display of solicitations. Neither of both treatments affected females` exploratory behavior in an ambulatory test. The increment of hops and darts and lordosis duration without affecting solicitations suggest that the low dose of cannabis might have enhanced females' sensitivity/reactivity to male's sexual stimulation rather than sexual motivation. On the other hand, the high dose of cannabis seems to reduce sexual receptivity and therefore sexual motivation. This differential effect of vaporized cannabis in females' sexual behavior according to the dose employed points toward a complex regulation of this behavior by the endocannabinoid system.

PS2.00170 STRESS EFFECTS ON MINERALOCORTICOID AND GLUCOCORTICOID SYSTEMS IN SPONTANEOUSLY HYPERTENSIVE RATS

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We evaluated stress-induced changes in mineralocorticoid and glucocorticoid systems of spontaneously hypertensive rats (SHR) compared with normotensive Wistar-Kyoto (WKY) rats. We used acute (2 hours) and chronic (daily 2 hours for one week) restraint stress paradigms. We measured plasma levels of corticosterone, ACTH and aldosterone and evaluated measured mRNA expression of genes involved in biosynthesis of corticosteroids in adrenal gland. Acute stress led to the elevation of plasma corticosterone in both strains, the effect being more pronounced in SHR. The corticosterone levels were also elevated in chronically stressed animals, but no difference between SHR and WKY was observed. On the other hand, acute and chronic stress increased aldosterone in both strains, the effect being more pronounced in WKY rats. Basal ACTH levels did not differ between stress naïve SHR and WKY. In chronic stress experiment, we found higher ACTH level in adapted SHR control (6x stress+24h rest). Restraint stress led to the increase of plasma ACTH in rats of both strains, the elevation during both acute and chronic stress was greater in SHR compared to WKY rats. We found increased basal expression of *Cyp11b1* gene, involved in biosynthesis of corticosterone, in adrenal gland of SHR compared to WKY. The basal expression of aldosterone synthase *Cyp11b2* was decreased in SHR. Response of plasma corticosterone and aldosterone to stress differ between spontaneously hypertensive rats and normotensive WKY rats. The differences can be explained by altered expression of genes involved in corticoid biosynthesis in adrenal gland of SHR.
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PS2.00171 THE HORMONAL AND CARDIOVASCULAR RESPONSES TO ACUTE AND CHRONIC STRESS IN FISCHER 344 AND LEWIS RATS

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In recent study we evaluated the effects of acute and chronic restraint stress on plasma levels of stress hormones and cardiovascular response in the stress hyper-responsive Fisher 344 (F344) rats and stress hypo-responsive Lewis (LEW) rats. Mean arterial pressure (MAP) and heart rate (HR) were measured in restrained conscious rats, using catheter inserted into the left femoral artery. We found no difference in basal plasma levels of ACTH, corticosterone and adrenaline between stress naïve rats F344 and LEW rats. As expected, both acute and chronic stress induced greater elevation of plasma levels of ACTH, corticosterone and adrenaline in F344 compared to LEW rats. Plasma levels of ACTH and adrenaline in acutely and chronically stressed F344 rats were similar. On the other hand, plasma levels of ACTH and adrenaline were

decreased in chronically stressed LEW rats compared to acutely stressed animals. MAP was slightly higher in F344 than LEW throughout the acute as well as the chronic stress session. We observed no strain differences in HR during acute stress, but chronic stress revealed substantial differences in HR between strains. While F344 rats reacted to chronic stress with slightly elevated HR compared to acutely stressed F344 rats, LEW rats responded to chronic restraint by lower HR response compared to acutely stressed LEW rats. Our data shows, that F344 and LEW rats differ not only in reactivity of hypothalamic-pituitary-adrenal axis, but also in plasma adrenaline and cardiovascular response to restraint stress. Supported by GACR16-10349Y.

PS2.00172 DEVELOPMENT AND USE OF "SNIFFER CELLS" TO DETECT THE PRESENCE OF NEUROPEPTIDES

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The brain is sensitive to Angiotensin II (AngII). However, neuropeptide release is difficult to study and it's currently unclear if AngII is utilized as a neurotransmitter within the brain. To address this question, our laboratory has adopted a relatively new approach to study the brain renin-angiotensin system – sniffer cells. Chinese Hamster Ovary (CHO) cells were transfected with plasmids to express angiotensin type 1a (AT1a) receptors and a genetically encoded fluorescent Ca2+ sensor (GCaMP or R-GECO). Sniffer cells were plated on glass cover slips and continually perfused with aCSF. Calcium imaging was performed and fluorescent intensity was measured in response to bath application of neuropeptides. Sniffer cells were also placed on the median preoptic nucleus (MnPO) in *in vitro* brain slices (produced using standard slice procedures) from male Sprague-Dawley rats. Fluorescent intensity was measured in response to electrical and optogenetic stimulation of the subfornical organ (SFO). Sniffer cells exhibit increases in fluorescence in the presence of AT1aR agonists ($n=38$, $p<0.01$) and increases in fluorescence were specific to AT1aR activation ($n=38$, $p<0.01$). Sniffer cells also showed dose dependent responses to AngII ($n=10$, $p<0.05$) and AngIII ($n=41$, $p<0.01$). We were able to detect spontaneous release of AngII in the MnPO in vitro ($n=4$). We were also able to detect evoked release of AngII with sniffer cells using both electrical stimulation ($n=3$) and optogenetic stimulation ($n=4$) of the SFO. Further studies using these sniffer cells will characterize the phenotype of AngII releasing neurons as well as potential changes in AngII release in sleep apnea.

PS2.00173 EFFECTS OF HYPERGRAVITY ON THE HYPOTHALAMIC FEEDING-RELATED NEUROPEPTIDES VIA VESTIBULAR INPUTS

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We examined the effects of hypergravity on the gene expressions of the hypothalamic feeding-related neuropeptides in vestibular lesioned (VL) mice in comparison with sham operated (Sham) mice. After VL and Sham mice were placed in 1*g* and 2*g* environments for 3 days, 2 weeks and 8 weeks, the gene expressions of corticotrophin-releasing hormone (*CRH*) in the paraventricular nucleus (PVN), pro-opiomelanocortin (*POMC*), cocaine- and amphetamine-regulated transcript (*CART*), neuropeptide Y (*NPY*) and agouti-related protein (*AgRP*) in the arcuate nucleus (Arc), melanin-concentrating hormone (*MCH*) and prepro-orexin (*orexin*) in the lateral hypothalamic area (LHA) were qualified by using semi-quantitative *in situ* hybridization histochemistry. Although *CRH* in the PVN was significantly increased in Sham mice but not VL mice after 3 days exposure in 2*g* environment compared to 1*g*, significant increases of *NPY*, *AgRP* in the Arc and *orexin* in the LHA and significant decreases of *POMC* and *CART* in the Arc were observed in both Sham and VL mice. After 2 weeks exposure in 2*g* environment, *POMC* in the Arc were increased significantly in Sham mice but not VL mice. After 8 weeks exposure in 2*g* environment, there were no significant changes of the gene expressions of the hypothalamic neuropeptides examined in Sham and VL mice. These results suggest that the gene expressions of the hypothalamic feeding-related neuropeptides may be affected by the exposed duration of the hypergravity via neural inputs from the vestibular system.

PS2.00174 ANXIETY AND DEPRESSION IN TYPE 2 DIABETIC PATIENTS OF KARACHI, PAKISTAN.

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The coexistence of diabetes and psychiatric morbidity (i.e. depression and anxiety) is not very uncommon and has a significant impact on health outcome and is associated with significant mortality & increased healthcare cost. The aim of this study was to measure the frequency of psychiatric morbidity (i.e. depression and anxiety) among diabetic patients and their healthy healthy individuals. A total of 800 subjects were enrolled for this study from the out-patient clinic out of which 783 responded to the questionnaire (97.8% respond rate) 420 diabetics

(males=284, females=136) were compared with 363 healthy individuals (males=263, females=100). Depression and anxiety (measured through PHQ9 and GAD7 questionnaire) were more significantly prevalee among diabetic patients (70.5%) & (69.8%) compared with healthy individuals (8.8%) & (7.2%) respectively, thus showing a far greater intensity of depression among diabetic patients as compared to healthy population. There was a significant correlation between the PHQ9 & GAD7 scale with diabetes with a correlation coefficient of 0.64 (*P* <0.001). Also, a significant correlation existed between PHQ9 & GAD7 scale and age, marital status ad family system (correlation coefficient, 0.508, *P* <0.001; 0.401; *P* <0.001; 0.27; *P* <0.001 respectively). Significant correlation were also observed between the PHQ9 and GAD7 (coefficient of correlation, 0.85; *P* <0.001) scores.These results alert clinicians and policy makers to diagnose and treat anxiety and depression as common components of diabetes care program to improve clinical outcomes and reduce the burden of illness. Further prospective studies are needed to develop the causal relationship and to test the impact of intervention.

PS2.00175 ALLOPREGNANOLONE REDUCES STRESS-INDUCED CORTISOL AND PROLACTIN SECRETION IN SHEEP

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In females, a hormonal response to stressful stimuli decreases during pregnancy. It requires adaptations at the levels of the anterior pituitary, hypothalamus and higher brain regions, in which a neurosteroid allopregnanolone is thought to play a crucial role. In this study, the effect of allopregnanolone, infused into the third brain ventricle (IIIv), on basal and stress-induced cortisol and prolactin secretions in non-pregnant sheep was investigated. Twenty four sheep were implanted with stainless steel guide cannula into the IIIv. The animals were randomly divided into 4 groups: i. infused with Ringer-Locke (RL) solution (C group); ii. infused with RL and treated with stressful stimuli (isolation and partial immobilization; S group); iii. infused with allopregnanolone and treated with stressful stimuli (AS group); and iv. infused with allopregnanolone alone (A group). Plasma cortisol and prolactin concentrations were assayed in samples taken for 4 h, every 10 min., by the RIA method. Stressful stimuli caused a significant (P<0.001) increase in both plasma cortisol and prolactin concentrations, which persisted until the end of the experiment in comparison with C group. In the AS group, the concentrations of both hormones in the second half of the experiment decreased to the similar level as in controls. Allopregnanolone infused alone did not change basal prolactin secretion, but in comparison with C group it significantly (P<0.05) reduced cortisol response to handling stress at the beginning of the experiment. Our results suggest that in sheep, allopregnanolone may reduce sensitivity to stress at the central nervous system level. NSC Grant 2015/19/B/NZ9/03706

PS2.00176 EARLY MATERNAL SEPARATION EFFECTS ON HYPOTHALAMIC BASAL AND HYPEROSMOLALITY-INDUCED AVP EXPRESSION

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We assess if the interplay between an early psychological stress, such as the maternal separation (MS), and late osmotic stress both associated with the activity of vasopressinergic circuits within the paraventricular (PVN) and the supraoptic (SON) nuclei would result in a differential osmoregulatory response in terms of vasopressin mRNA levels during adulthood. The aim of this work was to evaluate whether MS may induce a differential programming in the adult offspring vasopressinergic system in particular the hyperosmolality-induced vasopressin expression. Male Wistar rats were subjected to daily maternal separation for 4.5 hours during the first three weeks of life. At postnatal day 75, rats were intravenously infused with isotonic or hypertonic saline solution during 20 minutes. After 10 minutes, animals were decapitated. Thereafter, the PVN and SON were identified and collected by micropunch technique. Then, we determined the relative vasopressin expression by qPCR. In the PVN, as we expected, non-separated animals responded to hypertonic stimulation with a threefold increase in vasopressin levels ($p<0.05$). Surprisingly, MS rats did not respond to hypertonic solution, showing similar mRNA levels in PVN compared to rats infused with isotonic solution. Besides, separated rats showed higher vasopressin levels in the SON than non-separated rats ($p=0.045$). Our data suggest that psychological stress during the neonatal period may impair the vasopressin system activity provoking a reduced response in the offspring after an osmotic challenge. This could be the consequence of alterations in the central osmosensitive mechanism or in the activity of brain circuits involved in hydroelectrolyte homeostasis.

PS2.00177 PRENATAL GLUCOCORTICOID EXPOSURE PRODUCES SPECIFIC SIGNATURES IN SPERM MIRNA ACROSS MULTIGENERATION

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[Introduction] Prenatal glucocorticoid (pGC) is prescribed to women in preterm labour, to improve neonatal outcomes. However, pGC has been associated with neurodevelopmental impairment. Indeed, pGC alters offspring behaviour and brain gene expression across three generations following paternal transmission. miRNAs have been linked to brain development and transgenerational effects. We hypothesized that pGC results in a specific signature of miRNA expression in sperm for multiple generations. [Methods] Pregnant F0 female guinea pigs were injected with three courses of saline (VEH) or betamethasone (BETA). Male offspring were

mated with naïve females to produce 2 further generations of offspring that were euthanized on day 40. RNA-sequencing had been previously performed in the hypothalamic PVN, and this data was mined using miRNA prediction tool (miRDB) to identify target miRNA. Frozen semen from F1 and F2 breeders was separated into sperm and seminal plasma and miRNA quantified using qRT-PCR. [Results] We identified 12 miRNAs (based on PVN-RNA sequencing data) that target genes in the PVN that were altered across three generations. These miRNAs were previously related to stress/GC exposure, and germline transmission. miR-125b-3p was significantly ($p<0.05$) down-regulated in F1 and F2 sperm; miR-19b-3p and miR-96-5p were significantly ($p<0.05$) up-regulated in F1 but not F2 sperm; miR-204-5p was down-regulated in F2 sperm. These changes were not identified in seminal plasma. [Conclusion] We have identified a subset of sperm miRNAs that is affected by pGC exposure and that have the potential to regulate fetal brain development. These findings may provide new perspectives on mechanisms of transgenerational transmission.

PS2.00178 AVP AS A MARKER OF SURGICAL STRESS IN CANINE PATIENT

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Stress directly affects the physiological and psychological well-being of pet animals and may alter disease outcomes and prognosis. Traditionally, cortisol has been used in veterinary research as an indicator of stress, but with limited clinical use due to several factors affecting its release and levels. Arginine vasopressin (AVP), along with corticotrophin releasing hormone, synergistically activates the hypothalamic-pituitary-adrenal axis in response to stressful stimuli. Copeptin, a glycopeptide released stoichiometrically from the same parent hormone as AVP, has documented use as a measure of stress in human medicine. In the current study, we measured the impact of surgical stress on AVP and copeptin release in dogs. Plasma AVP and copeptin levels were measured in seven female canine patients (aged 6 months to 3 years) before and after elective surgery of ovariohysterectomy using enzyme immunoassay. Basal plasma electrolytes were measured to rule out influence on AVP release, and were within normal range. Plasma AVP levels were significantly higher post-surgery (0.44ng/ml), compared to pre-surgery (0.14ng/ml). However, in contrast to human studies, our results did not show a statistical difference in pre-surgery (0.40 ng/mL) and post-surgery (0.42 ng/mL) copeptin levels. This could be due to species differences, or analytical procedures followed, and should be pursued further. Although released stoichiometrically, an increase in AVP but not in copeptin release in response to surgical stress may emphasize the use of AVP, rather than copeptin as a reliable marker of surgical stress in canine patients.

PS2.00179 EFFECTS OF AQUATIC THERAPY ON SALIVARY CORTISOL IN AUTISTIC VERSUS NON-AUTISTIC CHILDREN

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Children with special needs, and particularly autism, struggle with many challenges throughout their everyday lives, such as changing environments and new situations. New methods to help these children build beneficial strategies to handle unexpected future experiences are required. Aquatic therapy is one such strategy that warrants further investigation as a potential alternative treatment in reducing anxiety. Working alongside Therapeutic Recreation faculty at Longwood University, a practice-embedded curriculum was designed in which undergraduate students could engage with special needs children from the surrounding school counties. These children were provided the opportunity to participate once a week in the aquatic therapy program or to opt into a dry lab therapy environment. At the beginning, mid-point, and end of the 10-week program, we collected saliva samples from the participating children before and after the daily therapy activities. Samples were subsequently analyzed via an enzyme-linked immunosorbent assay (ELISA) for anxiety-related hormones - namely cortisol - to provide quantitative assessment of the program. An assessment of the behavioral outcomes of the therapy was also recorded for comparison. In this presentation we describe the feasibility and acceptability of this study, we explore differences in salivary cortisol in autistic and non-autistic participants, and we discuss implication for therapy efficacy.

PS2.0018 IMPAIRED SEXUAL BEHAVIOUR IN A MOUSE MODEL OF PCOS.

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Polycystic ovary syndrome (PCOS) is the most common infertility disorder in women worldwide. In addition to infertility, recent epidemiologic studies indicate that PCOS is associated with decreased sexual desire, increased sexual dissatisfaction and gender dysphoria. Prenatally androgenized (PNA) animal model of PCOS exhibit an adult hyperandrogenism, impaired sensitivity to progesterone signalling in the brain and alterations in the gonadotropin-releasing hormone (GnRH) neuronal network linked to reproductive dysfunction. However, the impact of prenatal androgen excess and the development of PCOS features on sexual behaviour remains unclear. This study aimed to determine whether the PNA mouse model of PCOS exhibits typical female sexual behaviour. To model PCOS, female dams received injections of dihydrotestosterone, a non-aromatisable androgen (PNA n=8), or oil vehicle (VEH n=5) daily from gestational day 16 to 18. Adult female offspring were ovariectomized and implanted with a silastic capsule of estradiol to examine lordosis behaviour. PNA females exhibited an overall

reduction of the lordosis quotient compared to VEH females ($p<0.01$). These data suggest that increased androgen signalling during the perinatal period impaired sexual differentiation of the brain and behaviour in addition to other PCOS features. Ongoing experiments are 1) determining if the partner preference of female PNA mice is different from VEH and 2) investigating activated brain areas following sexual behaviour assessment to determine the specific neuronal targets potentially disrupted in PCOS-like females.

PS2.00180 SALIVARY CORTISOL RESPONSES OF WILDERNESS THERAPY CLIENTS AS AN EFFICACY MEASURE

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Wilderness therapy, a subset of the broader field of outdoor therapy, is used to treat clients with a range of issues from addiction to emotional struggles (Russell, 2001). The efficacy of wilderness therapy is unclear, however, as current evidence of effect relies heavily on self-reported measures. Clients do report beneficial outcomes related to substance abuse, behavioral disorders, and mood disorders when programs incorporate elements of nature and exercise as well as interactions with a licensed clinical practitioner (e.g., Bettmann et al., 2013; Hoag et al., 2014; Russell et al., 2015). Amidst changing health insurance coverage, enhanced research support and improved efficacy measures for alternative mental health treatments are needed. Anxiety disorders are typically comorbid, if not a primary diagnosis, for many patients seeking treatment through wilderness therapy, indicating that assessing changes in anxiety profiles may be one measure of efficacy. To meet this need, we studied mental health patients' neuroendocrine profiles during a wilderness therapy experience. In this project, we partnered with Blackwater Outdoor Experiences, a wilderness therapy group based in Midlothian, VA. We collected saliva samples and behavioral data at several time-points before, during, and after a 22-day wilderness therapy trip. Samples were assayed for stress-related hormones, namely cortisol and DHEA. Our findings represent the first integrative analysis of wilderness therapy clients that includes neuroendocrine data.

PS2.00181 COMPARISON OF CRF/UCN/CRFR1 VARIATION FROM MYOSPALAX CANSUS AND MICROTUS OECONOMOUS IN QINGHAI-TIBET

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Corticotropin-releasing factor (CRF) plays an important role in coordinating endocrine, autonomic and behavioral response to stress via its receptors. Animals living at Qinghai-Tibet plateau acclimatize to hypoxic environment . Here we reported CRF, UCN andCRFR1 variation and signal from *Myospalax baileyi* (*M.b.*) and *Microtus oeconomous*(*M.o.*), small mammals lived in the Qinghai-Tibet of high altitude environments. There is low sensitivity of HPA axis response to hypoxia, to investigate the animals HPA axis founction, we study the variation and signal of CRF, UCN and CRFR1. Hypoxia (8% O2, 8 h) significantly increases CRF, UCN and CRFR1mRNA in rat prefrontal cortex, there is no change in CRF, UCN and CRFR1 mRNA in the prefrontal cortex of *M.b. and M.o.*, but *M.c.* a lowland control (altitude of 800-1000m) seems a higher expression of CRF, UCN and CRFR1mRNA. CRF sequence is conserved among *M.b., M.c. and M.o.,* UCN contained variations at Asp2Asn and Pro4Ser in *M.b. and M.c.*; and variations at Asp1Asn, Phe37Leu, Asp38His in *M.o.,* there are some variations at potential binding-pocket and EC, TM, IC at wildtype-CRFR1s. By using site-mutagenesis in CHO cells transfected with series of CRFR1 receptors, CRF dose-dependent induced the different cAMP levels, EC50 of Mb-CRFR1, Mut379Mc-CRFR1, Mc-CRFR1, and rat-CRFR1 is 14.46, 23.36, 17.79 and 21.04nM respectively. 379aa of Mc-CRFR1 is confirmed to contribute the decreased binding of CRFR1 and CRFand cAMP signal. There are five AP-1 and eight NF-κB binding sites on the mouse-CRFR1 promoter by ChIP, and AP-1 inhibited and NF-κB increased the transcription of CRFR1 under hypoxia.

PS2.00182 EFFECTS OF POSTNATAL MATERNAL STRESS ON OFFSPRING ANXIETY DURING ADOLESCENCE AND ADULTHOOD IN RATS

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Intergenerational trauma involves the transmission of the effects of trauma from one generation to the next. Social transmission includes changes in parental behavior towards offspring and has been studied in a variety human populations. However, there is little research on this phenomenon using animal models. The present study examined the effects of postnatal maternal stress on offspring anxiety in rats, and explored differential effects of sex and age through the inclusion of both females and males in adolescence or adulthood. On postnatal day (P) 8, mothers were taken from their home nest, brought into separate rooms, and put into an empty aquarium for a 5-minute acclimation period. They were then exposed to predator odor (Stressed) or control odor (Control) for 30-minutes. This occurred once a day for three consecutive days. The offspring remained undisturbed in their home nests during the maternal stress paradigm. Pups were weaned at P26 and tested for anxious behavior using the light/dark test during adolescence (P39) or adulthood (P60). There was an interaction between age and maternal stress group on duration spent in the light portion of the apparatus with adult offspring of the Stressed group spending significantly less time in the light portion of the

apparatus compared to adult offspring of the Control group. Conversely, there was a trend for adolescent offspring of the Stressed group spending *more* time in the light portion compared to adolescent offspring of the Control group. Thus, postnatal maternal stress has an effect on offspring anxiety in an age-dependent manner.

PS2.00183 SEX DIFFERENCES IN TRAUMATIC STRESS RESPONSES: PTSD SYMPTOMS IN WOMEN RECAPITULATED IN FEMALE RATS

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Sex differences in traumatic stress responses are widely reported in clinical studies of post-traumatic stress disorder (PTSD), but the neurobiological basis for this is unknown, largely due to male bias in preclinical research. Using a rodent model of PTSD, single prolonged stress (SPS), we find sex differences described in humans reflected in adult rats. After SPS, males show hyper-responsiveness to subsequent mild stress, as measured by enhanced acoustic startle response and exaggerated negative feedback control of the stress hormone response, two presumed hallmark features of PTSD, but females do not. While these two measures may suggest female resilience to SPS, other measures commonly applied to depression studies (sucrose preference, social interaction) suggest females but not males are affected by SPS and indicate a more depressive-like phenotype for traumatized females. The results reported here further characterize sex differences in response to SPS, with specific attention to measures typically used in depression studies: forced-swim test, marble burying, novelty-induced hypophagia, and high-dose dexamethasone suppression test. Results continue to portray a hyper-responsive phenotype in males and a depressive phenotype in females. Additionally, PTSD is associated with changes in pain sensation/perception, and we find SPS-induced hyperalgesia only in female rats. We propose that the trauma response for female rats recapitulates the female bias in PTSD for internalizing symptoms and major depression in contrast to the externalizing symptoms of males. We conclude that males and females show fundamentally different responses to trauma that do not simply reflect differences in resilience.

PS2.00184 EFFECTS OF PRENATAL STRESS AND CHOLINE ON CENTRAL AMYGDALA NICOTINIC ACETYLCHOLINE RECEPTORS.

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Prenatal stress (PS) is associated with irregular brain development and expression of adult psychiatric disorders. These disorders are also associated with aberrant levels of alpha7

nicotinic acetylcholine receptors (nAChR), potentially contributing to behavioral abnormalities. While safe stress interventions during the prenatal period are lacking, rodent studies demonstrate that prenatal dietary choline-supplementation mitigates the deleterious PS effects on anxiety-related behaviors, social interactions, and memory function. However, whether choline mitigates these behaviors via central amygdala (CeA) alpha7 nAChR alterations is unknown. Pregnant female rats were assigned to one of 4 groups: non-stressed (NS)+control diet, PS+control diet, NS+choline diet, PS+choline diet. We hypothesized that choline supplementation will normalize stress-induced changes in CeA alpha7 nAChRs. Although PS did not impact overall levels of CeA alpha7 receptor levels, prenatal choline-supplementation preferentially increased CeA receptors in PS males compared to NS males. No effects of stress or diet on CeA alpha7 nAChRs were observed in females. Thus, CeA nAChRs may be a therapeutic target for prenatal choline-supplementation to mitigate PS-induced anxiety-related behavior in males. These results have translational implications for stress-related disorders such as schizophrenia, depression, and anxiety disorders.

PS2.00185 DIFFERENT PREDICTIVE STRENGTHS OF CORTISOL, OXYTOCIN, AND SLEEP FOR DIFFERENT PSYCHOLOGICAL SYMPTOMS

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Cortisol and oxytocin are both implicated in psychological health and disease, but with contrasting effects. Cortisol is associated with psychopathologies including stress and hostility, while oxytocin is inversely correlated with anxiety. However there is inconsistency in results, and comprehensive studies examining multiple hormones alongside additional measures such as sleep are lacking. We aimed to determine whether cortisol, oxytocin and sleep are differentially correlated with distinct symptom profiles of psychopathology in depressed and non-depressed individuals. Plasma cortisol and oxytocin concentrations were quantified from a morning blood sample in healthy participants and participants meeting DSM 5 criteria for major depressive disorder. Participants completed the Brief Symptom Inventory (BSI), a 53-item self-report measure of most major forms of psychopathology with nine domains (Somatization, Obsessive-Compulsive, Interpersonal Sensitivity, Depression, Anxiety, Hostility, Phobic anxiety, Paranoid ideation, Psychoticism) and three global indices. Cortisol was significantly positively correlated, and sleep was significantly inversely correlated, with all psychopathology domains. Oxytocin had a more discriminatory profile, with stronger correlations with the somatization, paranoid ideation, interpersonal sensitivity and anxiety domains; weaker correlations with depression, phobic anxiety and psychoticism; and no significant correlation with the obsessive-compulsive and hostility domains. Multiple regression analyses indicated that cortisol predicted 30%, and the combination of cortisol, oxytocin, age and sleep predicted 40-50%, of the variance of the BSI total score and the Global Severity Index. Overall, cortisol and sleep were stronger predictors of psychological symptom profiles, while oxytocin was more discriminatory, having

stronger correlations with psychopathologies characterised by distress pertaining to interpersonal relations and social situations.

PS2.00186 SIMULTANEOUS MEASUREMENT OF FREE MELATONIN AND CORTICOSTEROIDS BY AMBULATORY AUTOMATED MICRODIALYSIS

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Accurate description of circadian rhythms is time consuming and typically relies on invasive blood sampling or alternatively less precise methods such as saliva or urine analysis. We investigated the use of subcutaneous microdialysis as a minimally invasive technique for the determination of both cortisol and melatonin circadian rhythms. The participant was a 36-year-old healthy male volunteer. Samples of tissue fluid were collected for 20 hours by automated ambulatory microdialysis. This consisted of a low flow infusion pump perfusing a microdialysis membrane placed in the abdominal subcutaneous tissue, connected to a prototype fraction collector (U-RHYTHM device) worn on the waist. Fluid samples were automatically collected every 20 minutes, during which the participant returned home to continue regular activities including sleep. Dim light conditions (<30 lux) were maintained from early evening. Bed-time was at 2300. Activity and lux was monitored using a MotionWatch wrist actigraphy device. 58 consecutive microdialysis samples were analysed by triple quadrupole mass spectrometry. Free cortisol and cortisone, detected in all samples, showed a typical diurnal pattern with nadir concentrations overnight and peaks just prior to waking. Free melatonin became detectable from 2230 with the peak concentration at 0030, after the onset of sleep. We haved described simultaneous detection of multiple circadian hormones and the first reported detection of a free melatonin rhythm in subcutaneous fluid. We propose that automated ambulatory microdialysis is a powerful tool for the investigation of circadian biology and endocrine analysis.

PS2.00188 TSHR POLYMORPHISM IS STRONGLY ASSOCIATED WITH SEASONAL REPRODUCTION IN THE ATLANTIC HERRING

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Local activation of thyroid hormone within the mediobasal hypothalamus (MBH) by thyroid-hormone-activating enzyme (type 2 deiodinase, *DIO2*) regulates seasonal reproduction in birds

and mammals. The strongest genetic differentiation identified from our previous genome-wide comparison between spring- and autumn-spawning Atlantic herring overlaps the thyroid stimulating hormone receptor (*TSHR*) gene, including two non-coding SNPs and two missense mutations. Herring *TSHR* has a similar genomic structure with its orthologs comprising 10 exons with four transcript isoforms. Tissue profiling showed that herring *TSHR* is highly expressed in the hypothalamus and saccus vasculosus. A unique 66bp fragment coding for 22 residues near the C terminus of herring *TSHR* showed copy number polymorphism between the spring spawners (homozygous for three copies, 87%, n=38) and autumn spawners (homozygous for five copies or heterozygous for five and four copies, 91%, n=54). Two versions of herring *TSHR* including the two missense mutations and three copies or five copies of the 66bp repeated fragment were expressed in Chinese hamster ovary (CHO) cells. pGL4-CRE-luciferase reporter assay, which monitors the cAMP/PKA signaling pathway, indicated that the spring version of herring *TSHR* has a stronger constitutive activity than the autumn version. Regulations of the two versions of herring *TSHR* in other G-protein-coupled receptor (GPCR) signaling pathways and internalization are currently under investigation.

PS2.00189 PHOTOPERIODIC AND TRIIODOTHYRONINE REGULATION OF HYPOTHALAMIC DNA METHYLTRANSFERASE EXPRESSION

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Seasonal epigenetic modifications in the hypothalamus have been demonstrated to regulate timing of reproduction and energy balance. In adult Siberian hamsters, there is a significant reduction in DNA methyltransferase enzymes (e.g. *dnmt3a/b*) in the hypothalamus exposed to short-winter photoperiods. To date, the mechanisms that govern the photoperiodic control of *dnmt1, dnmt3a* and *dnmt3b* are not well defined. Our objectives were to investigate the effect of thyrotrophin-stimulating hormone (TSH) and triiodothyronine (T3) on the photoperiodic regulation of *dnmt* expression. If *dnmt1, 3a* and/or *dnmt3b* are regulated by the photoperiodic response, we hypothesized that either TSH or T3 in short day (SD) hamsters would stimulate hypothalamic expression. Study 1 examined the effect of TSH on *dnmt* enzyme expression in the rat hypothalamus. In study 2, we investigated whether T3 injections are sufficient to stimulate hypothalamic *dnmt* expression in adult female hamsters exposed to long day (LD) or SD conditions. First, P10 rat hypothalamic slices were treated with TSH or vehicle control for 48 h, followed by qPCR analysis. We did not detect a significant change in *dnmt1*, *dnmt3a* nor *dnmt3b*expression in response to TSH. Then, we examined the impact of daily T3 injections or saline on hypothalamic gene expression and female reproductive physiology. SD photoperiods were observed to reduce body and uterine weight. Unlike previous reports in male hamsters, daily T3 injections in SD females were ineffective to stimulate gonadal recrudescence. However, T3 was sufficient to induce *dnmt3a* expression in the female hypothalamus. These data suggest that the photoperiodic regulation

of *dnmt* expression is, in part, regulated by the local synthesis of T3. Moreover, it appears that additional cues are required for reproductive development in female hamsters.

PS2.0019 SELECTIVE VULNERABILITY AND EXCITOTOXICITY IN THE LATERAL HYPOTHALAMUS IN HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a fatal and hereditary CAG triplet repeat neurodegenerative disorder. HD is clinically characterized with the appearance of movement abnormalities (chorea) caused by selective degeneration of striatal neurons. Importantly, non-motor symptoms develop earlier in the course of the disease and include sleep and circadian rhythm disruptions, metabolic changes and psychiatric disturbances such as anxiety, apathy and depression. Ubiquitous expression of mutant huntingtin (HTT) selectively affects the striatum and the cerebral cortex and recent studies have pointed to the lateral hypothalamic area (LHA) as being another selectively affected brain region in HD. Although little is known about the origin and development of non-motor symptoms in HD, neuroendocrine disturbances in HD could be a consequence of LHA dysfunction. Excessive glutamate signaling (i.e. excitotoxicity) and disruption of synaptic glutamate homeostasis have been linked to the degeneration of striatal neurons in HD. Similar to the striatum, the LHA receives several glutamatergic synaptic inputs and excitotoxicity could thus be an important driving force in the development of LHA neuropathology. The aim of the work is to explore the properties and causative effects of selective vulnerability of the LHA in HD by investigating transcriptional changes in tissue derived from different HD mouse models as well as unique post-mortem tissue from HD patients and controls with a specific focus on possible changes in glutamate homeostasis. To further explore the involvement of excitotoxicity in LHA neuropathology, the sensitivity of the LHA in response to excitotoxic exposure is evaluated in different mouse models of HD.

PS2.00190 DIFFERENCES IN CIRCADIAN COORDINATION CAPTURED IN REAL TIME IN PEOPLE DURING THEIR DAILY ROUTINE

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Disruption of circadian clocks is an important risk factor for the development of multiple health conditions including cancer and metabolic diseases. Temperature cycles coordinate body cellular clocks outside the brain. In this study, we measured skin chest temperature and rest-activity rhythms in order to assess circadian clocks coordination in individual healthy people during their daily routine. This will allow us to develop further the biomarkers needed for precision chronomedicine.
Non-invasive real-time measurements of rest-activity and chest temperature rhythms were recorded during the subject's daily routine at home, using a dedicated mobile e-health platform (PiCADo). The chest sensor jointly measured accelerations, 3D-orientation and skin surface temperature every 1-5 min, and relayed them out to a mobile gateway via Bluetooth-Low-Energy. The gateway tele-transmitted all stored data to a server via GPRS every 24 hours. Circadian rhythms were e-monitored for 4 days to 4 weeks. Sampling-resampling spectral analyses enabled to compute rhythm parameters values, with their 90% confidence limits, and their dynamics in each subject. All the individuals displayed a dominant circadian rhythm in activity with maxima occurring from 12:09 to 20:25. In contrast, the dominant temperature period clustered around 24 h for 51 subjects (76.1%), and around 12 h for 13 others (19.4%). The circadian acrophase of chest temperature was located at night for the majority of people, but it occurred at daytime for 26% of the subjects, hence supporting important inter-subject differences in circadian coordination.

PS2.00191 SCN VIP NEURON SPONTANEOUS AND EVOKED ACTIVITY IN CIRCADIAN RHYTHMS AND ENTRAINMENT

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Circadian (~24 hr) rhythms influence nearly all aspects of physiology, including sleep/wake, metabolism, and hormone release. The suprachiasmatic nucleus (SCN) synchronizes these daily rhythms to the external light cycle, but the mechanism by which this occurs is unclear. SCN neurons that produce the neuropeptide vasoactive intestinal peptide (VIP) are the predominant contributor to synchrony within the SCN and are essential for the circadian regulation of downstream processes such as the hypothalamic-pituitary-adrenal and -gonadal axes. Importantly, exogeneous VIP administration can cause phase shifts similar to those produced by light, and *Vip*$^{-/-}$ mice show deficits in their behavioral responses to light. Thus, we tested the hypothesis that rhythmic VIP neurons mediate the phase-shifting response to light by using in vivo fiber photometry recording of VIP calcium activity and chemogenetic inhibition of VIP neuron activity. We found daily rhythms in VIP calcium events that peaked during the subjective day in a light-dark cycle and in constant darkness. Light pulses given throughout the day elicited large calcium responses with peaks in amplitude and duration occurring around subjective dusk. Finally, we found that while chemogenetically inhibiting VIP neurons at subjective dusk did not cause significant phase shifts in locomotor activity, inhibiting these neurons in combination with a phase-shifting light pulse greatly attenuated the resulting phase

delay compared to control animals. We conclude that VIP neurons participate in the circadian activity of the intact SCN and are necessary for the normal transduction of light signals to the SCN at times when light shifts daily rhythms.

PS2.00192 CIRCADIAN RHYTHMS OF MELATONIN AND BEHAVIOUR IN JUVENILE SHEEP IN FIELD CONDITIONS

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Entrainment of ovine circadian rhythms (CR) to the light dark cycle has been well described under controlled, experimental conditions. However, studies in rodents have reported that rhythms in the laboratory are not reproduced under field conditions. The aim of this study was to characterise the CR of sheep maintained under conditions of standard UK farm animal husbandry and to investigate the effects of environmental challenges presented by season, weaning and changes in housing. Male sheep (n=9) were kept at pasture, or in barns and CR in locomotor activity were monitored using accelerometry, and 24h patterns in plasma cortisol and melatonin were measured by ELISA. CR were measured before and after weaning, in summer and in winter, and at pasture and in barn housing. The sheep showed high amplitude, diurnal rhythms in locomotor activity that were disrupted by weaning and by barn housing. Rhythms in winter showed an interrupted night time activity pattern, but only when the sheep were kept at pasture. Cortisol and melatonin secretion followed typical circadian patterns in winter and summer. The CR of the sheep under the field conditions of this study were strikingly robust under basal conditions, but easily disrupted by environmental challenges. Interrupted patterns of activity in long nights of wintertime not previously reported for sheep kept in experimental conditions were recorded. Based on these findings, we propose that animals require exposure to more complex environments than the laboratory in order to exhibit their true circadian phenotype. This work was funded by the BBSRC (BB/K002821/1).

PS2.00193 FEEDBACK CONTROL OF TUBEROINFUNDIBULAR DOPAMINE (TIDA) NEURONS THROUGH AN ULTRA-SHORT GABA LOOP

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The role of GABA in neuroendocrine control remains mysterious, despite the ubiquity of this inhibitory transmitter in the hypothalamus. This question is particularly relevant in tuberoinfundibular dopamine (TIDA) neurons, which control the pituitary secretion of prolactin through tonic inhibition, and where GABA is a co-transmitter. Here, we address this issue in whole-cell recordings of TIDA neurons *in vitro*from juvenile male rats, where these cells exhibit robust network oscillations. In the presence of GABA, oscillation frequency remained unchanged but the de- and hyperpolarizing phases shifted temporal relationships; higher GABA concentration abolished oscillations. This effect could partly be explained by a $GABA_B$ receptor-mediated hyperpolarization (via GIRK channels). The selective $GABA_A$ agonist, muscimol also abolished the oscillation, but at a depolarized membrane potential. Pharmacological blockade of the $GABA_A$ receptor attenuated firing by shortening UP states, indicating endogenous release. Surprisingly, paired recordings failed to reveal evidence of inhibitory synaptic TIDA-TIDA connectivity. However, a tonic extrasynaptic $GABA_A$ current was identified. In the presence of THIP or DS-1, selective agonists for δ subunit-containing extrasynaptic $GABA_A$ -R, membrane potential was modulated such that relatively hyperpolarized TIDA neurons depolarized, and relatively depolarized neurons hyperpolarized towards a membrane potential of -70 mV. These data suggest that GABA provides a stabilizing influence on TIDA network activity, possibly providing feedback autoinhibition. This homeostatic control, rather than relying on hard-wired inhibitory recurrent axon collaterals appears to be encoded as fluctuations in ambient extrasynaptic GABA, analogous to what has recently been found for dopamine autoreceptor control in this system.

PS2.00194 SUPRACHIASMATIC- ARCUATE INTERACTION; AN ANALYSIS OF LEPTIN SIGNALING

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The suprachiasmatic nucleus (SCN), as the master clock, interacts with several hypothalamic regions to coordinate circadian rhythmicity. The interaction between the SCN and the arcuate nucleus (ARC) has been shown to be important in modulating several rhythms such as locomotor activity and temperature. Moreover, the ARC is an important metabolic sensor for circulating molecules, such as corticosterone and leptin, which act as feedback signals to the brain. Leptin, specifically, can act on AgRP and POMC neurons in the ARC to regulate food intake and energy expenditure, and alterations in leptin signaling in the brain can lead to obesity. On the other hand, many studies have shown that food intake during the resting phase

in rats promotes liver steatosis and visceral fat accumulation, among other metabolic disturbances. We hypothesized that these metabolic alterations in day-fed rats could be partially due to a differential leptin sensing by the ARC and other brain regions depending on the time the day. We have analyzed leptin signaling and brain activation in the ARC and several other hypothalamic regions after administrating leptin at different time points. These data could contribute to better understand the physiopathology of obesity and metabolic syndrome.

PS2.00195 NEURAL MECHANISMS UNDERLYING CIRCADIAN PATTERN OF COMPETITIVE SOCIAL VALUE DECISION

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Circadian rhythm regulates most of the mammalian physiology and cognitive functions. However, it remains largely unknown whether the circadian rhythm impacts social behavior. To address circadian regulation of social behavior, we utilize a behavioral paradigm offering a proxy to social value decision. In this paradigm, subject male mice freely explore a three-chambered arena to investigate two intrinsically attractive social targets, novel male mouse and familiar female mouse, each representing social novelty seeking and sexual preference, respectively. The decision of the subject, as reflected in the time investigating each target, exhibited a robust circadian pattern, where neither social novelty recognition nor sexual preference *per se* was affected by the time of the day. The behavior pattern of knockout mice lacking core clock element indicates the contribution of molecular clockwork to circadian pattern of social value decision. To understand neural mechanisms underlying the circadian bias of social value decision, we employ three independent strategies. First, we analyze the neural circuits connecting the suprachiasmatic nucleus (SCN) and the brain regions implicated in social behaviors. Second, we investigate the contribution of neuromodulators in the social decision. Third, we profile molecular clock in the brain regions critically implicated in the perception of social information. Taken together, social value decision of mice is regulated by circadian pattern through multiple underlying mechanisms. Further investigation of detailed neural mechanisms will offer an insight into how circadian clock machinery orchestrate social behavior of an individual.

PS2.00196 MUS MUSCULUS MOLOSSINUS: A NOVEL MODEL TO STUDY SEASONAL REPRODUCTION AND HYPOTHALAMIC DEVELOPMENT

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In seasonal mammals, melatonin regulates reproduction via a central network involving thyroid-stimulating hormone β (TSHβ) in the *pars tuberalis*, thyroid hormone deiodinase 2 (Dio2) in the tanycytes, and the RFamide peptides RFRP and kisspeptin in the hypothalamus. This study explored the photoperiodic regulation of the TSH/Dio2/RFamides pathway in various mice strains; the melatonin-deficient C57, the melatonin-proficient CBA lacking seasonal regulation of reproduction and the wild-derived *Mus musculus molossinus* (MSM) showing altered gonadal activity in response to melatonin. Adult male mice were kept for 6 weeks under long (LP: 16h light (L):8h darkness (D)) or short (SP: 8L:16D) photoperiod before gene expression analysis by *in situ* hybridisation. In LP, only MSM mice had larger reproductive organ size, but both CBA and MSM showed higher *TSHβ, Dio2* and *rfrp* expression as compared to SP, while *kiss1* expression remained unchanged. In C57, no photoperiodic variation was observed. Thus, photoperiodic variation in *rfrp* expression arises as a strong index for melatonin integration independently of the reproductive status. We further investigated how MSM pups integrate the maternal melatonin message *in utero* by keeping dams under LP or SP during pregnancy. At birth, *TSHβ* and *dio2* expression was higher in offspring gestated in LP than in SP. Adults gestated in LP showed higher gonadal size together with higher *TSHβ, Dio2* and *rfrp* expression as compared to SP-gestated animals. These results highlight MSM as a novel model to study the impact of melatonin on hypothalamic development and seasonal reproduction.

PS2.002 SEROTONIN 5-HT2C RECEPTOR REGULATES IMPULSIVE BEHAVIOUR

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Impulsive traits characterize many psychiatric conditions. These include binge eating disorder, attention deficit hyperactivity disorder, pathological gambling and substance abuse disorder. There are different types of impulsivity which rely on distinct behavioural processes and neuronal pathways. In general, impulsive behaviour can be divided into two categories: impulsive action and impulsive choice. While the 5-hydroxytryptamine 2C receptor (5-HT_{2C}R) has been implicated in the overall regulation of impulsive behaviour, its precise functions in its distinct components is less clear. Here we aim to clarify the role of the 5-HT_{2C}R in both facets of impulsive behaviour using two well validated operant conditioning tasks. We employed a differential reinforcement of low rates schedule to analyse impulsive action. In this response inhibition task, subjects were required to withhold a response for certain time until the reinforcer was available. In order to asses impulsive choice, we used an inter-temporal choice task where mice chose between 1 pellet delivered after a short delay (2 s), and 4 pellets delivered after a longer delay (28 s). We found that the 5-HT_{2C}R agonist lorcaserin reduced impulsive action, but increased impulsive choice in self-controlled individuals. To investigate the role of corticostriatal 5-HT_{2C} receptor expressing cells in this process, we employed a

chemogenetic approach to selectively manipulate these neurons in the prefrontal cortex (PFC), nucleus accumbens (NaC) and ventral tegmental area (VTA) of 5-$HT_{2C}R^{CRE}$ mice. Activation of VTA 5-HT_{2C} receptor expressing neurons, but not PFC or NaC, significantly reduced impulsive action. These studies identify the therapeutic potential of subsets of 5-HT_{2C}R expressing neurons in the management of impulsive action. Finally, we provide evidence that 5-HT_{2C}R activation has opposing effects on impulsive action and impulsive choice, suggesting different underlying neurological mechanisms.

PS2.0020 NEONATAL EXPOSURE TO SEX HORMONES AFFECTS GHRELIN RECEPTOR EXPRESSION IN VENTRAL TEGMENTAL AREA.

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Neonatal programming with sex hormones at postnatal day 1(PND-1) produces long-term functional changes on brain circuits related to reward and locomotion. The neonatal exposure to Estradiol Valerate (EV) at PND-1 increases the content of dopamine in Ventral Tegmental Area (VTA) and the expression of tyrosine hydroxylase at PND-60. Ghrelin is a stomach-synthesized peptide hormone which regulates the energetic state and growth hormone release. VTA Ghrelin administration increases ethanol intake, while antagonists of ghrelin receptor (GHSR) decrease this behavior. In addition, the plasma levels of ghrelin are higher in women than men, suggesting in humans a secretion sexually dimorphic. The aim of this study was to evaluate the effect of neonatal exposure to EV (0.1mg/50µL), TP (1mg/50µL), DHT (1mg/50µL) or the vehicle sesame oil (50µL) on the GHSR mRNA expression in VTA of adult rats that has been exposed or not to ethanol intake. In female, the GHSR mRNA expression was lower in EV and TP than control rats, while in male was in TP and DHT rats. In rats exposed to ethanol the higher ethanol intake was in EV) and TP rats regard to DHT and control) rats. Interestingly, the VTA expression of GHSR increased 5 times in EV female rats exposed to ethanol intake. These results suggest that the neonatal exposure to sex hormones affects the ghrelin system in the VTA. These results suggest that the highest GHSR mRNA expression could be an important factor in the large ethanol intake observed in EV rats. **Financial support:** FONDECYT Grant N°116-0398

PS2.0021 SELECTIVE MNPO INHIBITION ATTENUATES ANG II-INDUCED DRINKING BEHAVIOR AND NEUROENDOCRINE FUNCTION

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Angiotensin II (Ang II) is a peptide hormone that contributes to body fluid balance and hypertension. Forebrain circumventricular organs are sensitive to circulating AngII and project to the median preoptic nucleus (MnPO). The MnPO projects to the paraventricular nucleus (PVN) and contributes to elevated sympathetic tone and thirst. We used Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) to determine the role of the MnPO in drinking and neuroendocrine responses to peripheral Ang II in adult male Sprague-Dawley rats (250-300g). Rats were anesthetized with isoflurane and stereotaxically injected with an inhibitory (Gi) DREADD (rAAV5-CaMKIIa-hM4D(Gi)-mCherry; n=6) or control (rAAV5-CaMKIIa-mCherry, n=5) virus in the MnPO. After 2 weeks' recovery, each rat was administered 10 mg/kg of exogenous clozapine-N-oxide (CNO) to inhibit DREADD-expressing cells or vehicle ip followed by 2 mg/kg AngII sc twice per week for 4 weeks. DREADD-injected rats subsequently treated with CNO during Ang II exposure had a significantly attenuated drinking response compared to vehicle treatments and controls ($p<0.05$). Interestingly, DREADD-injected rats exhibited a diminished drinking response to Ang II during subsequent vehicle treatments after the first CNO treatment. Brain tissue was processed for cFos and mCherry immunohistochemistry. Overall, CNO-induced inhibition during Ang II exposure decreased cFos expression in the MnPO ($p=0.002$), PVN ($p<0.05$), and other downstream nuclei compared to controls. Ang II significantly increased plasma vasopressin, which was significantly attenuated during CNO-induced inhibition of the MnPO ($p=0.009$). The results indicate that CNO-induced inhibition of CaMKIIa-expressing MnPO neurons influences activity in downstream regions controlling drinking behavior, neuroendocrine and autonomic regulation.

PS2.0022 ANTAGONIZING DORSAL HIPPOCAMPAL DOPAMINE RECEPTORS SEX-SPECIFICALLY AFFECTS SOCIAL LEARNING IN MICE

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Dopamine (DA) is involved in the mediation of many behaviors including social learning, social behavior and feeding. Our previous work using systemic administrations of DA receptor antagonists implicate DA D1-type receptors in social learning, and DA D2-type receptors in feeding behavior in the social transmission of food preferences (STFP) in mice (Choleris et al., 2011). DA projections from the ventral tegmental area ascend directly to the hippocampus, a

structure that is implicated in the STFP. In our previous reports (Matta et al., 2016, 2017) we showed that D1-type and D2-type receptors in the dorsal hippocampus mediate social learning in the STFP differently in male and female mice. Specifically, we found that antagonizing dorsal hippocampal D1-type receptors with SCH23390 (1, 2, 4 or 6 μg/μL) 15 min before a 30 min social interaction blocks male social learning at the lowest and two highest doses, while female social learning was only blocked at the highest dose of SCH23390 (Matta et al., 2017, Neuropsychopharmacology). Conversely, antagonizing dorsal hippocampal D2-type receptors with Raclopride (10, 14, 18 or 20 μg/μL) 10 min prior to a 30 min social interaction blocked social learning in female mice (at the two highest doses), but not male mice (Matta et al., 2016). These results suggest sex differences in dorsal hippocampal DA receptor mediation of social learning: males rely only on D1-type receptors, whereas in females both D1-type and D2-type are involved. We will be emphasizing the possible involvement of gonadal steroids in these sex differences. Supported by NSERC.

PS2.0023 MATERNAL DIETARY ENVIRONMENTAL INFLUENCES ON THE BEHAVIOR OF NONHUMAN PRIMATE OFFSPRING

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In recent decades, prevalence of pediatric neurodevelopmental disorders has risen dramatically. Perinatal environmental factors such as poor maternal diet influence the risk of these disorders. In these studies, we compared the impact of maternal malnutrition (excess fat versus protein deficiency) on brain development and behavior of nonhuman primate offspring. We hypothesized that exposure to maternal high-fat diet or a protein-deficient diet impacts development of neural circuits critical in the regulation of behavior. We first examined offspring from female Japanese macaques that consumed either a control diet (13% of calories from fat) or a high-fat diet (HFD) (37% calories from fat) during gestation and lactation. In a second study, we examined rhesus macaque offspring from mothers that consumed either a control diet (26% protein) or a low-protein diet (LPD) (13% protein) diet during gestation and lactation. In both studies, offspring growth, energy balance, and behavior were tracked closely across development. Brain development was examined using a combination of histochemical and magnetic resonance imaging studies. Interestingly, both exposure to maternal HFD or LPD impaired offspring brain development and resulted in increased offspring anxiety and impairments in social behavior. Exposure to maternal HFD resulted in impairments in the development of the central serotonergic and dopaminergic system and alterations in functional connectivity. Exposure to a low-protein diet during gestation resulted in decreased white matter maturation. These findings indicate that inadequate maternal nutrition (either excess of

insufficient nutrient intake) initiates a fetal environment that results in neural reprogramming and predisposes offspring to pediatric neurodevelopmental disorders.

PS2.0024 PROCEPTIVE BEHAVIORS INDUCED BY OVARIAN STEROIDS ARE HIGHER IN HIGH-YAWNING RATS

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The female rat is the key stimulus for the display of sociosexual behaviors during mating. During estrous, female rat show characteristic behaviors including lordosis and proceptive behaviors such as: hopping, darting, and ear wiggling. The aim of the present study, was to analyze female sexual behavior in selectively bred for high- (HY) and low- (LY) yawning frequency, and Sprague-Dawley (SD) rats. All female rats were evaluated in pairs either with sexually experienced male rats from HY, LY or SD. Sexual receptivity was induced by sequential administration of estradiol benzoate (5 µg), and 44 h later by progesterone 2 mg diluted in 0.1 mL of olive oil and administered s.c. All subjects were tested 4h later with a sexual vigorous male of the same or different group of rats in circular Plexiglas arena (50 cm diameter). Our results showed that all female rats showed adequate lordosis quotient greater than 85% independently of the type of rats. However, the intensity of lordosis were higher in HY mean 2.1; respect to LY and SD with 1.77 and 1.80, respectively ($P<0.05$). Importantly, HY female rats showed more proceptive behaviors independently of the type of male rats tested ($P<0.05$). In conclusion, HY are more responsive to the effects of ovarian steroids to induced proceptive behaviors indicating that the hypothalamic nuclei involved in the display of different components of female sexual behavior diverge in HY rats. Partly supported by VIEP-BUAP 2018 and CONACyT grants 243333 and 243247 to CC and JRE. CONACyT fellowship 662091 to AMB.

PS2.0025 MEDIAL TEMPORAL LOBE STRUCTURAL INTEGRITY AND RECOGNITION MEMORY FOLLOWING OOPHORECTOMY

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Women who have oophorectomy prior to natural menopause (OPNM; prior to age 50) are at higher risk of Alzheimer's Disease (AD) later in life. We wondered if we would observe mid-life changes in brain regions sensitive to AD in women with OPNM. The perirhinal cortex (PRC), an

extra-hippocampal structure, is one of the first regions of the brain to demonstrate AD-related neurodegeneration. Additionally, impairments in visual recognition memory (VRM) are observed prior to the onset of clinical symptoms. Research in animals and post-menopausal women suggests that estrogens are neuroprotective. However, no studies to date have addressed the impact of estrogen loss on PRC and VRM. We hypothesize that women with OPNM will demonstrate smaller relative PRC volume and impaired VRM compared to age-matched controls (AMC), and that hormone use will attenuate this effect. In the current study, which is part of a larger longitudinal project, an OPNM group, AMC, and a naturally menopausal control group were given a VRM task and T2-weighted scans were collected perpendicular to hippocampal long axis from a 3T scanner. MTL volumes were obtained via manual segmentation by experimenters blind to group membership. Preliminary analyses support the idea that OPNM affects PRC volume and VRM. The results of this study have important implications for understanding the time course of AD-risk post-oophorectomy. This is the first study to examine the impact of spontaneous menopause and OPNM on PRC volume.

PS2.0026 CENTRAL OXYTOCIN RECEPTORS SUPPORT VOCALIZATIONS BETWEEN FEMALE DEGUS

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Oxytocin and related nonapeptides have been shown to play a role in the production of vocalizations across animal taxa (e.g. frogs, birds, and rodents). The importance of oxytocin for the expression and perception of vocalizations may contribute to the observed roles of this peptide in social behaviors ranging from mothering (Marlin et al., 2015) to mating (Floody et al., 1998). We examined the role of central oxytocin receptors in peer affiliation behavior by quantifying physical and vocal social interactions in recently separated and reunited, adult female degus. Female cagemates were separated for 24 hour periods and then administered intraventricular infusions of either an oxytocin receptor antagonist (OTRA) or artificial cerebral spinal fluid before recording behavior during a 20 minute "reunion" session. Unexpectedly, OTRA infusion was not associated with changes in the amount of physical interaction (e.g., face-to-face, allogrooming, or agonistic interactions); however, the drug did appear to cause a substantial and dose-dependent decrease in vocalizing. These results are consistent with evidence that the brain's oxytocin system plays a critical role in vocal communication, and also demonstrates that this function can be dissociated from social motivation more generally.

PS2.0027 PROFESSIONAL DEVELOPMENT THROUGH EVIDENCE-BASED PUBLIC ENGAGEMENT

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Engaging with the public is a professional responsibility, a civic duty, and expected by funding agencies. Although Americans have confidence in science and trust in scientists, science denial is rising across the political spectrum. Participation in public engagement, however, is often limited to "the dedicated few," as many are unaware that engagement 1) develops critical professional skills, including communication, teaching, and leadership; and 2) enriches understanding of scientists' own research. With support from the NSF BEACON Center for the Study of Evolution in Action, we developed evidence-based training modules to motivate, recruit, prepare, and match scientists with engagement opportunities. Our modules include background on STEM public engagement, how to engage effectively with different audiences, including policy makers, and more. We share our materials widely to be implemented at any institution and concurrently enhance professional development and public engagement. We also gathered data to learn more about the ways scientists already engage. Analysis of BEACON scientists revealed popular means of engaging (e.g., face-to-face), as well as underrepresented areas (e.g., policy). Individual engagement behavior also appeared patterned, where scientists tend to engage in activities with similar levels of investments, audiences, or format. These data allow us to plan for appropriate training and engagement opportunities and identify groups (academic and public) that are not being reached. Ultimately, we aim to fundamentally change how and why scientists learn to communicate with diverse and influential audiences. Effective public engagement may be central to ensuring the future of scientific research and, in turn, a thriving society.

PS2.0028 CAUSES AND CONSEQUENCES OF INDIVIDUAL VARIATION IN SOCIAL GROUPS

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Social groups often vary over time and across contexts due to variation within an individual and differences among individuals. This variation arises from different social, physiological, neuromolecular, and genetic factors, and has important consequences for social interactions and group structure, which ultimately can affect individual health and evolutionary fitness. *How do we comprehensively examine the causes and consequences of individual variation?* One way to develop an integrative understanding of individual phenotype is to examine co-variance of traits across multiple biological levels and within a dynamic social environment. We can subsequently identify the effects of the individual on the behavior and reproductive success of other members within the social group. Here, we uncover integrated phenotypes for individuals

within a group of highly social African cichlid fish, *Astatotilapia burtoni*, using measures of hormonal, neuromodulatory, and transcriptomic patterns associated with social behaviors. We monitored males and females from eight different groups over six weeks and found remarkable variation across groups in the behavior associated with territorial males, with corresponding changes in social interactions and group structure. Linking this behavioral variation with measures of sex steroid hormones and gene expression across brain regions critical to the regulation of social behavior allows us to understand the mechanisms that generate individual variation, including variation in evolutionary fitness. This research will contribute to a more detailed understanding of physiological and neuromolecular underpinnings of individual variation, and will provide causal insights into the effects of this individual variation on other members of a social group.

PS2.0029 THE ROLE OF ANDROGENS AND SLEEP APNEA ON BRAIN AND BEHAVIOR

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Sleep apnea (SA) affects a quarter of the population in the United States, and contributes to elevated oxidative stress (OS) and cognitive deficits. Sex differences exist in SA, suggesting a role for androgens and estrogens. To investigate how hormones influence the hypoxic events of SA on brain on behavior, our lab used chronic intermittent hypoxia (CIH) in male rats, which mimics a sleep apnea/hypopnea index of 8. We hypothesized androgen modulation of CIH induced OS leads to cognitive dysfunction. Male Long-Evans rats were divided into different hormone groups: gonadally intact, gonadectomized (GDX), GDX + testosterone (T), gonadally intact + T, or GDX + dihydotestosterone (DHT). Subcutaneous silastic capsules containing androgens were used. Following 7 days exposure to either room air or CIH, MWM testing was performed over 5 days. Both learning and memory behaviors were assessed. At the conclusion of behavior testing, rats were euthanized and samples collected for biochemical analysis. CIH only induced cognitive dysfunction in DHT rats. Regardless of CIH exposure, the GDX rats exhibited memory deficits. Interestingly, exogenous T administration to GDX rats blocked cognitive dysfunction. Results showed that CIH only increased OS and decreased T in gonadally intact rats and DHT rats. CIH did not affect OS in GDX rats or rats with exogenous T. In addition to circulating OS, DHT rats had higher OS proteins in the hippocampus, a brain region involved in learning and memory. This implies the sex differences observed in SA may be due to androgens elevating OS.

PS2.003 EFFECTS OF TESTOSTERONE, TIME-PRESSURE VS DELAY, AND A PERSONALITY RISK FACTOR ON MEN'S COOPERATION

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Recent evidence suggests that under time-pressure, humans are more likely to make cooperative decisions; when forced to deliberate, they are more likely to act in ways that are self-interested (Rand, 2012). Interestingly, testosterone has also been shown to predict processes relevant to impulsive behavior, such as reduced cognitive reflection (Nave et al., 2017), and reactive aggression following provocation (Carré et al., 2017). The present study was designed to test whether the predictions of the social heuristic hypothesis would be exaggerated when men received exogenous testosterone (versus placebo), and whether such effects would be moderated by a personality risk indicator. In the largest testosterone administration study to date, 400 men completed personality questionnaires, and then received 11 mg of testosterone nasal gel or equivalent placebo (between-subjects design), and later played a one-shot public goods game. Consistent with past work, a main effect of time-pressure predicted increased cooperation, but only among participants who followed the timing instructions. However, a drug x time condition x risk factor interaction showed that testosterone reduced cooperation under conditions of forced-delay (but not under time-pressure), with effects specific to men who were high on the personality risk factor (i.e., those with high trait dominance, low self-control, and independent self-construals). Results are discussed within an evolutionary framework suggesting that in the absence of information from social partners, testosterone may promote self-interested behaviours, and particularly among those with self-interested dispositions.

PS2.0030 EFFECT OF GONADAL STEROID HORMONES ON ABSENCE SEIZURES IN THE MYELIN MUTANT TAIEP RAT.

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Absence seizures are characterized by spike-wave discharges (SWD) in the cerebral cortex in the myelin mutant *taiep* rat. SWD are sexually dimorphic pattern being males more susceptible than female rats. So, thalamo-cortical circuit must be modulated by gonadal steroids and it is important to determine the role of estrogens and progesterone on SWD. The aim to this study was to analyze the effects of estradiol and progesterone on the frequency and duration of SWD. We implanted 3 cortical electrodes under deep anesthesia (ketamine/xylacine mixture) 9

months old female rats, one week later were ovariectomized also under deep anesthesia. Then after 5 days of recovery period we s.c. diluted in olive-oil administered estradiol benzoate (10 µg/day) or progesterone (20mg/Kg) and 24 h later evaluate frequency and duration of SWD in continuous 24h electroencephalographic recordings using Harmonie system (Canada). Our results show after estradiol administration significantly reduced the duration of SWD ($P<0.05$), but not their frequencies. However, progesterone produced the opposite effect increasing the duration of SWD ($P<0.05$), but the frequency of SWD were similar to control (oil-treated) rats. In conclusion, ovarian steroids change the duration of SWD in this model of absence seizures suggesting that thalamo-cortical network is susceptible to these hormones that could be the base for new treatment options in this type of epilepsy. Partly founded by CONACYT grants 243333 and 243247 to MCC and JRE, respectively and also by VIEP-BUAP 2018, and CA Neuroendocrinología BUAP-CA-288

PS2.0031 HIGH-YAWNING DAMS CHANGE THE YAWNING AND PENILE ERECTION FREQUENCIES INDUCED BY QUINPIROLE

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Systemic administration of dopaminergic D2 agonists-induced an increase in yawning and penile erection frequencies. We obtained by inbreeding a subline from Sprague-Dawley (SD) rats with a high-yawning (HY) frequency. However, the display of masculine sexual behavior differ in HY because they showed longer inter-intromissions intervals that delayed ejaculation. HY dams also showed a careless maternal care because they constructed low quality nests and showed different retrieving display. In base of these differences the aim of this study was to analyze the yawning and penile erection frequencies induced by quinpirole a D2 agonists in cross-fostered HY and SD rats at 3 months of age. HY male rats nurture by HY dams had significantly higher frequencies of yawning and penile erection induced by three different doses of quinpirole (25, 50 and 100 µg/Kg) respect to that obtained in SD rats ($P\leq0.05$). Importantly, when HY dams nurture SD male rats they significantly increased their yawning and penile erection frequencies induced by 50 µg/Kg of quinpirole ($P\leq0.05$). When SD nurture HY or SD rats there were a significant reduction on yawning and penile erection frequencies ($P\leq0.05$). In conclusion, maternal care is capable to change the magnitude of quinpirole-induced both behaviors, probably acting on the paraventricular hypothalamic nucleus a key structure for the induction of yawning and penile erection in rats. Supported by VIEP-BUAP 2018 and CONACyT grants No. 243333 and 243247 to CC and JRE. MADN was CONACyT fellowship No. 662091.

PS2.0032 MATERNAL CARE CHANGE EJACULATORY FREQUENCY IN HIGH-YAWNING AND SPRAGUE- DAWLEY RATS

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Ejaculation frequency vary among male rats with a Gaussian distribution being 1% non-copulators (NC), 10% sluggish (S), 10% premature (P) and 69% average (A) copulators. We had a subline from Sprague-Dawley (SD) rats with a high-yawning (HY) frequency. These animals had higher percentage of NC and the dams showed different maternal care because they spend less time in the nest, and has re-retrieving and atypical retrieving of the pups. In base of this, the aim of this study was to determine the role of maternal care on the percentage distribution of ejaculatory distribution when tested with ovariectomized female rats with estrous induced by sequential administration of estradiol and progesterone. We used the cross-fostering between HY and SD rats to determine the effects on male sexual behavior. Our results showed that HY male rats raised by their biological mothers were 18% P, 27% A, 36% S and 19% were NC. However, if HY pups were raised by SD dams the distribution was the following 54% P, 1% A, 45% S and none NC. SD male rats raised by SD dams showed the following proportions 6% P, 69% A, 25% S and 0% NC rats. Finally, SD raised by HY dams showed 8% P, 61% A, 31% S and 0 NC. In conclusion, SD dams significantly improve sexual performance of HY males suggesting change in the hypothalamic neural circuits involve in male sexual display during early life.

PS2.0033 EFFECTS OF EXOGENOUS TESTOSTERONE ON RATINGS OF ATTRACTIVENESS: DOES RELATIONSHIP STATUS MATTER?

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Previous work indicates that men in committed relationships devalue the attractiveness of potential alternative mates. Some have suggested that devaluation of potential alternative mates may represent a cognitive mechanism that promotes relationship maintenance. Notably, a wealth of evidence in both animal models and humans suggests that testosterone may regulate mating effort. Also, recent evidence in the monogamous California mouse indicate that testosterone increases mating effort among single males, but decreases mating effort among paired males. Here, using a within-subject, placebo-controlled, pharmacological challenge experiment (n = 92), we examine whether testosterone has similar divergent effects in human males, as indexed through their ratings of attractiveness of women. Although preliminary analyses did not provide support for these divergent effects (p = .68), follow-up analyses restricted to the most and least attractive women (as previously established by an independent

set of raters) revealed a more complex three-way interaction. Whereas testosterone increased the perceived attractiveness of less attractive women in single men, it increased the perceived attractiveness of more attractive women in paired men. Therefore, testosterone may modulate the perceived attractiveness of women, but these effects seem to depend on both the male's relationship status, and the attractiveness of the woman being evaluated.

PS2.0034 ULTRASTRUCTURAL ANALYSIS OF THE PEPTIDERGIC NEURONS MEDIATING ITCH SENSATION

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Neuropeptide systems, including those of gastrin-releasing peptide (GRP), are widely distributed throughout the central nervous system and are significantly involved in various physiological functions and behaviors. Recently, the spinal GRP/GRP-receptor system has been identified as an itch-specific mediator in the somatosensory system, and we focused on this GRP as an itch neuronal marker. Immunohistochemical analysis showed that the GRP was expressed in the small-sized primary afferents. The GRP-immunoreactive terminals projected to the superficial layers of the spinal cord and localized in close proximity to GRP-receptor in this region. Pharmacological and behavioral analysis showed that scratching behavior induced by peripheral itch mediator was inhibited by intrathecal injection of GRP-receptor blocker. We then analyzed the ultrastructure of GRP-immunoreactive terminals using a variety of electron microscopies combined with immunohistochemistry. Transmission immunoelectron microscopy showed that GRP-positive terminals contained various excitatory neurotransmitters and dense-cored vesicles. Furthermore, we used 3-dimentional scanning electron microscopy (3-D SEM) combined with immunohistochemistry to analyze the 3-D ultrastructure of the itch-mediating synaptic formation. This 3-D SEM analysis revealed that GRP terminals formed consecutive varicosities and connected with more postsynaptic components (e.g., dendrite, axon, and glial cells) than expected. Thus, the 3-D SEM allows for 3-D visualization of itch-mediating synaptic structures / networks composed of peptidergic neurons.

PS2.0035 THE INTERACTION OF GHRELIN & ENDOCANNABINOID SYSTEMS IN THE VTA IS CRITICAL FOR MODULATING FEEDING

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Ghrelin and endocannabinoids (eCBs) are known to promote feeding behaviours within feeding related brain regions such as the hypothalamus (HYP) and ventral tegmental area (VTA) by binding and activating growth hormone secretagogue receptors (GHSRs) and cannabinoid receptors (CB-1Rs), respectively. Within the HYP, ghrelin and eCB systems jointly modulate feeding behaviours as inhibiting receptors of either system attenuates the orexigenic capacity of ghrelin and/or endocannabinoids when administered alone. Recent data suggests that a similar interaction may also be important for modulating feeding behaviours within the VTA of rats. To explore this further, we set out to test if pharmacological blockage of CB-1R within the VTA would influence ghrelin's capacity to increase feeding following its infusion into the VTA. To this end, we implanted male rats with cannulae aimed at the VTA (ML= 2 mm, DV= 7.8 mm, AP= 5.6 mm) and assigned them to one of four treatment groups: vehicle/saline, rimonabant (0.5 μg)/saline, vehicle/ghrelin (1 μg), rimonabant (0.5 μg) /ghrelin (1 μg). On test days, rats were pre-treated with vehicle or rimonabant and then infused with either saline or ghrelin (1 μl total volume). Acute food intake and locomotor activity of each animal was subsequently monitored. Although locomotor activity did not differ between treatment groups; inhibition of the eCB system within the VTA attenuated intra-VTA ghrelin induced feeding. These data support the hypothesis that ghrelin and eCB systems collaboratively regulate feeding within the VTA.

PS2.0036 THE EFFECT OF CO-TWIN GENDER ON SEXUAL AND AGONISTIC BEHAVIOR IN THE FEMALE GOAT (CAPRA HIRCUS)

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Gender of a female's sibling in utero may affect sexual differentiation and subsequent sexual dimorphism of her adult behavior. Other than freemartins with ovo-testes (genetic chimeras), this phenomenon has not been investigated in non-litter bearing animals such as goats. To determine if females (does) that have a male twin (1M does) are behaviorally masculinized compared to does that have a female twin (0M does), multiple experiments were conducted during three breeding seasons utilizing 14 1M and 14 0M adult Alpine does. Sexual and agonistic behaviors performed by 1M and 0M does, as well as the does' social affiliation differences, were studied. In estrus, 1M does tended to spend more time than 0M does in close proximity to a confined male (buck) ($P=0.07$). 1M does also tried to gain access to a buck ($P<0.05$), and tended to present towards the buck, more frequently than did 0M does ($P=0.08$). 1M does tended to urinate in front of a buck more than 0M does ($P=0.1$). Receptivity did not differ between the groups. Buck (n=17) preference for 1M versus 0M estrous does was also examined. Bucks courted 1M females more than 0Ms ($P<0.05$), but they did not spend more time with 1M does. When away from bucks, 1M does engaged in more female-female sexual behaviors than did 0M does ($P<0.05$). Both near and away from bucks 1M females engaged in more agonism ($P<0.05$). We conclude that prenatally, partial masculinization of both sexual and agonistic behavior occurs in female goats that are twins with males.

PS2.0037 POSSIBLE INVOLVEMENT OF THYROID HORMONE IN SEX DIFFERENTIATION OF RAT BRAIN

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Although it has been demonstrated that thyroid hormone plays an important role in normal development of brain, little is known how thyroid hormone influences on sexual differentiation of the mammalian brain. In this study, we examined effects of Thiamazole (MMI), a thyroid hormone synthesis inhibitor, during the critical period of brain sexual differentiation on adulthood sexual behavior in male rats. MMI was treated to ED18 – PD6 male rat pups via drinking water of their dams at doses of 0 (CONT), 0.002% (LOW) and 0.02% (HIGH). In Experiment I, they were orchidectomied and simultaneously implanted with a Silastic capsule containing testosterone at 4 weeks old (isoflurane anesthesia). After maturation, they were weekly subjected to preference tests for conspecific odors (male vs. receptive female) and copulation tests, indicating that HIGH males showed mild impairment of male-type preference and significantly decreased number of intromissions in copulation test. In Experiment II, they were orchidectomied at 4 weeks old, and implanted with a Silastic capsule containing estradiol at 9 – 10 weeks old followed by weekly olfactory preference tests and female sexual behavior tests with stud males. In the olfactory preference test, CONT males but not LOW and HIGH males preferred male odor to receptive female odor. In sexual behavior tests, HIGH males showed smaller number of rejections and significantly high lordosis quotients than CONT males. These results indicate a possible involvement of thyroid hormone in sexual differentiation of mammalian brain. Thyroid hormone may promote masculinization and/or defeminization brought by sex steroids during the perinatal critical period.

PS2.0038 DEVELOPMENTAL EFFECTS OF SYNERGISTIC ENDOCRINE DISRUPTION ON PARTURITION AND BEHAVIOR IN THE RAT

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In classic work by William C. Young's laboratory, it was established that organization of mammalian brain morphology is guided by expressed gonadal hormones *in utero* and that specific enzymatic alteration of gonadal hormones can occur in the undifferentiated neuron. Experiments manipulating embryonic environments in *oviparous* avian and fish models have demonstrated phenotypic sex expression opposite of genotype is possible in body morphology and in brain organization. However, currently no *viviparous* organisms with induced phenotypic sex opposite of genotype have been demonstrated, likely because of chemical complexities associated with internal gestation. Here, we report on effects of prenatal exposure to a chemical cocktail (tamoxifen, thiouracil, and corticosterone) at environmentally relevant levels that synergistically influence early development and alter the mammalian intrauterine environment in ways that may affect morphology and behavior. Initially, parturition difficulties

were observed in our dams exposed to this chemical cocktail. Administration of this chemical mixture to the intrauterine environment appears to have impacted gestation in ways that have altered development resulting in changes in morphology of the anogenital region and impacted righting responses as well as other functional measures of growth. With a reduced exposure to the chemical cocktail, we have had dams who have successfully completed parturition with extant pups. Examination of these pups has revealed anogenital differences, as well as other neuromuscular and behavioral effects. By comparing control and treatment populations at the neural, morphological, reproductive, and behavioral levels, we hope to gain deeper insight into the mechanisms driving sexual differentiation in mammals.

PS2.0039 NEONATAL TESTOSTERONE EXPOSURE MEDIATES SEX DIFFERENCES IN IMPULSIVE ACTION IN ADULT RATS.

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Previous work in our lab has demonstrated that neither pubertal nor adult gonadal hormones mediate an observed adult sex difference in impulsive action in rats. The goal of the current work was to examine the influence of neonatal gonadal hormones on sex differences on measures of impulsive action. Three groups of rats were used in the study. To serve as controls, one male group and one female group received subcutaneous injections of sesame oil vehicle on postnatal days 0 and 1. The third group, an experimental group of females, received 150 µg of testosterone propionate delivered subcutaneously in sesame oil vehicle on postnatal days 0 and 1. All three groups were gonadectomized prior to puberty (at 28 d of age). Beginning at ~100 d of age, all rats were trained on the 5-choice serial-reaction time task (5-CSRTT), a test for impulsive action. The task requires rats to identify (via nose poke) the location of a brief light stimulus among five possible locations. When training was completed, impulsive action, as measured by premature responding, was assessed during sessions in which the onset of the stimulus was unpredictably lengthened. Under testing conditions, control males and females given neonatal testosterone, indistinguishable from one another, made significantly more impulsive action responses than did control females. Results indicate that this sex difference results from organizing actions of testosterone during the neonatal period.

PS2.004 TRANSPLANTING IMMORTAL OREXIN CELLS IN NARCOLEPSY

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Narcolepsy is a sleep disorder caused by loss of orexin neurons in the lateral hypothalamus. By the nature of this disorder it could be reasoned that reinstating orexin transmission in the central nervous system (CNS) will lead to a recovery of behaviour. The primary objective of cell transplantation is to treat disease by reinstating lost transmission, but is dependent on the availability of cells with phenotype of those lost. Hence, the aim of this study is to investigate a novel orexin cell line and to perform CNS cell transplants using a mouse model of narcolepsy. To do this, we used a cell line isolated from transgenic mice (m) expressing green fluorescent protein (GFP) in orexin (ORX) neurons, isolated from the adult (A) hypothalamus (Hypo)—the mHypoA-ORX/GFP4 cell line. We performed immunocytochemistry and performed a live cell secretion assay, coupled with enzyme immunoassay. Next we performed cell transplant surgeries with these cells in a mouse model of narcolepsy (orexin-knockout, KO). All (100%) of neurons in the mHypoA/ORX-GFP4 (#cells=379; n=3) cell line expressed both orexin-A and GFP antigens. Using a secretion assay on the mHypoA-ORX/GFP4 cell line, we detected orexin-A secretion at baseline (0.276±0.030ng/ml; n=3; 5.0mM glucose media). We found that challenging mHypoA-ORX/GFP4 cells with hypoglycemia (0.2mM glucose media) significantly enhanced orexin-A secretion (0.337±0.031ng/ml; t-test; n=3; $p<0.01$). GFP+ cells were identified in the rodent brain at 2 (#cells=731±382; n=4), 14 days (#cells=1186±603; n=6) and 30 days 963±192 cells (mean±SEM; n=6) post-transplant. This experiment highlights the potential of using immortal cell lines in cell replacement therapy for narcolepsy.

PS2.0040 ZINC AS A THERAPY IN AN EXPERIMENTAL MODEL OF AUTISM PRENATALLY INDUCED BY VALPROIC ACID

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Autism is a complex developmental disorder characterized by numerous behavioral impairments, such as communication, socialization and cognitive inflexibility. In rodents, exposure to VPA during the pregnancy induced symptoms similar to those found in the human condition of autism.Exposure to VPA may alter zinc metabolism resulting in a transient deficiency of zinc. Therefore, we selected zinc as prenatal treatment to prevent VPA-induced deficiencies. In a rat model of VPA-induced autism we tested the treatment with zinc supplementation. Female rats received either saline solution or VPA (400 mg/ kg,i.p) on gestation day (GD) 12.5. In order to test the effect zinc supplementation, after 1 h of treatment with saline or VPA, a dose of zinc (2 mg /kg) was injected. The offspring was tested for abnormal communication behavior on lactation day 11 (LD), repetitive behavior and cognitive ability was tested on 29 LD and social interaction was tested on 30 LD. We also evaluated tyrosine hydroxylise protein (TH) expression. VPA group showed decreased ultrasonic vocalization, repetitive/restricted behavior, cognitive inflexibility, impairment of socialization,

characterized by decreased play behavior and reduction in striatal TH levels as compared with saline control group. Zinc supplementation generated behavioral improvement in the cognitive inflexibility, attenuated the VPA-induced deficit on social play behavior, and restored the vocalization pattern. However, we found no evidence of zinc effect on the VPA-induced reduction of the TH striatum levels. The persistence of low TH expression in VPA-Zn group suggests that Zn-induced behavioral improvement in autistic rats may not depend on TH activity.

PS2.0041 EFFECTS OF PERINATAL EXPOSURE TO A CONTAMINANT MIXTURE ON THE MATERNAL BRAIN: FOCUS ON THE MPOA

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Maternal behaviour is a collection of behaviours by the mother that can increase offspring survival. Estrogens bind and activate estrogen receptors in the Medial Preoptic Area (MPOA) and ventral Bed Nucleus of the Stria Terminalis to promote and facilitate maternal behaviors. We have previously shown that perinatal exposure to an ecologically relevant toxicant mixture (organochlorine pesticides, polychlorinated biphenyls and methyl mercury) increases the number of neurons in the CA3 region of the hippocampus as well as the number of glial cells in the CA3 but also in the MPOA. The current work seeks to characterize the effects of exposure to this mixture on the number of estrogen receptors in an area directly relevant to maternal behaviour. Pregnant rats were randomly assigned to either 1) full mixture (4.00mg/kg/day or 0.04mg/kg/day doses), 2) methyl mercury (1.0mg/kg/day or 0.01mg/kg/day) or corn oil vehicle. At postpartum day 21, brains were fixed, collected and sectioned at 30 μm. Immunohistochemistry for estrogen receptor alpha was completed and bilateral photographs were taken. Immunopositive cells were counted using Image J. While our earlier results show no effects of this toxicant on estrogen receptor numbers in the VTA, we anticipate a reduction in these numbers in the MPOA - final analyses are underway. Results from this study will deepen our understanding of estrogen sensitivity in the MPOA and how changes in receptor expression, from perinatal exposure to toxicants, may alter mother–infant interactions.

PS2.0042 EFFECTS OF INTRANASAL VASOPRESSIN AND OXYTOCIN IN A RODENT MODEL OF POSTPARTUM DEPRESSION/ANXIETY

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Postpartum depression and anxiety, which affect 10-20% of mothers, have negative effects on the health of both mother and offspring through effects on maternal behavior, yet little is known about the etiology of these disorders and specific treatment options are extremely limited. The hormones oxytocin (OXT) and vasopression (AVP) are both potent mediators of maternal behavior and have been implicated in depression and anxiety etiology. Furthermore, postpartum OXT is now recommended for all births in the US to prevent postpartum hemorrhage. One of the strongest predictors for depression and anxiety disorders is exposure to early life chronic social stress (ECSS). This project tested the efficacy of intranasal (IN) OXT and AVP in a ECSS based rodent model of postpartum depression and anxiety. The hypothesis was that these IN treatments would prevent ECSS induced depression of maternal care and increase in maternal anxiety. Maternal rats exposed to ECSS and non-stressed controls were administered intranasal saline, AVP, or OXT on day 2 of lactation and tested for maternal care and anxiety. Results indicate that IN AVP effectively prevented the ECSS induced depression of maternal care and increased maternal anxiety. In contrast, IN OXT treatment was ineffective at preventing the adverse effects of ECSS and increased measures of maternal anxiety. These results are interesting given our recent clinical epidemiology report of a positive association between peripartum pitocin exposure and depression and anxiety. The data support the hypothesis that intranasal AVP may be an effective preventative measure and/or treatment for postpartum mood disorders, and underscore the need for additional work on the effects of perinatal OXT.

PS2.0043 IMPACT OF ESTRADIOL TREATMENTS ON SUBCELLULAR LOCALIZATION OF HIPPOCAMPAL ERALPHA IN AGING OVX RATS

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Work from our lab has demonstrated that previous midlife estradiol treatment improves memory and results in lasting increases in levels of estrogen receptor alpha (ERα) in the hippocampus of aging ovariectomized rats months after hormone exposure has ended. Traditionally, ERα acts as a nuclear transcription factor. However, membrane ERα can also result in non-genomic, rapid acting effects. In an initial experiment, we found that previous midlife estradiol exposure increases ERα specifically in the nuclear compartment of hippocampal cells. However, it is currently unknown how this increase in ERα following previous estradiol exposure compares to that of ongoing estradiol exposure. The goal of the current work is to compare the subcellular localization of ERα after previous and ongoing estradiol exposure following ovariectomy. Middle-aged rats were ovariectomized and implanted with capsules containing either estradiol or vehicle. Forty days later, all capsules were replaced. Rats initially receiving vehicle capsules received another vehicle capsule (OVX controls), and rats initially receiving estradiol capsules received either another estradiol capsule (Ongoing E) or a vehicle capsule (Previous E). One month later, hippocampi were dissected and processed for subcellular fractionation. Hippocampal lysate was separated into cytosolic,

membrane, and nuclear compartments using a commercially available kit. All compartments were processed for western blotting for ERα. Results indicate that both ongoing and previous estradiol treatment increase nuclear levels of ERα, but do not alter cytosolic or membrane levels. These data suggest that the increase in ERα following ongoing and previous estradiol may result in transcriptional changes that impact hippocampal function.

PS2.0044 LEPTIN REGULATES VOCAL EFFORT IN ALSTON'S SINGING MICE: DATA FROM LAB AND FIELD

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When animal displays seem extravagant and expensive, they are often thought to indicate the body condition of the signaler. Nevertheless, we know little about how signalers adjust effort based on condition. To further examine this relationship, we first characterized physiological condition and acoustic displays in a wild rodent with elaborate vocalizations: Alston's singing mouse, *Scotinomys teguina*. We found two major axes of variation in condition – one defined by short-term fluctuations in caloric nutrients, and a second by longer-term variation in adiposity. Among song parameters, a major dimension of variation we labelled "song effort" was characterized by high song rates and longer songs. Song effort was highly correlated with measures of adiposity (leptin, adiponectin, insulin and residual body mass; R^2=0.49). Among these, leptin predicted display effort especially strongly (R^2=0.39). A casual exploration of leptin on song effort followed. Unfasted, lab-reared adult male mice received intraperitoneal injections of either saline or 1.0mg/kg leptin. Next, mice heard three stimuli in randomized order: silence, conspecific song, and frequency-matched tone. Leptin increased overall singing behavior. The relationship between song and playback, however, was complicated by an order effect: mice were more likely to sing to tone controls (but not silence) if they had recently heard a song. We interpret this to suggest that mice were updating responses to ambiguous stimulus based on social context. Overall, our results indicate that leptin plays an important role modulating display effort in male *Scotinomys teguina*. Given the broad conservation of both energy balance and social behavior circuits, we hypothesize that this may be a general pattern in the regulation of vertebrate display.

PS2.0045 EFFECTS OF DEVELOPMENTAL AIR POLLUTION ON INFLAMMATION, BEHAVIOR AND NEUROANATOMY IN RATS

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There is convincing evidence that living close to major roadways and highways is associated with increased morbidity and mortality. Data indicate that traffic-related air pollution (TRAP) contributes to these adverse health outcomes. Two pollutants in TRAP that are elevated near major roadways are particulate matter (PM) with a diameter of 2.5 um of less (PM2.5) and ultrafine particles (UFP; <100 nm). Animal toxicology studies and recent human epidemiology suggest that exposure to PM affects cardiovascular health and the nervous system. There is epidemiological evidence of neurological effects including white matter loss, impaired cognition in the young and old, depression and anxiety, and teenage delinquency. It is apparent that etiologically relevant animal studies are needed to strengthen the case for the biological plausibility of the epidemiological associations. Therefore, we have developed a novel rodent model of developmental exposure to TRAP associated PM where gestating and lactating rats are chronically exposed to epidemiological levels of PM from Boston area traffic tunnels. Following this exposure, the behavior, peripheral immune activity, and neuroanatomy (ex vivo anatomical and diffusion tensor neuronal track fMRI) of juvenile male offspring were assessed. Compared to low ambient PM exposed controls, TRAP PM exposed offspring exhibited increased fear/anxiety, impaired group and pairwise social behavior, deficient nest building, an altered gastrointestinal response to novelty stress, and white matter neuronal track changes. Immune assessments are ongoing. It is concluded that this is a valuable model to test the adverse effects of TRAP PM on neurobehavioral health, and increased focus on neuroendocrine / neuroinflammatory mechanisms is warranted.

PS2.0046 THE ROLE OF SPECIFIC VASOPRESSIN CELL POPULATIONS IN THE REGULATION OF SOCIAL COMMUNICATION

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The neuropeptide arginine-vasopressin (AVP) has long been implicated in the regulation of social behavior and communication in diverse taxa but the source of AVP release relevant for behavior has not been precisely determined. Potential sources include the extrahypothalamic cell groups in the bed nucleus of the stria terminalis (BNST). To address if AVP cells in the BNST are important for male mouse social communication, we used viral delivery of a Cre-dependent caspase-9 cell-death construct to delete BNST-AVP cells in male AVP-iCre positive mice or AVP-iCre negative littermate controls, and assessed levels of urine marking (UM), ultrasonic vocalizations (USV), and social investigation of male and female conspecifics. Substantial lesions of BNST AVP cell population (90-100%) decreased social investigation of a live male and male urine and increased UM in presence of a live female, without altering USV production, resident-intruder aggression, copulatory behavior, anxiety, or investigation of females or their odor

cues. These results suggest that AVP released from the BNST normally stimulates investigation of competitors and regulates courtship urine marking.

PS2.0047 SEX-SPECIFIC REGULATION OF ER-ALPHA IN THE SOCIAL BEHAVIOR NETWORK OF JUVENILE SIBERIAN HAMSTERS

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The prepubertal ovary is often said to be quiescent. However, our experiments using seasonally-breeding Siberian hamsters have found that photoperiod regulates juvenile social and reward-associated behaviors, in part through a prepubertal ovarian mechanism. Long day lengths (LDs) inhibit, whereas short day lengths (SDs) and prepubertal gonadectomy facilitate, juvenile social play (males and females) and novelty seeking (females only). Photoperiod could regulate ovarian contributions to juvenile behavior by altering the production/secretion of ovarian hormones or by altering the sensitivity of the juvenile brain to these hormones. In this experiment, we tested the hypothesis that photoperiod alters the sensitivity of juvenile female hamsters to estradiol by regulating estrogen receptor-α (ERα) levels in the amygdala, a central node in the social behavior network with connections to the mesolimbic reward pathway. Male and female hamsters reared in a LD or SD were sacrificed at postnatal day 30, and their brains processed by immunohistochemistry for ERα. ERα-immunoreactive (ir) cells in the amygdala were largely restricted to the medial amygdala. LD-reared females had more ERα-ir cells in the posterior medial amygdala than LD-reared males. Notably, this prepubertal sex difference was absent in SD-reared hamsters due to a selective decrease in ERα-ir cells in SD-reared females. No sex or photoperiod differences were detected in the anterior medial amygdala. These findings are consistent with our hypothesis that photoperiod regulates the behavior of female juveniles by altering the sensitivity to prepubertal estradiol. These findings add to a growing literature that challenges the current view of a quiescent prepubertal ovary.

PS2.0048 SPECIES DIFFERENCES IN AGGRESSION, NEURAL ACTIVITY, AND GENE EXPRESSION IN LAKE MALAWI CICHLID FISH

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In Lake Malawi, two ecologically distinct lineages of cichlid fishes (rock- versus sand-dwelling ecotypes, each comprised of over 200 species) evolved from a single ancestral population within the last million years. The rock-dwelling species (Mbuna) are aggressively territorial year-round and males court and spawn with females over rocky substrate. In contrast, males of sand-dwelling species are not territorial and instead aggregate on seasonal breeding leks in

which males construct courtship “bowers” in the sand. First, we demonstrate differences between species in both the quantity and quality of aggressive behavior using mirror-elicited aggression tests in seven species. Rock-dwelling species attack their reflection faster and perform more frontal attacks. In contrast, sand species perform more lateral displays (orienting laterally and displaying their colors). Second, using one rock species (*Petrotilapia chtimba*, Petro), and one sand species (*Mchenga conophoros*, MC), we compare neural activity following mirror-elicited aggression using immunolabeling for pS6. Finally, we use phosphorylated ribosome immunoprecipitation of mRNA from whole brain followed by RNA-sequencing to compare the gene expression patterns activated by mirror-elicited aggression across the two species. This work lays the foundation for future experiments using this emerging genetic model system to investigate the genomic basis of evolved species differences in both brain and behavior.

PS2.0049 FAST, NEUROMODULATORY EFFECTS OF ESTRADIOL ON NETWORK AND INTRINSIC PROPERTIES IN SONGBIRD CORTEX

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In the sensory cortex, social and environmental context can dynamically shift the response properties of neurons to sensory stimuli. Neuromodulators like dopamine, serotonin, oxytocin, and norepinephrine provide context-dependent information to shift the network and intrinsic properties of sensory neurons, in many species and cortices. One prominent neuromodulatory system shared by humans and songbirds is the dense expression of the enzyme aromatase in neurons within the auditory cortex. We have developed evidence that neuroestrogens modulate the gain and coding properties of neurons in the songbird auditory cortex *in vivo*. We now hypothesize that these changes in auditory coding depend on rapid regulation of the network and/or intrinsic properties of auditory neurons. Here, we test this hypothesis using in vitro whole-cell patch clamp recordings from the songbird auditory cortex. We find that one class of neuron, characterized by high input resistance, exhibits stable and unchanging membrane properties in response to 17-beta-estradiol (E2). By contrast, a second class of neuron, characterized by low input resistance, exhibits a host of changes in response to E2 which manifest within minutes. Fast changes in both passive and active membrane properties (e.g., spike latency, jitter, input resistance) together support an E2-dependent increase in the temporal precision of neuronal firing. Using voltage clamp recordings we also observe that E2 rapidly suppresses network inhibition by ~30% in the same cortical region. Therefore, on a neuromodulatory timescale, neuroestrogens regulate both network and intrinsic properties of cortical neurons to enhance timing precision. Support from NIH R01NS082179 and NSF IOS1354906.

PS2.005 BEHAVIORAL FLEXIBILITY IN TASK-SWITCHING WAS ENHANCED BY IMPRINTING AND THYROID HORMONE IN CHICKS.

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Experience affects the brain, resulting in the behavioral changes during the sensitive or critical period for learning. Through experience, individual animals can adjust themselves to their environment in the early development. In the case of imprinting of domestic chicks, we previously showed that thyroid hormone (T_3) which increases in the brain during imprinting training is a starter of the sensitive period. Once T_3 increases in the chick's brain during the sensitive period, the sensitivity to a novel moving object preserves beyond the end of the sensitive period. Also, chicks which were already imprinted to one object can be flexibly imprinted to another novel object in the absence of once-imprinted object. We call the effect induced by T_3 "memory priming" (MP) (*Nature communications*, 2012). We hypothesized that MP may affect the flexibility in later learning other than imprinting. We examined whether imprinting and T_3 enhances the flexibility in the task-switching paradigm. In this learning paradigm, chicks have to adjust to a switch from the color discrimination task to the position discrimination task. As a result, the scores of chicks which experienced MP by T_3 or imprinting were significantly greater than those of chicks without MP. Whereas, in the non-switching paradigm, the effect of MP was not observed. These results indicate that MP enhanced the flexibility in the learning paradigm, and that it may lay the foundation for future learning during development.

PS2.0050 SOCIAL REWARD PLAYS DIFFERENT ROLES IN MATE AND PEER RELATIONSHIPS IN PRAIRIE VOLES

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Prairie voles (*Microtus ochrogaster*) are widely studied for their reproductive pair-bonds, but individuals also demonstrate selective preferences for familiar same-sex peers. The mechanisms underlying pair-bonds in this species may differ from those underlying peer relationships, as reproductive partnerships and parental behaviors are highly motivated. We examined the role of reward and motivation in prairie vole peer relationships compared to prairie vole mate relationships through pharmacological manipulations of dopamine signaling as well as operant conditioning. Blockade of dopamine receptors with haloperidol did not alter selective preferences for familiar same-sex partners, suggesting that dopamine neurotransmission is not necessary for the formation of prairie vole peer relationships, unlike mate relationships. Voles were trained to press a lever to gain access to different types of social stimuli. Female voles pressed at higher rates to gain access to a mate than to a familiar same-

sex peer. Once in the social chamber, females huddled significantly more with familiar mates or peers than with opposite- or same-sex strangers. These data support distinct roles of dopamine and motivation in peer relationships relative to pair-bonds: Although they are necessary to form and maintain pair-bonds, they are not necessary for peer relationships. The fact that a reproductive bond is mediated differently from a non-reproductive one suggests that peer relationships need to be further investigated in addition to pair-bonds to fully elucidate mechanisms of social behavior.

PS2.0051 EFFECTS OF MANIPULATING ADOLESCENT GONADAL HORMONE LEVELS ON BEHAVIOURAL DEVELOPMENT IN RATS

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Adolescence is characterised by sexual maturation and by changes in behaviour, such as increased novelty-seeking behaviour. In addition, the brain undergoes substantial re-organisation during this period, and gonadal hormones, such as testosterone and estrogen, are thought to have long-term effects on brain development and behaviour during adolescence. Experimental studies with laboratory rodents can shed light on the link between adolescent gonadal hormones, behaviour and brain development. In a set of studies using Lister hooded rats (*Rattus norvegicus*), we have investigated the effects of manipulating adolescent gonadal hormone levels on behavioural development. We have shown that suppressing gonadal hormone levels in adolescent male rats, using a GnRH antagonist that binds to GnRH receptors in the pituitary, reduces novelty-seeking behaviour compared to control males. In addition, prepubertally castrated male rats (i.e. that have not been exposed to gonadal hormones during adolescence) spend more time in exposed areas of novel environments in adulthood than post-pubertally castrated males. In contrast, treatment of female rats with a GnRH antagonist during adolescence does not influence response to novel objects. Future studies will investigate the effects of manipulating adolescent gonadal hormones in female rats on responses to novel environments and novel social partners, and we will examine the effects on brain development, e.g., amygdala structure. Our findings are consistent with the hypothesis that adolescence is a sensitive period of development when gonadal hormones can have long-term, 'organisational' effects on behavioural development. This research is relevant to understanding the increased susceptibility of adolescents to some mental health disorders.

PS2.0052 CHARACTERIZATION OF ESTROGEN RECEPTOR BETA EXPRESSING NEURONS IN NEWLY DEVELOPED TRANSGENIC MICE

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Two types of estrogen receptors, ER-α and ER-β are differently involved in the estrogenic regulation of various types of social behaviors. In contrast to ER-α dependent action, mechanisms of hormonal action through ER-β have remained unclear mainly due to difficulties to identify ER-β expressing cells in the brain. Here, we have generated transgenic mice, ER-β-RFPtg, in which red fluorescent protein (RFP) was inserted downstream of ER-β BAC promotor. We first verified RFP signals as ER-β based on 1) comparison of ER-β mRNA on brain tissues collected by fluorescence-activated cell sorting (FACS) for the levels of RFP, 2) correlation between ER-β and RFP mRNA levels in hypothalamic tissues, and 3) cellular co-localization of ER-β and RFP in the pareventricular nucleus of the hypothalamus (PVN). Immunohistochemical assays revealed that RFP expressing cells were mainly localized in the PVN, olfactory bulb, cingulate cortex, island of Calleja, medial preoptic area (MPOA), bed nucleus of stria terminalis (BNST), medial amygdala (MeA), granule cell layer of ventral hippocampus, and dorsal raphe nucleus. Double immunohistochemical staining revealed that RFP co-localized with oxytocin, arginine vasopressin, tryptophan hydroxylase-2 and progesterone receptors in a manner consistent with previously reported findings. Furthermore, we could identify neuronal sub-populations those express both types of ERs and those express exclusively ER-α or ER-β in the MeA and MPOA. These findings collectively suggest that ER-β-RFPtg mice can be a powerful tool for future studies on ER-β function in the estrogenic regulation of social behaviors.
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PS2.0053 PARTNER-SPECIFIC SOCIAL BUFFERING ON STRESS RESPONSES IN PRAIRIE VOLES

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Social support, especially from close relationships, can help lower the stress response and its subsequent negative health outcomes – a phenomenon described as “social buffering”. In the present study we used the socially monogamous prairie vole (*Microtus ochrogaster*) model and examined the stimulus-specific preventative social buffering effects on anxiety-like behavior induced by immobilization (IMO) stress. Female voles were divided into three groups that were exposed to an IMO restrainer tube containing a previously pair-bonded male partner (Partner), a male stranger (Stranger), or nothing (Control) for 60 mins, during which the females’ behaviors were recorded. Following the 60-min test, anxiety-like behaviors were assessed for the IMO males using an elevated plus maze (EPM) test. Our data indicate that females in the

Partner group spent significantly more time interacting with the restrainer than ones in the Stranger and Control groups. Control females showed increased levels of locomotor activity and rearing/self-grooming compared to the other two groups. Furthermore, IMO males that had a female partner in the arena entered the open arms more and tended to spend more time there in the following EPM test, indicating decreased anxiety-like behaviors than IMO males that were exposed to a female stranger or just an empty arena. Interestingly, the time that females spent interacting with the restrainer was positively correlated with decreased anxiety-like behavior only for partners, but not for stranger males. These data not only reveal stimulus-specific induced social behaviors, but also indicate potential roles of those behaviors in buffering stress responses in prairie voles.

PS2.0054 NEUROPEPTIDERGIC REGULATION OF SOCIALITY AND PHYSIOLOGY IN JUVENILE MARMOSET MONKEYS

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Developing and maintaining high-quality social relationships is fundamental to both physiological and psychological well being across the lifespan. The neuropeptides oxytocin (OT) and vasopressin (AVP) have critical and pervasive roles in reproduction and physiology, and have attracted enormous interest as neuromodulators of social and cognitive functioning. While it is clear that these two hormones have important roles in the initiation of social interactions in adulthood, less attention has been given to whether OT and AVP regulate sociality during early development. Thus, the goal of these studies was to determine the extent that OT and AVP modulate social preferences, familial affiliation, and physiological and behavioral responses to stress in prepubertal marmoset monkeys across multiple developmental time-points. The expression of familial affiliation, including the initiation of social approach [$F(2,12)=13.0$, $p=.001$] and total time spent in social proximity [$F(2,12)=4.78$, $p=.03$], decreased as prepubertal marmosets progressed to independence. Neuropeptide treatment also interacted with age to modulate the initiation of social approach [$F(4,24)=2.5$, $p=.07$]; ten-month old marmosets that received AVP experienced the largest decline in social approach behavior. We also show that AVP enhances stress recovery, but not stress reactivity, following social isolation in juvenile marmosets [$F(6,60)=2.14$, $p=.06$]. These findings indicate that neuropeptides differentially modulate affiliation and the physiological stress response in juvenile marmoset monkeys. Ultimately, these results inform the design and application of selective therapeutic treatments for neuropsychiatric disorders that include maladaptive social functioning by providing further clarity on the age- and context-specific roles of OT and AVP in modulating sociality across development.

PS2.0055 OXYTOCIN AFFECTS MEADOW VOLE SOCIAL PREFERENCES DIFFERENTLY BY BRAIN REGION AND DURATION

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Oxytocin is implicated in aspects of social behavior from maternal bonding to mating behavior. Oxytocin is also important for behaviors outside the reproductive context, including social recognition, aggression, and affiliation with same-sex peers. Meadow voles are seasonally social during the winter months, or short photoperiods in the lab. In short photoperiods they form selective preferences for familiar same-sex peers, and engage in some stranger-directed affiliative behavior. Oxytocin signaling modulates social behavior in female meadow voles. Early studies indicated that same-sex partner preferences are strengthened by chronic central oxytocin administration. However, acute administration of oxytocin into the lateral septum decreased partner preference. Acute administration of oxytocin to the central nucleus of the amygdala also led to a significant decrease in partner huddling, while acute central administration did not alter preferences. Site-specific administration to any of these regions did not affect total huddling time. Here I present results of these past and current studies of oxytocin together, as a way of building an integrated framework for understanding the effects of oxytocin across brain regions and time-scales. Further investigation into the influence of oxytocin administration location and timing will help shape our understanding of the role of oxytocin in behavior.

PS2.0056 M. VACCAE AMELIORATES SURGERY-ELICITED NEUROINFLAMMATION AND COGNITIVE DYSFUNCTION IN AGED RATS

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Advanced age is a major risk factor for developing postoperative cognitive dysfunction (POCD). Peripheral immune stimuli (e.g. infection, injury, surgery) cause an exaggerated pro-inflammatory response in the aged brain and this “primed” inflammatory response likely underlies vulnerability to POCD. Overly sterile conditions are a modern environmental divergence that can prime immune responses and prior exposure to specific micro- and macro-organisms can quell inflammatory priming. Peripheral immune signals are communicated to the central nervous system, suggesting microbial based treatments may ameliorate age-associated neuroinflammatory priming. Here we hypothesized that treatment with *Mycobacterium vaccae* (*M. vaccae*) would reduce neuroinflammatory priming and associated cognitive impairments in aged rats following surgery. Aged (24 mos) and adult (3 mos) male F344XBN rats

received three weekly subcutaneous injections of heat-killed *M. vaccae* and then underwent either a laparotomy or sham (anesthesia control) procedure. Prophylactic treatment with *M. vaccae* protected aged rats from surgery induced-cognitive impairments. Furthermore, *M. vaccae* treatment shifted the aged pro-inflammatory neuroenvironment (increased IL-1beta and NFKBIA) towards an anti-inflammatory phenotype (increased IL-4 and Arg1). Microglia may represent the cellular source of inflammatory priming in the aged brain: microglia isolated from aged rats showed heightened inflammatory response to *ex vivo* immune stimulation and treatment with *M. vaccae* reduced exaggerated inflammatory responses in microglia. Overall, the present findings suggest that *M. vaccae* can re-direct a primed neuroimmune environment in aged rats and prevent cognitive dysregulation following surgery.

PS2.0057 PREADOLESCENT OXYTOCIN INFLUENCES SOCIAL INVESTIGATION DEPENDENT ON SEX&MATERNAL FLUOXETINE EXPOSURE

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Postpartum depression(PPD) affects approximately 15% of women. Selective serotonin reuptake inhibitors(SSRI's) are commonly prescribed to treat PPD, yet their effects on child development are largely unknown. SSRI's can enter breastmilk and cross the placental and blood-brain barrier, exposing the fetus/breastfeeding infant to increased serotonin levels. SSRI exposure during development has been associated with increased rates of autism spectrum disorder(ASD), and anxiety. Oxytocin, a hormone thought to promote prosocial behaviours, is in trials to mitigate social deficits associated with ASD. However, oxytocin does not readily cross the blood-brain barrier. In this study oxytocin was coupled with Triozan™, which may facilitate peptides across the blood-brain barrier. This study used a corticosterone-induced rodent PPD model along with a concurrent SSRI, fluoxetine. This study aimed to determine maternal corticosterone and/or fluoxetine effects on offspring development, and if deficits can be mitigated with pre-adolescent offspring oxytocin administration. We hypothesized fluoxetine would alter offspring anxiety and social behaviour, which would be mitigated by oxytocin. Corticosterone and/or fluoxetine were administered to the dams from postpartum day 2-23. Oxytocin+Triozan™, were administered to the offspring postnatal day(PND) 25-34. Offspring social investigation, and anxiety behaviour testing were conducted on PND 35-37 and PND 70-73. Oxytocin+Triozan™ increased social investigation in females after maternal fluoxetine exposure but decreased social investigation after maternal vehicle exposure. These data suggest Triozan™ facilitates oxytocin's effects when administered peripherally in females. Moreover, oxytocin has sex and context-specific effects. Maternal corticosterone and fluoxetine decreased adolescent offspring anxiety. These results may have implications for PPD and ASD oxytocin treatment.

PS2.0058 ANDROGEN RECEPTOR OVEREXPRESSION DOESN'T PREVENT AGE-RELATED DECLINE IN SEXUAL REWARD IN FEMALE MICE

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Age-related declines in sexual function have been causally linked to androgen insufficiency in women. Here we use mice to examine whether reward associated with genital stimulation declines with age, and whether increasing androgen signaling by androgen receptor overexpression can prevent this decline. Rewarding properties of genital stimulation were assessed using a conditioned place preference paradigm (CPP), an associative learning task. In this task, initial preference for an asymmetric test chamber is established, and potentially rewarding stimuli (conditioning stimuli) are paired with the initially unpreferred side and control stimuli are paired with the initially preferred side. CPP is assessed once all animals have completed 5 conditioning sessions and reward manifests as an increase in time spent in the initially unpreferred side. We find that young adult (2-3 month old) but not older (8-10 month) female C57BL6 mice are able to develop a significant CPP in response to this regimen of tactile genital stimulation. Furthermore, androgen receptor overexpression (CMV-AR transgenic mice) did not increase CPP in older females. Acknowledgements: Funded by NSERC discovery grant RGPIN-2016-06302 (AM)

PS2.0059 BEHAVIORAL AND NEUROENDOCRINE RESPONSES OF HIGH-FAT FED RATS TO ANTIHISTAMINERGIC DRUG PROMETHAZINE

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Histamine H_1 receptors (H_1-Rs) are found in peripheral tissues and in regions of the hypothalamus that are concerned with regulating body composition. In the present study, we investigated the detailed mechanisms of histamine H_1-Rs in the development of obesity using high fat diet and repeated injections of antihistamine Promethazine (2.5mg.kg^{-1}) in rats. Male Albino Wistar rats (n=48) were purchased from Dow University of Health Sciences, Pakistan. Two groups (n=24: (1) Controls (Standard lab chow-SLC; fat content 10% in kcal) control, (2) Test (High fat diet-HFD 35% in kcal) were treated for four weeks in accordance with NIH guidelines and Institutional Committee.. On 5th week, Promethazine (2.5mg.kg^{-1}) was injected intraperitoneal daily (between 9:00am-10:00am) for 14 days (n=12) each group). Weekly changes in physiological and behavioral responses were monitored equally. Statistical analysis was determined by one-way ANOVA and 2-factor ANOVA with repeated measures design using SPSS version 14.0 software. P<0.05 values were considered statistically significant. HF fed rats treated with promethazine (2.5mg.kg^{-1}) weighed 35% more (P<0.01) than SLC rats. Weekly

changes in Food intake (F=50.5 P<0.0) and body weights (F=35.3 P<0.01) were also high. Impaired cognitive functions and anxiogenic-like effects were also observed. Hyperglycemia (F=10.8 P<0.01), insulin resistance (F=9.0 p<0.01), increased brain serotonin and plasma corticosterone levels were also observed. We concluded that 14 day promethazine (2.5mg.kg^{-1}) administrations in HF fed rats triggered hyperphagia and exacerbated the feeding behavior dmonstrated obese syndrome consistent with the view that H1-Rs play a promising role in regulating appetite and possible neuroendocrine control over metabolic disorders.

PS2.006 SEXUAL AROUSAL-ASSOCIATED C-FOS INDUCTION IN THE SAGITTALIS NUCLEUS OF THE HYPOTHALAMUS

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During extended observation of estrogen receptor (ER) α-immunoreactive neurons in the hypothalamus, we previously identified a novel nucleus, the Sagittalis Nucleus of the Hypothalamus (SGN), in the interstitial area between the arcuate nucleus and the ventromedial hypothalamic nucleus in rats. The SGN exhibits sexual dimorphism in its volume and cell count, and estrous cycle related variations in ERα-immunoreactive neuronal number. These characteristics suggest a contribution of the SGN to sexually differentiated brain function. In this study, we examined correlation of the SGN with sexual behavior. Immunohistochemical staining of c-Fos, a marker of neuronal activity, revealed that a significant increase in the number of c-Fos-positive neurons was seen following administration of an estrus-inducing dose of estrogen and progesterone in female rats. In male rats, c-Fos-positive cell number in the SGN was elevated with only exposure to chemosensory cues of estrous females and significantly increased after the first mount. These findings may indicate that neurons in the SGN are associated with sexual arousal in both males and females.

PS2.0060 EFFECTS OF A NOVEL METHOD TO INCREASE MATERNAL CARE ON CORTICOSTERONE LEVELS IN RAT PUPS

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In human neonates, increased skin-to-skin contact with the mother can improve development and health stability in at-risk infants which may be mediated through decreased levels of infant cortisol. Further, animal models using natural variation in maternal care have shown that

increased licking and grooming behavior can significantly influence the offspring's adult phenotype. The goal of the current project is to investigate the acute effects in our novel rodent model of increased maternal care on the offspring's stress response system. For this, half the pups from five litters will be covered in a palpable food (Nutella®), which will entice the dams to lick these pups significantly more compared to their untreated littermates as our previous pilot data has shown. In the current study, pups will be exposed to Nutella® four times a day from postnatal day (PD) 2-5 and maternal care (i.e. licking and grooming behavior) will be observed during each session. Male and female pups will be sacrificed via live decapitation immediately following the last Nutella® session on PD 5. Blood will be collected for ELISA assays for corticosterone levels and brains will be processed with high-pressure liquid chromatography for several neurotransmitter levels. We hypothesize that the increasing licking and grooming will results in lower baseline corticosterone and altered dopamine levels in male and female pups. Increasing maternal care using this translational model can be used in future studies to investigate long-term developmental effects in rat pups exposed to early-life stressors such as neonatal pain.

PS2.0061 THE ROLE OF OVARIAN HORMONES 17B-ESTRADIOL AND PROGESTERONE IN MEMORY BIAS IN FEMALE RATS

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While navigating a maze, rats can use one of two types of memory while seeking a reward: place or response memory (Tolman et al., 1946). Female rats shift between using place and response memory in a maze depending on their circulating levels of estradiol (E2) and progesterone (P) across their estrous cycle (Korol, 2004). Seventy-two ovariectomized female Long-Evans rats were split into 3 groups: low E2 (n =24), high E2 (n=24), and high E2+P (n=24). All rats were tested in an ambiguous T-maze to differentiate whether they were predominantly using place or response memory while navigating. No statistically significant differences were found between the three hormone conditions. However, a pattern emerged from the data showing that 62.5% of rats in the Low E2 and High E2 + P groups used response memory. In the high E2 group, instead, there was a 62.5% bias towards the use of place memory. These findings suggest that when P is combined with high E2, that it may reduce the odds of being biased toward switching to using place memory.

PS2.0062 EXPOSURE TO SYNTHETIC PROGESTIN DURING DEVELOPMENT ALTERS DECISION MAKING IN ADULTHOOD

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17α-hydroxyprogesterone caproate (17-OHPC) is a synthetic progestin commonly administered to women considered at risk for preterm birth, despite little understanding of the potential effects of exposure to 17-OHPC on the developing fetus. 17-OHPC administration typically begins during the second trimester and coincides with critical periods of development of the mesocortical dopaminergic pathway, which originates in the ventral tegmental area (VTA) and projects to the medial prefrontal cortex (mPFC), mediating executive functioning. In rodent models, neonatal exposure to 17-OHPC induced changes in both the innervation patterns and density of dopaminergic fibers at postnatal day 7, as measured by tyrosine hydroxylase immunoreactivity (THir). Progesterone receptors (PR) are expressed in dopaminergic cells of the VTA that project to the mPFC. In addition, 17-OHPC exposure during neonatal life impaired cognitive flexibility in adulthood, a complex cognitive behavior regulated by dopamine activity in the mPFC. Impulsive decision making is also dependent on the mesocortical pathway. In this study, we examined the effects of administration of 17-OHPC during postnatal life (P1-14) on performance in a delay-discounting task, in which animals choose between a larger delayed reward or a small reward delivered immediately. Rats treated with 17-OHPC were significantly more likely to wait for the larger, delayed reward and were significantly less likely to choose the small, immediate reward with increasing delays. Interestingly, 17-OHPC treated rats were significantly more likely to not respond at all (omissions). Together, these results suggest that 17-OHPC may decrease impulsivity, but may also interfere with general decision making abilities.

PS2.0063 EFFECTS OF 17B-ESTRADIOL AND PROGESTERONE ON THE BEHAVIOURAL RESPONSE TO KETAMINE.

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Ketamine, an NMDA antagonist, has been shown to have anti-depressant effects among individuals with treatment resistant depression. Although women are two times more likely to suffer from depression the majority of research on the neurobiology of ketamine's effects has used males. The purpose of this study was to examine the role of 17β-estradiol (E2) and progesterone (P) on the behavioural response to ketamine. Here 91 ovariectomized female Wistar rats were split into 3 hormonal conditions (low E2, high E2, and high E2 + P) and 5 ketamine conditions (0, 2.5, 5, 10, & 20 mg/kg). All rats were tested in the Porsolt forced swim test and the open field test. Early results show that high E2 + P replacement rats responded to

ketamine by spending the least time immobile at the 20mg/kg condition however, they spent more time immobile compared to the other hormone conditions. The low and high E2 replacement rats paradoxically spent more time immobile in the swim test following all ketamine doses relative to saline. While there was an effect of hormones on the open field test, i.e. the low E2 group spent more time in the middle than the high E2+P, there was no effect of ketamine. Future research will investigate the neurobiological mechanisms of any interaction that may exist between ketamine and these female sex hormones.

PS2.0064 AROMATASE INHIBITION IN THE SONGBIRD AUDITORY CORTEX IMPAIRS LEARNING IN A NOVEL BEHAVIORAL TASK

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The caudomedial nidopallium (NCM) is a secondary auditory region in the songbird association cortex. It is a key neural locus for song perception, juvenile song learning, and associations between songs and behaviorally relevant consequences. NCM is enriched with aromatase and is a site of action of neuromodulators including estradiol (E2), norepinephrine and dopamine, consistent with its prominent role in neuroplasticity. However, NCM's role in adult auditory learning has not been clarified. We have shown previously that systemic suppression of aromatase activity disrupts learning in an auditory-dependent operant task. Here, we show that local inhibition of aromatase activity in NCM during the auditory learning task produces similar deficits, indicating that E2 signaling specifically in NCM supports auditory learning. We also show that aromatase-positive neurons are often associated with tyrosine hydroxylase-positive fibers and dopamine receptor-positive neurons. Furthermore, whole-cell patch clamp recordings show that NCM neurons exhibit long-duration, high-amplitude NMDA receptor-dependent currents that are likely targets for neuromodulation. These experiments suggest that NCM plays a central role in auditory plasticity and memory formation, perhaps driven by an interaction between neuroestrogens and dopamine. Support from NIH R01NS082179, NSF IOS1354906, and LASPAU/CAPES-Brazil.

PS2.0065 ANDROGEN RECEPTORS CONTROL DISTINCT TRAITS: EVIDENCE FROM TRANSGENIC CICHLID FISH

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Dominance hierarchies are ubiquitous in social species. In *Astatotilapia burtoni,* dominant (DOM) male fish maintain territories through aggressive interactions and court females, while non-dominant (ND) males do not. DOM males are brightly colored, have higher testosterone (T) and 11-ketotestosterone (11-KT; a fish specific androgen), and possess larger testes compared to ND males. When given the opportunity, ND males become brightly colored and attempt to establish a territory and court females, a process called social ascent. We have shown previously that blocking androgen signaling in ND fish abolishes courtship during social ascent to DOM status; however; the molecular mechanisms underlying social ascent and behavior in *A. burtoni* is not clear. *A. burtoni* possess two ARs encoded by two different genes, *aralpha* and *arbeta*. *aralpha* and *arbeta* show distinct expression patterns throughout the *A. burtoni* brain, suggesting they control distinct aspects of behavior. Using CRISPR-Cas9 genome editing, we have generated *A. burtoni* homozygous for frame-shift alleles encoding *aralpha* (*aralpha-/-*) and *arbeta* (*arbeta-/-*). We have observed striking disruptions in male-typical traits. *arbeta-/-* males are drably colored like females and possess smaller testes compared to wild-type, sibling males. On the other hand, *aralpha-/-* males show bright coloration typical of their wild-type counterparts, yet possess much larger testes. Preliminary studies suggest *ara-/-* males court females less than wild-type males.
Therefore, *araalpha* and *arbeta* govern distinct aspects of that normally characterize DOM social status, suggesting these two genes have been subfunctionalized. Investigating the distinct roles played by ARs in *A. burtoni* will provide insight into the fundamental molecular mechanisms of social behavior.

PS2.0066 SEXUALLY DIMORPHIC NUCLEUS IN THE PREOPTIC AREA REINFORCES SEXUAL MOTIVATION IN MALE RATS

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Appropriate modulation of sexual activity is important to enhance social communication with conspecifics of the opposite sex. Successful mating experiences make males proficient in sexual behavior, resulting from reinforced sexual motivation. However, it is largely unclear how sexual activity is modulated by sexual experience. The medial preoptic area regulating sexual motivation and sexual behavior in male rats contains a nucleus exhibiting male-biased morphological sex differences, which is known as the sexually dimorphic nucleus of the preoptic area (SDN-POA). In this study, we examined the roles of the SDN-POA in reinforcing sexual motivation with sexual experience in male rats. A genome-wide gene expression analysis identified *Vgf* as a gene whose expression was higher in the SDN-POA of male rats following the first copulation than the second copulation. Knockdown of *Vgf* in the SDN-POA disrupted reinforcement of sexual motivation by copulation in males before acquisition of sexual

experience, but it was invalid in males that had acquired sexual experience and had higher sexual motivation. Additionally, activity of the SDN-POA neurons determined by a c-Fos analysis was higher in males copulating for the first time than in males copulating for the second time. Spine density in the SDN-POA was reduced after males copulated with females. These findings suggest that the SDN-POA of male rats is functioning during the first copulation to reinforce sexual motivation with copulation experience. The SDN-POA may switch the brain from a sexually naive state to a sexually proficient state in male rats.

PS2.0067 BNST VASOPRESSIN CONTRIBUTES TO EXPRESSION OF SICKNESS BEHAVIORS IN MALE MICE

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Central arginine vasopressin (AVP) modulates autonomic process, anxiety-related, and social behaviors, all of which are altered during sickness, pointing at AVP as a factor central to sickness responding. To understand the role of AVP in sickness, we targeted AVPergic cells in the bed nucleus of the stria terminalis (BSNT). BNST AVP is involved in fever regulation during sickness, social processing, and is expressed in a sex-dependent manner. Male AVP-Cre mice and WT littermates were injected with Cre-dependent caspase-9 cell death construct in the BNST. This leads to ablation of BNST-AVP cells in AVP-Cre mice only. Subsequently, animals were injected with either 1mg/kg lipopolysaccharide (LPS) or vehicle, which was counterbalanced one week later. Three hours after each injection, anxiety-related and locomotor behavior was tested within open field and elevated zero maze apparatuses. Depressive like behavior and anhedonia were tested with a 24-hour sucrose preference test and a tail suspension test the day after injection. Sociability was also tested with a three chamber social choice apparatus the day after injection. LPS caused sickness behavior (defined as heightened anxiety-related and depressive-like behaviors, diminished sociability and locomotion) in both AVP-Cre and WT animals. AVP-Cre animals had an increased preference for sucrose, driven by overall sucrose consumption. AVP-Cre animals also showed less sickness behaviors after LPS administration in the open field and tail suspension tests. These data suggest that BNST AVP contributes to reward processing and anxiety behavior during sickness. The data are surprising as earlier studies had suggest that BNST AVP diminishes sickness behavior.

PS2.0068 LACTATION AND STRESS ALTER DEPRESSION-LIKE BEHAVIOR AND HIPPOCAMPAL NEUROGENESIS IN POSTPARTUM RATS

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Women who do not breastfeed or discontinue breastfeeding early have a higher risk of developing postpartum depression (PPD) compared with women who breastfeed exclusively. Further, stress is a major risk factor for depression. Thus, we sought to determine whether different lactational experiences would alter the susceptibility to stress-induced changes in depression-like behavior and hippocampal neurogenesis. Adult female Sprague-Dawley rats underwent thelectomy (thel; surgical removal of teats), sham surgery, or no surgery (control). Thel and sham rats were yoked and litters were rotated every 12 h postpartum days (PD) 0-26 (thus yielding a higher nursing demand for sham rats). Control litters were rotated between paired control rats. Rats received chronic variable stressors or no stressors PD 2-25. Stressors were presented in a semi-random order without separating dams from litters. Control rats spent less time with offspring compared with sham and thel rats, regardless of stress. Stressed rats spent more time with offspring compared with non-stressed rats, regardless of nursing condition. Nursing and stress interacted to alter immobility in the forced swim test: among non-stressed rats, thel rats spent more time immobile than sham rats. Stress increased immobility in control and sham rats, but unexpectedly, reduced immobility in thel rats. Preliminary data also suggest that lactational experience interacts with stress to alter hippocampal neurogenesis. These data suggest that nursing does not necessarily yield resistance to stress-induced changes in depression-like behavior or neurogenesis. Thus, the relationship between the absence of breastfeeding and PPD could be independent of stress susceptibility in non-breastfeeding women.

PS2.0069 THE ROLE OF HYPOCRETIN 1 IN MATERNAL MOTIVATION IN A TMAZE IN LACTATING DAMS

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Hypocretin-1 (HCRT-1) is a hypothalamic neuropeptide associated with arousal wakefulness in rats and humans. HCRT-1 also enhances signaling in the mesolimbic pathways associated with increased motivation and reward-seeking behavior in various paradigms. During lactation, there is an increase in HCRT-1 neuronal activity in lactating dams, however if HCRT-1 is associated with postpartum changes in reward-related behavior and maternal motivation is not known. The aim of this study was to observe the effects of a HCRT-1 receptor antagonist (HCRTR1A) on the latency of lactating dams to retrieve pups a in a novel environment (T-maze). Dams were given either a HCRTR1A (n=13) or vehicle control (n=11) and latency to leave goal box and

retrieve pups as well number pups retrieved were recorded. There were no significant differences in pup retrieval, though retrieval rates were low. However, there was a strong trend towards a longer latency to leave the goal box in the HCRTR1A group than vehicle (p=0.06). In a second study, to optimize pup retrieval, it was found that when mothers were deprived of pups of 2 hours, they retrieved more pups ($F(1, 18) = 7.43$, $p = .01$) and faster ($F(1, 18) = 4.858$, $p = 0.04$) than the non-deprived control group. Dams also retrieved pups quickest on the pospartum day 4 relative to day 1. This work suggests HCRT-1 may play a role in behavior in novel environments, but more work incorporating the role of deprivation and HCRT is needed to elucidate the role of HCRT in maternal motivation.

PS2.007 VARIATIONS IN REARING EXPERIENCES INFLUENCE OPEN FIELD TEST PERFORMANCE IN PRAIRIE VOLE PARENTS

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A wealth of studies demonstrates the various ways in which early life social experiences and stress impact the development of offspring brains and behavior. The quality and quantity of care provided by parents in the natal nest have lasting consequences on offspring physiology, neuroendocrinology, and anxiety-like behaviors. However, comparatively fewer studies have investigated how variation in parental experiences impacts the parents themselves. In the wild, socially monogamous prairie vole pups (*Microtus ochrogaster*) are either reared by both mothers and fathers, or by their mothers alone. Raising pups as a single mother may increase the demands of parental caregiving and provisioning, which may subsequently be a more stressful parenting experience compared to rearing a litter with the help of a male partner. Presently, we asked how distressing conditions experienced by parents during the postnatal rearing period (induced by removal of the male partner, and by handling manipulations) impacts parental performance in an open field test. We found that mothers that were handled moved a significantly greater total distance in the test compared to unhandled mothers. Handled mothers, as well as mothers who reared young biparentally, visited the open center of the apparatus more frequently than unhandled and single mothers. Unlike mothers, fathers did not show any behavioral differences as a function of handling manipulations. These data provide evidence that anxiety-related conditions experienced during the rearing period can differently impact the behaviors of parents.

PS2.0070 EFFECTS OF MATERNAL EXPERIENCE ON THE PUP-INDUCED ACTIVATION OF APPROACH AND AVOIDANCE CIRCUITS

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Mothering behaviors tend to occur by default in nulliparous C57BL/6J mice when foster pups are presented in a familiar environment. In a novel context, however, pups are more likely to elicit indifferent, cautious or fearful responses from naïve virgin female mice. We have previously reported that the repeated experience of interacting with pups was linked to a reduction in the relative expression of immediate early genes in the anterior hypothalamic nucleus (a region that critically regulates pup avoidance), as well as an increase in the relative expression of immediate early genes within the ventral tegmental area (a region that critically regulates pup approach) in response to pup presentation in a novel context. These data suggest that maternal experience alters the relative activation of neural pathways known to mediate approach or avoidance behavioral responses. Thus, pups activate an otherwise latent neural pathway regulating indifferent or avoidant behavior in naïve virgin mice within a novel context. In contrast, experience with pups seems to set mothering behavior as default regardless of the context in which pups are presented. It is presently unclear how increased experience with infants alters the pattern of neuronal activation in these approach and avoidance circuits to mediate long-term changes in maternal responsiveness. To begin to investigate this question we asked if the phenotype of cells activated across repeated pup experiences is altered within the several regions of the approach and avoidance pathways. This work has been supported by R01 HD087709.

PS2.0071 FACTORS CONTRIBUTING TO DISPERSAL IN EUSOCIAL NAKED MOLE-RATS

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Naked mole-rats are eusocial mammals native to sub-Saharan Africa. Their hierarchy consists of one breeding female, 1-3 male consorts, and their reproductively suppressed offspring, called subordinates. Subordinates are divided into two subcastes: soldiers and workers. A third "disperser morph" subcaste has been suggested with a subset of subordinates exhibiting motivation to leave their natal colony and mate with unfamiliar conspecifics. To determine whether colony variables (e.g., population density, sex ratio, queen temperament) influence the incidence of dispersal, we evaluated dispersal behavior in 17 colonies. Queen aggression significantly predicted the presence of female but not male dispersers though dispersers were not themselves recipients of queen aggression. We also compared in-colony and out-pairing behavior between putative dispersers and their sibling workers. Most in-colony behaviors did

not differ between dispersers and workers though dispersers were more aggressive towards familiar subordinates; both sexes were targets of female disperser aggression. Following out-pairing, dispersers and workers produced litters at similar rates demonstrating that motivation to leave the colony, and not reproductive maturation per se, is the key to successful dispersal. Collectively, these data suggest that dispersal in naked mole-rats is driven by aggressive queens and that putative dispersers show traits consistent with successful breeders (e.g., aggression).

PS2.0072 ESTRADIOL REGULATION OF GENE EXPRESSION IN THE BRAIN

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Estradiol, the primary endogenous estrogen, modulates diverse neural functions including memory, anxiety, activity level, and appetite. Estradiol is also the master regulator of sexual differentiation of the brain: in rodents it organizes and modulates the neural circuits that control sex-typical social behaviors including parental care and aggression. Estrogens such as estradiol primarily act through their cognate nuclear receptors ERα/ERβ to regulate gene expression; upon ligand binding these receptors undergo conformational changes and recruit transcriptional machinery to DNA. However, we still lack a clear picture of how estrogen regulates gene expression in the brain. No neural-specific target genes of estrogen receptors have been identified, due to the technical challenges posed by the complexity of the mammalian brain, and the sparse expression of these receptors. To address these challenges, we have assessed gene expression and chromatin accessibility specifically within ERα neurons from female and male mice. We have identified genes acutely regulated by estrogen, as well as putative regulatory elements that impart sex-specificity to gene expression. To complement these *in vivo* studies, we carried out chromatin immunoprecipitation (ChIP)-seq for ERα in a hypothalamic cell line and found ERα binding sites that correspond to open chromatin regions in ERα neurons. Collectively our studies describe the first direct transcriptional targets of estrogen signaling in neurons, and implicate specific transcription factors in the modulation of ERα function in the brain.

PS2.0073 EFFECTS OF EDCS ON REPRODUCTIVE BEHAVIOR AND SUCCESS IN SECOND GENERATION ZEBRA FINCHES

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Endocrine-disrupting chemicals (EDCs) are synthetically produced chemicals that interact with the body's hormone receptors. Bisphenol A (BPA) is a commonly found EDC present in many

plastics and has been shown to have estrogenic activity, but there are many others. EDCs may influence expression of transgenerationally inherited traits, so exposed parents may show few or no direct effects and several generations may pass before the complete effects are realized. This study sought to investigate the transgenerational effect of EDC exposure on reproductive behaviors and success in second generation zebra finches. Birds in the parental generation (F0) were administered drinking water via one of the following: BPA-positive plastic bottle, BPA-negative plastic bottle, glass bottle, or glass bottle supplemented with estrogen (0.25 nmol/g body weight/animal). The F0 generation was allowed to produce a second (F1) generation, which were all administered water via glass bottles. Preliminary results show altered reproductive and parental efforts with significantly more F2 offspring produced by the E2+ treated birds and fewer in the BPA+ treatment. F1 BPA-exposed parents produced lighter nests when compared to those from the E2+ and NEG groups ($p=0.0182$ and $p=0.0247$, respectively), had more unhatched eggs ($p=0.0359$), and showed altered timing in reproductive behaviors like incubation duration and chick rearing functions. F1 parents in the BPA- group demonstrated abnormal nest building by building nests on the roof of nests built by other parents. Nest temperatures from the E2+, BPA+, and BPA- treatments reveal altered incubation efforts and increased female:male offspring ratios when compared to NEG controls.

PS2.0074 VASOPRESSIN EXACERBATES THE DEVELOPMENT OF HYPERTENSION

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Hypothalamic magnocellular neurons in the supraoptic nucleus secrete vasopressin into the systemic circulation at the posterior pituitary gland to maintain blood pressure by increasing renal water reabsorption and by vasoconstriction. When blood pressure rises, baroreflex activation normally inhibits vasopressin neurons via activation of GABAergic inputs. However, plasma vasopressin levels are paradoxically elevated in several models of hypertension and in some patients with essential hypertension, despite increased blood pressure. We have previously shown that vasopressin neuron activity is increased early in the development of moderate angiotensin II-dependent hypertension via blunted baroreflex inhibition of vasopressin neurons in Cyp1a1-Ren2 rats (that have mouse Ren-2 cDNA fused to a cytochrome P450 (Cyp1a1) promoter) inserted into the Y-chromosome. Here, we show that subcutaneous V1 receptor antagonist administration slows the progression from moderate to severe hypertension in Cyp1a1-Ren2 rats and that intra-supraoptic nucleus administration of a $GABA_A$ receptor antagonist inhibits vasopressin neurons during, but not before, the onset of hypertension. Taken together, our data suggest that vasopressin-mediated vasoconstriction drives the progression from moderate to severe hypertension and that this is caused by blunted baroreflex inhibition of vasopressin neurons due to an excitatory shift in their response to endogenous GABA signalling.

PS2.0075 AUTOSOMAL AND GENETIC SEX DETERMINED INCREASES IN BODY WEIGHT ARE CORRELATED WITH AGRP EXPRESSION

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In order to understand the control of growth we have demonstrated that the largest autosomal genetic effect on body weight in chickens is the CCK receptor A (CCKAR) locus on chromosome 4. Animals carrying the high growth allele have lower CCKAR expression and 20% larger body mass. This phenotype is correlated with higher expression of hypothalamic *AGRP*. However, there is an even larger genetic effect on body mass than CCKAR and that is sex, the possession of one (female) or two (male) Z chromosomes. We have tested the hypothesis that this genetic effect is also correlated with differences in the expression of *AGRP* and other genes in the hypothalamic feeding centre. We examined this in rapid and slower growing chickens in conjunction with quantitative versus qualitative food restriction. Expression of *AGRP* ($P<0.001$) and *NPY* ($P=0.003$) in rapid growing broilers was higher in males than females and in a slower growing chicken, males had higher *AGRP* mRNA ($P=0.002$) expression than females, suggesting the observed sex difference were not only apparent in one strain. *AGRP* ($P<0.001$) and *NPY* ($P<0.001$) expression were both significantly lower in *ad-libitum*- compared to quantitative or qualitative restricted fed chickens which were not statistically distinguishable from each other. In conclusion, expression of orexigenic peptides in the avian hypothalamus are correlated with sex differences in growth and body mass. The differences in gene expression between sexes provides further evidence of AGRP expression being correlated to growth potential. Results also suggest that gut-fill alone does not reduce orexigenic gene expression.

PS2.0076 IDENTIFICATION OF MONOSYNAPTIC INPUTS TO THE NEUROPEPTIDE Y NEURONS IN THE DORSOMEDIAL HYPOTHALAMUS

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Neuropeptide Y (NPY) is a neural peptide distributing in the brain widely and has various functions in each region. The dorsomedial hypothalamus (DMH) is a region where there are NPY-expressing neurons. The expression of NPY in the DMH is not constitutively but increase only during chronic hyperphagic conditions such as lactation and chronic obesity. Lesion of DMH caused hypophagia and loose body weight in various animals, suggesting that the DMH

NPY neurons are involved in food intake under hyperphagic conditions. Obtaining more knowledge about DMH NPY neurons, in the present study, we investigated neural input to the DMH NPY neurons using a first-infected cell specific monosynaptic retrograde tracing. Two cre-dependent adeno-associated virus (AAVs) expressing TVA-mCherry (receptor for the avian sarcoma leucosis virus glycoprotein (EnvA) fused with mCherry) and SADG (envelope glycoprotein of rabies virus) were injected into the DMH in NPY-Cre mice. Two weeks after AAVs injection, we injected EnvA-enveloped HEPdG-GFP (High egg passage-fluey (HEP) strain rabies virus possessing GFP gene instead of gene for glycoprotein) into the same region and examined the brain one week later. We detected GFP and mCherry double positive cells only in the DMH, probably showing first-infected NPY neurons. We also found that GFP- but not mCherry-positive cells in the DMH, preoptic area, bed nucleus of the stria terminalis, amygdala, and ventral tegmental nucleus. This result suggests that DMH NPY neurons receive, at least in parts, neural inputs from these neural nuclei.

PS2.0077 LAMINA TERMINALIS, OXYTOCIN NEURONS AND THE RAT OSMOTIC HOMEOSTASIS. A COMPUTATIONAL MODEL.

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Magnocellular vasopressin and oxytocin neurons in the rat supraoptic nucleus project their axons to the posterior pituitary, where they secrete their product into the bloodstream. Once in plasma, vasopressin and oxytocin contribute to the osmotic homeostasis of the body fluids by promoting natriuresis, increasing blood pressure and inhibiting diuresis. Both vasopressin and oxytocin magnocellular neurons are osmoresponsive. Their membrane dynamics change depending of the osmolality of their extracellular fluid. They integrate that proximal information with inhibitory (IPSPs) and excitatory input afferents (EPSPs) from the organum vasculosum of the lamina terminalis (OVLT), the subfornical organ (SFO) and the median preoptic nucleus (MnPO). The SFO and OVLT contain osmoreceptive neurons that project to magnocellular neurons directly and also indirectly, via the MnPO. In this work we used a previously published computational model (1) that mimics the spiking and secretion activity of oxytocin neurons to simulate the oxytocin plasma response to different osmotic and volumetric challenges. Integrating this oxytocin model with a model of the inputs that oxytocin neurons receive from the circumventricular organs and the MnPO, our results suggest that inhibitory inputs and excitatory inputs are co-activated by osmotic stimuli. We have studied how the gain of osmotically stimulated oxytocin release changes in the presence of a hypovolemic stimulus, showing that this is best explained by an inhibition of an osmotically-regulated inhibitory drive to the magnocellular neurons. 1. Maícas-Royo J, Leng G, MacGregor DJ. A predictive, quantitative model of spiking activity and stimulus-secretion coupling in oxytocin neurons. *Endocrinology*. January 2018.

PS2.0078 POPULATION-BASED SIGNAL PROCESSING AND STIMULUS-RESPONSE IN OXYTOCIN NEURONS

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Oxytocin neurons are known for their role in milk-ejection and in homeostatic regulation of osmotic pressure, but they also function as part of appetite regulation, signalling satiety in response to the gut hormone CCK. They respond to multiple signal types, secreting oxytocin both peripherally into plasma, from posterior pituitary axonal terminals, and centrally, via dendritic secretion. Blood plasma oxytocin gives the most accessible measure of oxytocin neuronal activity, but the relationship between input stimulus and plasma output response is complex and highly non-linear. In milk-ejection, where a large short pulse of oxytocin is required, the neurons act as a coordinated network, but in other roles spiking activity is asynchronous, and the heterogeneous neurons act as a population of independent cells producing a summed output signal. The oxytocin neurons must act both individually and as a population to process noisy synaptic inputs into a robust output signal. We have previously developed an integrated input, (integrate-and-fire based) spiking, secretion, and plasma diffusion single oxytocin neuron model, accurately simulating *in vitro* and *in vivo* response. This model showed how intrinsic mechanisms such as the after-hyperpolarisation (AHP) act to reduce signal noise. Here we study a model neuron population, with varied levels of heterogeneity and independence of input signals in order to understand the relationship between single cell properties and action as a population in producing a robust signal response. Maícas-Royo J, Leng G, MacGregor DJ. A predictive, quantitative model of spiking activity and stimulus-secretion coupling in oxytocin neurons. *Endocrinology*. January 2018.

PS2.0079 EVOLUTION OF DPP4 SUSCEPTIBILITY IN VERTEBRATE PEPTIDE YY

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PP-fold peptides fulfil complex and diverse signalling roles in vertebrate energy homeostasis. The satiety action of peptide YY (PYY) is classically potentiated by dipeptidyl peptidase-4 (DPP4) cleavage and altered receptor specificity. Examinations of non-mammalian PYY molecules are limited, but DPP4 resistance is evidenced in some species. As this distinction might explain intricate contrasts in energy balance, we aimed to characterise cleavability of PYY across vertebrates. Known and presently-derived vertebrate PYY precursor sequences were examined for susceptibility to DPP4 cleavage. Parallel phylogenetic analysis was performed to describe evolution of DPP4 cleavage. Other than few spurious examples, DPP4-cleavable PYY seems to be an exclusively mammalian trait, affecting all mammalian clades. Susceptibility of PYY to DPP4

cleavage therefore likely arose around the time of mammalian divergence, representing a relatively novel development of the involvement of PP-fold signalling in vertebrate energy homeostasis. Ancestral vertebrate PYY was clearly resistant to cleavage by DPP4. This resistance persists in most extant non-mammalian vertebrates. The satiety action of PYY is therefore ostensibly exclusive to mammals and might explain some unique features of mammalian energy homeostasis, for example comparatively low glucose tolerance and susceptibility to type 2 diabetes (T2D). These findings broadly support recent observations of a critical pancreatic signalling role for PYY in mammalian glucose homeostasis. Aberrant regulation of PYY cleavage and signalling may therefore play a role in progression of T2D and somewhat explain the antidiabetic action of DPP4 inhibitor drugs. Exploration of the role of PYY in glucose homeostasis might yield novel therapeutic approaches for T2D.

PS2.008 MECP2 REGULATES INHIBITORY PLASTICITY UNDERLYING MATERNAL BEHAVIOR IN SENSITIZED VIRGIN FEMALE MICE

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Cohabitation of pups and mother with virgin mice induces neural circuitry plasticity in such a way that those virgin mice display maternal behavior towards the pups. Non-hormonal factors are thought to be important in mediating that plasticity, likely through chromatin remodeling of specific neural circuitry. We have previously shown that virgin females deficient in Methyl CpG-binding protein 2 (MECP2), an epigenetic chromatin regulator, display inefficient pup gathering behavior. We found that the auditory cortex of MECP2-deficient females had increased numbers of PNNs, extracellular matrix protein structures that function as structural barriers for plasticity, which contributed to the inefficient pup gathering behavior. Currently, we use whole brain immunostaining and imaging studies to identify neural circuitry responsible for various features involved in efficient pup gathering behavior.

PS2.0080 CHARACTERIZATION OF AGRP NEURONS KNOCK OUT MOUSE FOR THE CARNITINE PALMITOYL TRANSFERASE 1A (CPT1A).

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In the acruate nucleus of the hypothalamus, a key region in the energy homeostasis regulation, the Agouti related peptide (AgRP) neurons were largely described to be involved in this function. These neurons receive various metabolic signals including nutrients and hormones. Growing evidence displayed a role of the fatty acid metabolism in these neurons in their

integration. The Carnitine Palmitoyl Transferase1a is a key enzyme involved in the pool of acetyl-CoA in the mitochondria by permitting the long-chain fatty acyl-CoA to enter into the matrix and undergo the beta-oxidation. Previous works have shown that this enzyme is involved in the food intake and glucose homeostasis. To determine the role of CPT1a in the energy homeostasis, we breeded $AgRP^{CRE}$-IRES mice with $CPT1a^{floxed/floxed}$ mice to specifically deleted CPT1a in the AgRP neurons compared to the wild type ($AgRP^{WT}$) mice. A complete phenotypic characterization was undertaken (body weight, food intake, body composition, plasma assays, metabolic parameters). Data were analyzed by Student's t-test, two-way anova or two-way anova repetead measure. Although the AgRP $CPT1a^{-/-}$ mice presented sligh higher food intake no difference in the body weight and body composition were observed compared to the $AgRP^{WT}$ mice. In parallel, in fed and fasted conditions no difference was observed for the energy metabolism and plasmatic parameters. However, the AgRP $CPT1a^{-/-}$ mice presented a higher plasma insulin secretion induce by the glucose. The next step will be to focus on the hypothalamic impact of the knockout as well as the AgRP neurons sensitivity to the insulin.

PS2.0081 ACTIVATION OF THE RAT SUPRAMAMMILLARY NUCLEUS FOLLOWING THE MOTIVATED CONSUMPTION OF FOOD

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Understanding of the complex crosstalk between the reward-based feeding system and the brain regions involved in the homeostatic control of the food intake is currently the challenging issue in the field of energy balance regulation. The supramammillary nucleus (SuM) is a posterior hypothalamic region with a potential role in goal-oriented behaviour (Pan et al., Prog. Neurobiol, 2004). However, the role and connectivity of SuM in the context of feeding or reward control was until recently unexplored. We tested the hypothesis that motivated eating behaviour would be associated with activation of the SuM. To determine the effects of motivation for palatable food, we conditioned satiated rats to consume 5 ml sweetened condensed milk (SCM) daily (15 min/day for 7 days) and used c-Fos to map neuronal activity in regions involved in appetite control and food reward. Compared to control animals, we saw an increase in c-Fos expression in the SuM. Furthermore, the immunohistochemical double staining experiments for c-fos/ tyrosine hydroxylase (TH) suggest that the dopamine component of the SuM could be involved. Interestingly, we detected a simultaneous c-fos activation in areas involved in energy balance regulation (dorsomedial hypothalamus and lateral hypothalamus) and in the reward system (ventral tegmental area). Using injections of viral/non-viral fluorescent tracers and a transgenic rat line model expressing cre-recombinase in TH neurons (Witten et al., Neuron, 2011), tracing experiments are performed to determine if the SuM is connected to these regions and to identify the chemical nature of these putative pathways.

PS2.0082 FAT, CARBOHYDRATE OR PROTEIN BY ORAL GAVAGE EFFECTIVELY REDUCE APPETITE IN THE SPRAGUE DAWLEY RAT.

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The aim of this study was to assess whether a difference existed between macronutrients in their ability to activate afferent neuronal cells of the hindbrain in the gut-brain-axis which constitutes the primary input from the intestinal tract leading to satiation. A scheduled feeding paradigm was used whereby food was presented at ZT6 and ZT11 for 2h to ensure prompt eating behaviour on food presentation. On the 8th day after scheduled feeding started, 30min prior to ZT6, rats were gavaged with an isocaloric (8kcal), isovolumetric solution containing one of three macronutrients or isovolumetric saline solution. Thirty minutes after gavage food intake was assessed by providing a scheduled meal of AIN-93M. Rats which received saline consumed a similar amount of AIN-93M compared to pre-gavage intakes indicating that gavage does not affect subsequent food intake. However, rats which had received a gavage containing a caloric solution of macronutrient, all reduced intake of AIN-93M by a similar amount (18-20kcal), demonstrating that all macronutrients were equally effect at reducing food intake. Interestingly, the caloric reduction in food intake was greater than the caloric value of the macronutrient solution gavaged. Analysis of *c-fos* expression in the hindbrain by *in-situ* hybridization 90min after food presentation showed similar increases in *c-fos* expression in the NTS. Given that gavage per se does not affect subsequent food intake, these data would suggest that when delivered directly to the stomach each macronutrient elicits a similar response to reduce appetite. Funded by the EU Seventh Framework programme (Full4Health - grant number 266408).

PS2.0083 DEGENERATION OF THE DOPAMINERGIC NEURONS IN A RAT MODEL OF CHRONIC HYPERGLYCEMIA.

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An accumulating number of epidemiological studies support a link between diabetes and neurodegenerative disorders. Indeed, hyperglycemia is known to cause oxidative stress in vulnerable tissues, including the nervous system. Glucose is the obligate energy substrate of adult neurons. Owing to the preponderant expression of glucose transporter 1 (GLUT1) at the blood-brain barrier and glucose transporter 3 (GLUT3) at the neuron plasma membrane, uptake overwhelmingly occurs in an insulin-independent fashion. Recently, our team established that elevated levels of glucose lead to oxidative stress and apoptosis in cultivated dopaminergic neurons and several early studies report dopaminergic alterations in diabetes or acute hyperglycemia. Nevertheless, the state of entire dopaminergic pathways has not been inquired.

Considering the paucity of literature addressing dopaminergic alterations in hyperglycemia, the aim of this study was to characterize the effects of long-term hyperglycemia in dopaminergic pathways. In the nicotinamide-streptozotocin rat model of hyperglycemia, the nigrostriatal motor pathway and the reward-associated mesocorticolimbic pathway, both composed of dopaminergic neurons, were specifically investigated. Neuronal and glial alterations were evaluated 3 and 6 months after hyperglycemia induction. Our results demonstrate preferential degeneration of the nigrostriatal pathway associated with astrogliosis and loss of microglial cells after 6 months. These results provide refreshing insight on the higher occurrence of Parkinson's disease in diabetic patients increasingly acknowledged by medical authorities.

PS2.0084 GHRELIN RESCUES SKELETAL MUSCLE CATABOLIC PROFILE IN THE R6/2 MOUSE MODEL OF HUNTINGTON'S DISEASE

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Background Huntington's disease (HD) is an inherited neurodegenerative disease and accumulating evidence suggests altered energy metabolism as a key feature in HD pathology. Hyper-catabolism, including weight loss and muscle atrophy, is seen in HD patients and HD mouse models. Metabolic hormones are key players also in neurodegenerative processes. The R6/2 mouse model of HD mirrors human HD and the mice exhibit progressive weight loss, skeletal muscle atrophy, altered glucose metabolism and body composition. Ghrelin, a gut peptide-hormone, plays an important role in regulating energy metabolism and stimulating appetite. It has been suggested that the use of Ghrelin and analogues may be beneficial for many clinical problems such as muscle wasting, cachexia, cognitive decline and metabolic problems. ***Aim*** In this study, we targeted energy metabolism in R6/2 mice using ghrelin administration, with the primary aim to delay weight loss and reduce muscle atrophy. We also evaluated glucose metabolism and behaviour. ***Methods*** We treated mice with ghrelin (subcutaneous 150 e/kg daily injections) for 2, 4 or 6 weeks and evaluated effects in circulation using antibody based assays and in target tissues using real-time PCR and western blot. ***Results*** We here demonstrate that ghrelin administration, reversed the catabolic gene expression profile seen in R6/2 mouse skeletal muscle. Skeletal muscle morphology was also improved with ghrelin, and importantly, behavioural deficits in R6/2 mice was normalized. ***Conclusion*** Taken together, our findings encourage further studies targeting metabolism in HD. ***Future plans*** Effect of ghrelin will be further investigated. ***Funding*** EHDN seed fund and Swedish research council.

PS2.0085 DORSAL RAPHE SEROTONIN NEURONES RESPOND TO DIETARY NUTRIENTS

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Genetic and pharmacological research has identified the 5-hydroxytryptamine (5-HT; serotonin) system as a fundamental regulator of energy balance and body weight. We hypothesise that circulating nutrients act as endogenous regulators of the 5-HT appetitive brain circuit. Using c-fos immunohistochemistry as a marker of neuronal activation, here we demonstrate that a subset of 5-HT neurones in the dorsal raphe nucleus (DRN) of mice increase their activity in response to the ingestion of food. Next, we used a gavage technique to remove variables associated with the sight, smell and taste of food in addition to mastication and gastric distension and found that nutrients increased the activity of a subset of DRN 5-HT neurones compared to a gavage of water. Using *ex vivo* slice electrophysiology in a line of mice with fluorescently labelled 5-HT neurones, we also reveal that DRN 5-HT neurons possess K_{ATP} channels, which suggests that they are capable of directly responding to nutritional cues. Finally, we determined that individual nutrients directly affect the firing rate of a subset of DRN 5-HT neurones, *ex vivo*. These findings suggest that 5-HT neurones are directly activated by dietary nutrients and this may be an essential mechanism through which 5-HT regulates appetite and body weight.

PS2.0086 ELEVATED PLASMA CONCENTRATIONS OF SOLUBLE (PRO)RENIN RECEPTOR IN OBSTRUCTIVE SLEEP APNEA

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Obstructive sleep apnea (OSA) is characterized by recurrent episodes of respiratory disturbance during sleep and excessive daytime sleepiness, and is frequently associated with obesity, hypertension and diabetes mellitus. (Pro)renin receptor ((P)RR), a receptor for renin and prorenin, is widely expressed in various organs including brain and kidney. (P)RR has been shown to be related to the pathophysiology of cardiovascular, renal diseases including hypertension and diabetes mellitus. Soluble (P)RR (s(P)RR) consisting of the extracellular domain is present in blood, and the plasma levels are elevated in patients with chronic kidney disease and pregnant women with hypertension or diabetes mellitus. The aim of the present study was to clarify the relation between plasma s(P)RR concentrations and the severity of OSA. Plasma s(P)RR concentrations were studied in 289 subjects (196 men and 83 women). Plasma s(P)RR levels were significantly elevated in OSA patients, compared to non-OSA subjects. s(P)RR levels were significantly correlated with apnea hypopnea index (a marker for OSA severity), with a higher r value found in male subjects. OSA patients with type 2 diabetes mellitus or

chronic kidney disease showed higher s(P)RR levels. By contrast, hypertension had negligible effects on s(P)RR levels. The s(P)RR levels were significantly decreased by continuous positive airway pressure treatment in 41 OSA patients. In conclusion, plasma s(P)RR levels were elevated in OSA patients and a higher association with the severity of OSA was found in male subjects. Hypoxia and subsequent oxidative stress, particularly in brain, may cause the elevation of plasma s(P)RR concentrations in OSA patients.

PS2.0087 PRE-PREGNANCY OBESITY AND EXCESSIVE MATERNAL WEIGHT GAIN ALTER FETAL HEART RATE ACTIVITY

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Pre-pregnancy obesity and maternal weight gain during pregnancy can lead to adverse effects in the newborn but also to metabolic, cardiovascular and even neurological diseases in older ages of the offspring. The present study therefore uses heart activity as a proxy for the activity of the autonomic nervous system (ANS) with the aim to evaluate the effect of pre-pregnancy weight and maternal weight gain on the ANS of the fetus in healthy pregnancies. Fetal Magnetoencephalography (fMEG) allows to record fetal and maternal heart rate and fetal brain activity. Fetal heart activity was recorded from 184 healthy pregnant women in second and third trimester pregnancies. The pre-pregnancy body mass index (BMI) and maternal weight gain during pregnancy was recorded. The study showed a higher fetal heart rate in obese mothers compared to normal weight mothers (p=0.04). Both, high pre-pregnancy BMI and excessive weight gain in healthy pregnancies was associated with changes in fetal heart activity, indicating alterations of the ANS. These findings support the concept of fetal programming and add knowledge about the important influence of intrauterine environment on the developing ANS and the possible programming of obesity.

PS2.0088 STABILIZATION OF BETA-CATENIN IN MOUSE HYPOTHALAMIC CELL LINES

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Beta-catenin is a signalling molecule in the Wnt-signalling pathway, which has typically been associated with embryogenesis and tumorigenesis. More recently, new lines of evidence suggest that it may also be involved in the pathogenesis of type-2 diabetes. In its active form, beta-catenin acts together with the transcription factor T cell-specific transcription factor-7-like-2 (TCF7L2) to activate target genes of the Wnt-signalling pathway. Impairment in this signal transduction pathway both in the pancreas and in the hypothalamus may contribute to the development of type-2 diabetes. Here, we sought to identify possible mechanisms of feeding-induced stabilization of β-catenin in adult mouse hypothalamic cell lines that express the phenotype for various neuropeptides and receptors involved in central regulation of metabolism. We surveyed a variety of potential hormone factors that can simulate the effect of feeding: forskolin, exendin-4 and MTII (an α-MSH analogue). After treatment, we firstly measured NPY and AgRP secretion. Treatment with these factors did not affect the secretion of either neuropeptide. After applying KCl to depolarise the cells, however, there was significantly greater release of both AgRP and NPY from treated cells compared with vehicle-controls, indicative of an effect on synthesis or vesicle trafficking. We next treated these cell lines with forskolin and discovered that in one of the cell line, all the Wnt-responsive genes analyzed were markedly up-regulated. These data suggest that the treatment has increased the pool of neuropeptide available for release. These results are consistent with the role of β-catenin in regulating a feeding response through a central mechanism.

PS2.0089 CIRCULATING OSTEOCALCIN AND VERBAL MEMORY PERFORMANCE IN PEOPLE WITH TYPE 2 DIABETES MELLITUS.

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Background: Osteocalcin circulates in carboxylated (cOCN) and undercarboxylated (ucOCN) forms, and ucOCN is understood to have widespread neuroendocrine effects. In type 2 diabetes mellitus (T2DM), circulating ucOCN concentrations are lower, and cognitive decline is accelerated. We aim to determine whether ucOCN is associated with verbal memory performance in people with T2DM. Methods: Fasting serum concentrations of cOC and ucOCN were assayed using isoform specific ELISAs. Verbal memory performance was assessed using the California Verbal Learning Test, 2nd Ed (CVLT-II), from which a composite Z-score was calculated from verbal learning, short-delayed free recall and long-delayed free recall. Results: In 30 people with T2DM (age 63.3±8.9, 60% women, HbA1c 7.64±0.01%, duration of diabetes 8.2±8.6 years), ucOCN (β=0.423, p=0.019), but not cOCN (β=0.052, p=0.789) or total osteocalcin (β=0.193, p=0.319), was associated with memory performance, in models controlling for age and gender. ucOCN was associated with fasting insulin concentrations (β=0.454, p=0.021);

however, fasting insulin was not significantly associated with verbal memory performance (β=0.278, p=0.181). Conclusions: The results suggest the possible relevance of a bone-derived neuroendocrine mediator to memory performance in people with T2DM.

PS2.009 COMPARING FEEDING AND EXPLORATORY BEHAVIORS BETWEEN WT AND ER ALPHA KO MALE AND FEMALE MICE

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The reproductive steroid hormone, 17β-estradiol (E2), control feeding and exploratory behaviors associated with mood disorders. The loss of circulating E2 puts menopausal woman at a increased risk for developing obesity and mood disorders when compared to premenopausal woman. Therefore, it is critically important to understand the role of sex steroids and their receptors in the neuroendocrine control of feeding and mood. The goal of this project is to understand the role of estrogen response Element (ERE)-dependent and ERE-independent ERα signaling on behavior by characterizing feeding patters and exploratory behaviors in male and female mice lacking either total ERα signaling or lacking ERE-dependent ERa signaling. We hypothesize that ERE-independent ERα is partially sufficient to restore feeding and exploratory behaviors that are lost in total ERα knockout mice. We tested three strains of mice: two ERa transgenic models, a total ERa knock out (ERKO) and a novel ERa knock in/knock out (KIKO) that lacks a functional DNA-binding domain) and their wild type (WT) C57 littermates using a real-time feeding behavior monitoring system and series of standard behavior tests (open field tests, elevated plus maze, forced swim test). Each experiment was initially done with intact animals and then again repeated in ovariectomized (OVX) animals split into either an oil treated control group or an E2-treated group. By using these ERα transgenic mouse models, we will investigate the contribution of ERE-mediated ERα signaling in controlling feeding and exploratory behaviors.

PS2.0090 ANALYSIS OF THE MELANOCORTIN SYSTEM IN THE REGULATION OF PITUITARY GLAND FUNCTION IN MICE

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The five melanocortin receptors (MC_{1-5}) are G-protein-coupled receptors (GPCRs), expressed in the central nervous system and peripheral tissues, which are activated by melanocyte-stimulating/adrenocorticotrophic hormones and modified by melanocortin receptor accessory proteins (MRAP1 and 2). MCs have well-characterised roles in the regulation of appetite, immune and stress responses, but their expression in a range of tissues suggests they may have other roles in modifying physiology. We have characterised MC and MRAP expression in the female murine pituitary and found expression of MC_3, MC_4 and MC5, as well as MRAP1 and MRAP2, which changes with age. This suggests that MC expression may mediate coordination of pituitary gland axes, as well as alterations in pituitary gland functional output across lifespan. Further analysis with highly specific chromogenic RNA *in situ* hybridisation (RNAscope®) shows that a proportion of somatotroph cells co-express MC_3, consistent with our previous studies showing that loss of this receptor leads to alterations in the GH axis. Since it is becoming apparent that interactions of MCs and MRAPs with other GPCRs can modify signalling, we are testing the effects of their co-expression with established receptors regulating the GH axis both in the presence and absence of MC ligands. GPCR function is being tested both by directly monitoring specific GPCR activation and their down-stream signalling pathways. These studies will have implications for fundamental understanding of pituitary axes crosstalk, as well as potential off-target effects of pharmacological interventions.

PS2.0091 SURVEYING THE HETEROGENEITY OF HYPOTHALAMIC AGRP/NPY NEURONS BY SINGLE-CELL RNA SEQUENCING

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Over the past two decades, insights from human and mouse genetics have illuminated multiple pathways within the brain that play a key role in the energy metabolism. We now know that the leptin-melanocortin signalling within the hypothalamus is central to the control of food intake, with genetic disruption of components of this pathway resulting in severe obesity. In the hypothalamic arcuate nucleus there are two distinct but rare populations of leptin responsive neurons, namely agouti-related peptide and neuropeptide Y (AgRP/NPY) neurons, and Pro-opiomelanocortin and Cocaine- And Amphetamine-Regulated Transcript (POMC/CART) neurons, where they play opposite roles in the regulation of food intake. Here, we isolated NPY-expressing cells from NPY-eGFP reporter mice using fluorescence-assisted cell sorting (FACS), followed by encapsulation of cells into oil droplets. These oil droplets contain unique molecular barcodes (UMI) and reagents required for reverse transcription and cDNA amplification, and thus allow us to generate uniquely tagged transcriptomes for each of the captured cells. Using this technique, we captured a total of 4246 cells. Based on their transcriptomic profiles, we splitted these cells into 2 neuronal and 9 other cell clusters, with each expressing a distinct set of characteristic markers. Understanding the heterogeneity of AgRP/NPY neurons and differential roles of each subtype will help explain and provide further insights into the interplay between various nutritional stimuli and neural circuits in the brain. The identification of

different subtypes (and their response) will also enable us to discover potentially novel and perhaps more specific drug targets to improve the treatment of nutritional disorders.

PS2.0092 ROLE OF OLFACTORY MARKER PROTEIN IN HIGH FAT DIET INDUCED OBESE MOUSE

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Obesity is a major cause of diabetes, hyperlipidemia, stroke, hypertension and is known to be a chronic disease that increases mortality. Recent studies have shown that olfactory receptors are widely expressed in various tissues including kidney, heart, and liver muscle. This study revealed that olfactory marker proteins are expressed in adipose tissue and are more expressed in adipose tissue of obese mice. In mice in which the olfactory marker protein (OMP) was removed, the body weight gain due to the high fat diet was significantly lower and the UCP-1 expression was increased in the white fat and brown fat. In addition, it was confirmed that the formation of fatty liver due to the high fat diet was significantly reduced in the $OMP^{-/-}$ mice. This is the result showing that olfactory marker proteins can also be expressed in metabolic tissues and participated in energy metabolism and body fat regulation. Loss of olfactory function promotes beige localization of white fat, promotes thermogenesis through the expression of UCP-1 in brown adipose tissue, and decreases the expression of lipogenesis-related markers in liver tissue. Thus, the loss of olfactory function is caused by the consumption of fat accumulated in the body as an energy source, thereby reducing body fat and preventing obesity.

PS2.0093 ASSOCIATION OF SERUM INSULIN-LIKE GROWTH FACTOR-1 WITH BRAIN MICROSTRUCTURE IN PARKINSON'S DISEASE

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Background: Growing evidence shows that impaired signaling of Insulin Growth Factor-1 (IGF-1) is associated with neurodegenerative disorders such as Parkinson Disease (PD) and is proposed as a potential early PD biomarker. Although there is still controversy regarding its proinflammatory or neuroprotective function. In this study, we aimed to discover the relation between serum IGF-1 levels in Drug-naïve early PD patients and microstructural changes in white matter tracts in order to better understand central associations of this factor. Method: Through connectometry approach we tracked the connectivity patterns in white matter diffusion MRI of 85 Drug-naïve and non-demented early PD patients with only motor

manifestations and 58 age and sex-matched healthy controls, to investigate the association of subcomponents of neural pathways with serum IGF-1 levels measured in a fasting state. Result: The connectometry analysis proved significantly (FDR= 0.0131004) negative correlation between serum IGF-1 levels and the connectivity in following fibers while controlling for age, sex, BMI, mood and cognitive status and disease duration in multiple regression analysis: middle cerebellar peduncle, cingulum, genu, and splenium of the corpus callosum only in PD patients. There was no significant association between whole brain white matter connectivity of healthy controls and levels of IGF-1. Conclusion: elevated IGF-1 is contributed to neural damage in the pathways with the significant role in prodromal PD and its debilitating motor and cognitive disturbances, which may propose IGF-1 as a prodromal serum marker and predictor of worse outcome. More studies are needed to investigate this suggestion.

PS2.0094 THE HYPOTHALAMIC FEEDING-REGULATING NEUROPEPTIDES IN THE STREPTOZOTOCIN-INDUCED DIABETIC RAT

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We examined the hypothalamic feeding-regulating neuropeptides gene expressions in the paraventricular nucleus (PVN) and the arcuate nucleus (ARC) with/without fasting in the diabetic rats administered streptozotocin (STZ). STZ (80 mg/kg) was administered intraperitoneally (i.p.) in adult male Wistar rats. Rats were divided into 3 groups: Normal glucose (<300 mg/dl at light period) tolerance (N), Impaired glucose (≧300 mg/dl at light period and < 200 mg/dl after fasting for dark period) tolerance (I), and Diabetes (D) (≧300 mg/dl at light period and > 200 mg/dl after fasting for dark period). Two weeks after i.p. administration of STZ, they were decapitated after fasting for 12 hours. The gene expressions of *corticotrophin releasing hormone (CRH), thyrotropin-releasing hormone (TRH), proopiomelanocortin (POMC), cocaine- and amphetamine-regulated transcript (CART), neuropeptide Y (NPY), agouti-related protein (AgRP)* in the ARC were quantified by using *in situ* hybridization histochemistry. *POMC* and *CART* were significantly decreased in I and D compared to N. On the other hand, *NPY*, *AgRP and TRH* were significantly increased in D but not I compared to N. The gene expressions of *TRH* but not others were significantly decreased in insulin<0.45 ng/ml group compared to insulin≧0.45 ng/ml group. The gene expression of the hypothalamic ARC anorexigenic neuropeptide decreased in the rats with hyperglycemia after STZ administration but not hyperglycemia after fasting, and no significant change was observed in the orexigenic

neuropeptide. The gene expressions of *TRH* might have relationship with insulin level in plasma of diabetic rats.

PS2.0095 MOLECULAR PHENOTYPE OF TEMPERATURE AND PRESSURE-SENSITIVE NEURONS IN THE OVLT

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The OVLT is a circumventricular organ ~600 um wide by ~700 um high (horizontal plane) that lies over the third ventricle where detects variations in systemic osmolality carried by the cerebrospinal fluid (CSF) (Prager-Khoutorsky & Bourque 2015). Preliminary unpublished work in our lab has shown synaptic control of the supraoptic nucleus (SON) magnocellular neurosecretory cells (MNCs) by thermosensitive neurons in the *organum vasculosum lamina terminalis* (OVLT). However, the molecular identity of these neurons, their thermosensitivity profile, and their specific location in the OVLT was unknown. Here, using a mix of electrophysiology, single-cell RT-PCR, pharmacology, and temperature stimulation protocols we explored the distribution of the neurons containing *Trpv1* (TRPV1 WT) or *Trpv1dn* (DN-TRPV1) on a surface ~500 um wide and ~440 um high of OVLT tissue. Our results show a large distribution of thermosensitive neurons in the OVLT that do not circumscribe to the middle line. Detailed analysis of these populations shows three temperature responsive behaviors. The molecular component shows a well-defined distribution of neurons expressing *Trpv1dn* across the middle line coexpressing with *Avpr1a*; this distribution correlates with the response to negative pressure demonstrated by these neurons and the significant reduction in the firing activity after SB366791 is added in the bath. Meanwhile, neurons expressing the *Trpv1* transcript locate mostly around the middle core, and they correlate with the areas where neurons do not respond to negative pressure. We also report here the finding of an unexpected population of *Avpr2* expressing neurons surrounding the inner core.

PS2.0096 EFFECTS OF CENTRAL OXYTOCIN ON CISPLATIN-INDUCED ANOREXIA IN RAT

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During chemotherapy of cancer, some drugs such as 5-HT_3 receptor antagonists usually have been used to control with nausea, vomiting and anorexia. However, these drugs cannot entirely control them. Oxytocin (OXT) is a well-known neuropeptide related with appetite. We examined the effects of OXT in cisplatin-induced anorexia in rats, using immunohistochemistry for Fos. Fos-like immunoreactivity (Fos-LI) was expressed in the supraoptic nucleus (SON), paraventricular nucleus (PVN), area postrema (AP) and nucleus of the solitary tract (NTS) after intraperitoneal (ip) administration of cisplatin. Double-immunostaining for Fos and OXT showed that some OXT-LI cells coexisted with Fos-LI in the SON and PVN after administration of cisplatin. We also examined the OXT-monomeric red fluorescent protein 1 (mRFP1) fluorescence intensities after ip administration of cisplatin in OXT-mRFP1 transgenic rats. The mRFP1 fluorescence intensities were significantly increased in the SON and PVN 12 hours after administration of cisplatin. In the NTS, the mRFP1 fluorescence intensities were significantly increased 24 hours after administration of cisplatin. The food intake was significantly decreased 2 hours after administration of cisplatin. The cisplatin-induced anorexia was abolished by the pretreatment with OXT receptor antagonist (OXTR-A). Finally, we examined the effects of OXTR-A in cisplatin-induced anorexia in rats, using immunohistochemistry for Fos. Cisplatin-induced Fos expressions in the SON, PVN, AP and NTS were significantly suppressed by pretreatment of OXTR-A. In the OXT-LI cells, cisplatin-induced Fos expressions in the SON and PVN were also suppressed by pretreatment of OXTR-A. These results suggested that central OXT may be involved in cisplatin-induced anorexia in rats.

PS2.0097 SANSEVIERA LIBERICA METHANOLIC ROOT EXTRACT AMELIORATES INSULIN RESISTANCE IN TYPE 2 DIABETIC RATS

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This study was aimed to evaluate the effects of *Sanseviera liberica methanolic root extract* on serum insulin, lipids levels and beta cells in fructose-fed streptozotocin induced Type 2 diabetes. Eight-week-old Albino rats (160.56 ± 15.60 g) were divided into three groups, the normal control, diabetic control and *Sanseviera liberica methanolic root extract* (200 and 400 mg/kg) treated group. All animals were fed with a regular pellet diet and additionally received a 20% of D-Fructose respectively in drinking water for 2 weeks prior to induction of diabetes. Diabetes was induced by a single dose subcutaneous injection of freshly prepared streptozotocin and dissolved in 0.1M citrate buffer (pH=4.5) at the dose of 40 mg/kg body weight and injected intraperitoneally. Blood glucose level was measured by using one-touch glucometer. Insulin levels was measured using ELISA method. Additionally, insulin resistance was calculated using a Homeostasis Model Assessment of Insulin Resistance (HOMA-IR). The lipids levels were determined using Randox diagnostic kit. *Sanseviera liberica methanolic root extract* (200 and 400 mg/kg) treated group showed a significant effect ($p < 0.05$) in the levels of blood glucose, serum insulin, lipids levels and beta-cells when compared with the normal

control and diabetic control groups. The histological assessment of the pancreas of *Sanseviera liberica methanolic root extract* (200 and 400mg/kg) treated group showed normocellular islets surrounded by normal appearing exocrine acini and no necrosis was seen. This study has established a possible mechanism of action of *Sanseviera liberica methanolic root extract* in fructose-fed streptozotocin induced type 2 diabetic rats.

PS2.0098 RESISTANCE OF THE AGING LOU RAT TO DIET-INDUCED METABOLIC DISTURBANCES

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OBJECTIVES: Type 2 diabetes and obesity increase the risk of Alzheimer's disease (AD) and may promote cognitive decline. Here, we studied the effect of a diet, known to induce obesity and insulin resistance, in LOU/C/Jall (LOU) rats. This strain is considered a model of healthy aging with increased longevity, low body fat mass throughout life, low incidence of age-related diseases and maintenance of cognitive functions in advance age. **METHODS:** Six- and 24-month-old male LOU rats were metabolically challenged using a long-term HF/HG diet (16 weeks, 60% calorie intake from high fat chow + 10% glucose in water). Control groups had access to standard diet and tap water. Body weight (BW) and food/water consumption were measured regularly. Glycaemia was monitored during the diet and at sacrifice. Recognition and spatial memory and body composition were assessed at the end of the diet. **RESULTS:** The HF/HG diet neither affected caloric intakes nor glycaemia but led to some changes in fat mass and BW, and in the regulation of the hypothalamic leptin axis. In spite of a high daily consumption of glucose, glycaemia and memory performances remained unaltered and comparable to those of young and old controls. **CONCLUSION:** These results indicate that the LOU rat can resist to the effects of diets inducing severe metabolic disturbances in common laboratory rat strains. Identification of molecular targets linked to this phenotype should help developing novel pharmacological strategies to preserve metabolic health and cognitive abilities in aging.

PS2.0099 MIR103/107 DETERMINE POMC-EXPRESSING PROGENITORS CELL FATE AND IMPACT LIFELONG GLUCOSE HOMEOSTASIS

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The hypothalamic melanocortin system, which includes neurons that produce proopiomelanocortin (POMC)-derived peptides, is a major negative regulator of energy balance. A distinct developmental property of POMC neurons during embryonic life is that they can adopt an orexigenic neuropeptide Y (NPY) phenotype. Here, we demonstrated that POMC neurons express Dicer, an essential enzyme for miRNA maturation and that the loss of Dicer in POMC neurons causes metabolic defects associated with an age-dependent reduction in the number of *Pomc* mRNA-expressing cells. Moreover, lack of Dicer in Pomc-expressing progenitors favors the acquisition of a NPY phenotype. During the prenatal development, miR-103/107 are highly expressed in Pomc-expressing cells but displayed a low level in NPY neurons that derived from Pomc-expressing progenitors. In vitro, miR-103/107 inhibition leads to a reduction of Pomc mRNA-expressing cells and to an increase in the proportion of Pomc-expressing progenitors that differentiate into NPY neurons. In utero, miR-107 silencing impaired lifelong glucose homeostasis. Together, these data suggest new role for miRNAs, and particularly miR-103/107, in the timely maturation of POMC neurons. They also provide new insights into the developmental mechanisms responsible for the regulation of energy balance in adults.

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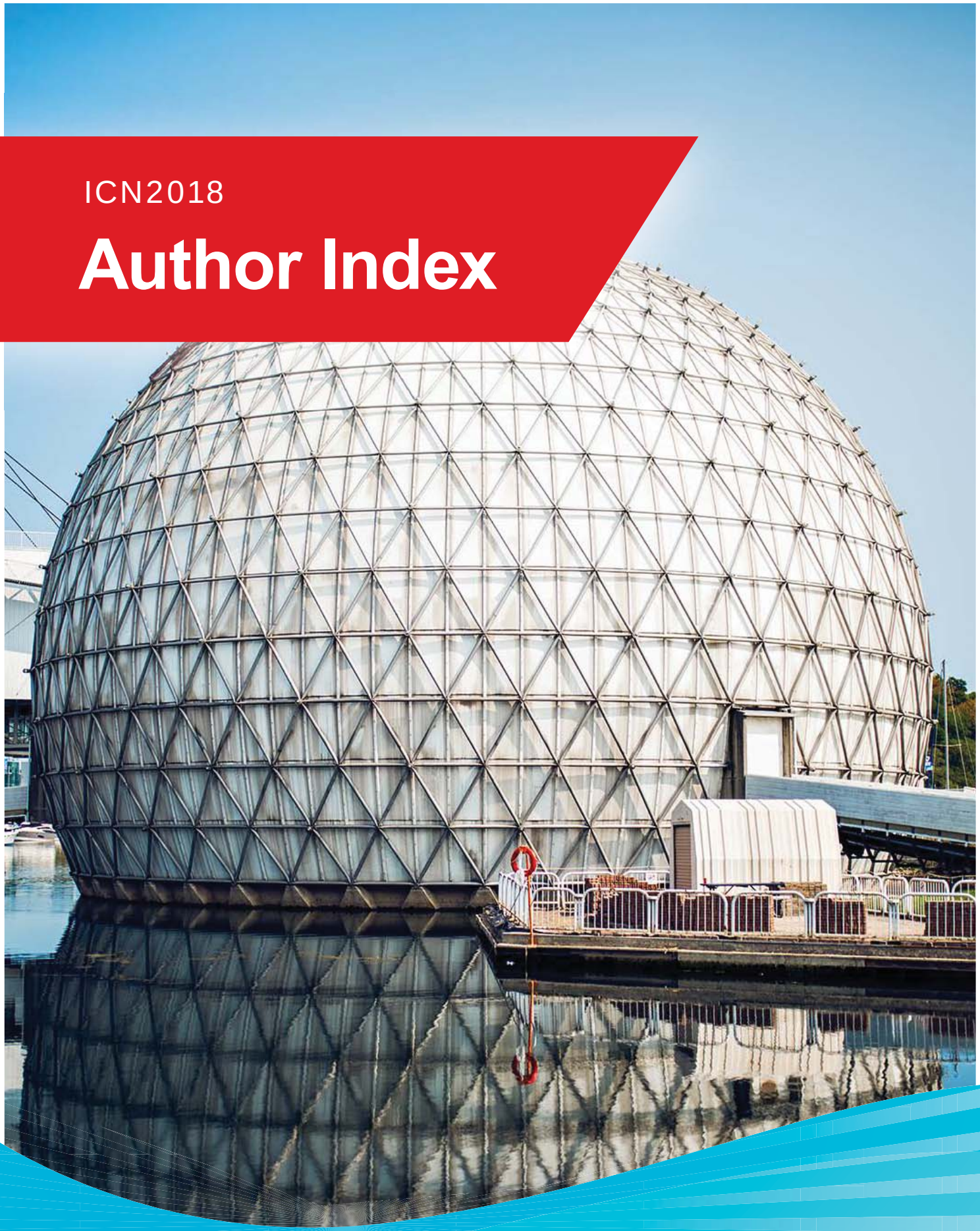

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Arnal Jean-François	**CS3R.002**
Asari Yuko	**PS2.00155**
Ashton Anna	**PS2.00189**
Asling Hayley A.	**PS1.006**
Atwell Jonathan	**PS1.00161**
Au April	**BOP2B.006**
Aucagne Vincent	**PS1.00124**
Audinat Etienne	**PS1.0069**
August Avery	**PS1.00177**
Augustine Rachael A.	**PS2.00121, PS2.00141**
Augustin Hellmut	**CS2R.002**
Avan Paul	**PS1.0055**
Avendaño Marisol	**BOP1R.003, PS1.00140**
Aylwin Carlos F.	**CS1R.002, PS2.00131**
Azam Amber B.	**PS1.0037**
Bachelor Martha	**PS2.00172**
Bäckhed Fredrik	**CS4M.002**
Baek Songjoon	**PS1.0071**
Bagci Esra	**PS1.0080**
Bagley Jennifer	**PS2.0023**
Bailbé Danielle	**BOP2B.001**
Bailey Craig D..C.	**BOP2S.003**
Bains Jaideep	**CS3S.001**

Baker Cydni N.	**PS2.00137**
Baker Matthew R.	**PS2.00149, PS2.00150**
Baker-Sullivan Elizabeth A.	**PS2.0025**
Bake Tina	**PS1.003**
Bakker Julie	**YA3.001**
Balabanov Ivaylo E.	**PS2.006**
Balapattabi Kirthikaa	**BOP2S.001**
Baldo Barbara	**PS2.0019**
Bale Tracy	**CS1M.003**
Banerjee Jineta	**BOP1M.001**
Bano Riffat	**PS1.00141**
Barabás Klaudia	**PS1.00157**
Baran Marta	**PS2.00148**
Baran Nicole M.	**PS2.0048**
Barantin Laurent	**PS1.00155**
Barbosa Alan S.	**PS2.00127**
Barcelos Lucíola D.S.	**PS2.00127**
Bardet Pierre-Luc	**PS1.0032, PS1.0035**
Barrett Catherine E.	**CS3B.004**
Barrett Perry	**CS4T.002, PS2.00130, PS2.0082**
Barroso Romero Alexia	**BOP1R.003, PS1.00134**
Bassareo Valentina	**PS2.0083**
Bassett Anne	**PS1.0096**
Bassett Paul	**PS1.00103**
Bass Noah	**CS1B.001**
Basso Julia C.	**YA3.003**
Batool Farhat	**PS2.0059**
Bauer Carolyn M.	**PS1.00161**
Bauer Ilena A.M.	**PS2.0087**
Baumgartner Nina E.	**PS2.0043**
Bayerl Doris	**PS1.00164**
Bayne Mitchell	**PS1.0083**
Beach Katherine	**PS1.00159**
Beach Linda Q.	**PS1.00163**
Beales P L.	**PS1.00101**
Beau Jacques	**PS2.00190**
Beaulieu Jimmy	**PS2.0083**
Beaumatin Nicolas	**PS2.00190**
Bech Paul	**PS1.00103**
Becker Susan E.	**PS2.0036**
Bedenbaugh Michelle N.	**PS1.00125, PS1.00127, PS1.00142**
Been Laura	**PS1.00159**
Beery Annaliese K.	**CS2B.001, PS2.0050, PS2.0055**
Behuliak Michal	**PS2.00170, PS2.00171**
Belal Marziyeh	**PS2.00100**
Bellingham Michelle	**PS1.0021, PS2.00169, PS2.00192, YA3.002**

Belsham Denise D.	**BOP2M.006, PS1.00100, PS1.0062, PS1.0075, PS1.0076, PS1.0096, PS1.0097, PS1.0098, PS2.00114, PS2.004**
Beltramo Massimiliano	**PS1.00124**
Benani Alexandre	**PS1.0081**
Benavidez Michelle	**PS2.00124**
Benketira Sarah	**PS2.0063**
Benrick Anna	**PS1.00116**
Bergström Ulrika	**PS1.003**
Berland Chloé	**BOP2M.001**
Berland Marco A.	**CS4R.004**
Bernard Daniel J.	**BOP2R.006, PS1.00148, PS2.00107**
Bernardi Maria M.	**PS2.0040**
Berreby Else-Yona	**PS1.0081**
Berrhamoune Hind	**BOP2M.005**
Berrio Alejandro	**BOP1B.003**
Berruien Nasrin	**PS2.0090**
Bettler Bernhard	**PS2.00106**
Betz Stephen	**CS3TR.004**
Beymer Matthew	**BOP1R.002, PS2.00196**
Bian Jiang Hui	**PS2.00181**
Biello S M.	**PS2.00192**
Biga Peggy	**PS2.00101**
Bird Brian M.	**PS1.0024, PS2.003, PS2.0033**
Bishop Olivia	**PS2.0038**
Bishop Valerie	**PS1.00179**
Bitar Mahmoud	**PS2.0058**
Bizzozzero Hiriart Marianne	**PS1.00149, PS2.00106, PS2.00115**
Björkqvist Maria	**PS2.0084**
Blacher Silvia	**BOP2B.003**
Black Natasha	**PS2.0057**
Blackshaw Seth	**PS1.00109**
Blaustein Jeffrey D.	**AW7.001**
Blondeau Nicolas	**PS1.0074**
Bochukova Elena G.	**BOP2M.002**
Boehm Ulrich	**BOP2R.006**
Boerboom Derek	**PS1.00148**
Bohlooly-Y Mohammad	**PS1.0081**
Bolborea Matei	**PS1.00195, PS1.0060, PS2.00190**
Booth April	**PS1.00156**
Borland Johnathan M.	**PS1.0046**
Bosch Oliver J.	**PS1.00164**
Bossong Frank	**PS2.00178**
Boswell Timothy	**PS2.0075**
Bo Ting-Bei	**PS1.0095**
Bouchahda Mohamed	**PS2.00190**
Boulos Vanessa	**PS2.0063**

Bouret Sebastien	**BOP2M.002, PS2.0099**
Bourguignon Jean-Pierre	**BOP2B.003**
Bourguignon Nadia S.	**PS2.00106**
Bourne Rebecca	**PS1.00177**
Bourque Charles	**PS2.00160**
Boutin Jean A.	**CS3TR.001**
Boutin Stan	**BOP1S.001**
Bouwer Greg	**BOP2R.005, PS2.00118**
Bovee Sonny	**PS2.0076**
Boyd Hannah	**PS2.0064**
Bradburn Steven	**PS2.0042**
Brake Wayne G.	**PS1.0011, PS2.0061, PS2.0063**
Brandenburger Matthias	**CS2T.002**
Brandon Nicholas J.	**PS1.0017**
Brau Frédéric	**PS1.0069**
Breda Gabriele	**PS2.00190**
Bredewold Remco	**PS1.005**
Breedlove S. M.	**PS2.00183**
Breen Kellie M.	**PS2.00116, PS2.00117**
Breuer Joseph A.	**PS1.00119**
Brinie Matthew T.	**PS1.0071**
Broberger Christian	**BOP1B.001, PS2.00193**
Brocklehurst Sarah	**PS2.0075**
Brodin Birger	**PS1.00111**
Bronstein Robert	**PS2.0072**
Brown Colin H.	**PS1.00153, PS2.00121, PS2.00141, PS2.0074**
Brown Gillian	**PS2.0051**
Brown Rosie S.E.	**AW5.001, PS1.00131, PS1.00132**
Brubaker Patricia L.	**PS1.00100**
Brucker Sara	**PS2.0087**
Brugge Doug	**PS2.0045**
Brûlé Emilie	**PS2.00107**
Brummelte Susanne	**CS3B.001, PS2.0060**
Brunaud Laurent	**PS2.00168**
Brunton Paula J.	**PS1.00164, PS2.00154, PS2.00167**
Buckinx An	**PS1.0053**
Buckley Stephen	**PS1.00111**
Bugliani Marco	**PS1.00103**
Buijs Ruud M.	**PS2.00194**
Bunn Stephen	**PS1.00130**
Burdakov Denis	**BOP1B.004**
Burke Mary	**BOP2M.001**
Burkhard Tracy	**PS2.0044**
Burn David	**PS2.0014**
Butler Michael	**PS1.00108**
Cador Martine	**BOP2M.001**

Caglayan Ahmet Burak	**PS1.00115**
Caillé-Garnier Stéphanie	**BOP2M.001**
Cairns Mark	**PS2.00161**
Calisi Rebecca M.	**PS2.00143**
Camacho Francisco	**PS2.0016**
Campbell Rebecca E.	**CS2R.001, PS1.00143, PS1.00152, PS2.00118, PS2.00120, PS2.0018**
Campbell-Yeo Marsha	**CS3B.002**
Campideli-Santana Ana C.	**BOP2R.002, PS1.00117**
Cansell Céline	**PS1.0069, PS1.0074**
Cao Bei-Bei	**PS1.00188, PS1.0044**
Cao Ye	**PS1.0087**
Capuron Lucile	**BOP2B.001**
Cara Alexandra	**PS1.0059**
Carcea Ioana	**PS1.0039**
Cardoso Thais S.R.	**PS2.00127**
Cariboni Anna	**CS2R.002**
Caron Emilie	**PS1.0064**
Carrasco Rodrigo A.	**PS2.00139**
Carrat Gaelle	**PS1.00103, PS1.00106**
Carré Justin M.	**PS1.0024, PS2.003, PS2.0033**
Carroll Quinn E.	**PS1.0050, PS2.0047**
Carroll Rona S.	**PS2.00119**
Carson Paige	**PS1.00159**
Carstens Kelly E.	**BOP1S.002**
Carter Jasmine	**PS2.0089**
Carter Kirsten M.	**PS1.0020**
Carter Sara N.	**PS2.0015**
Carter Sylvia D.	**PS2.00151**
Caruso Michael	**PS1.00177**
Casals Nuria	**PS1.00140**
Casanueva Laura	**PS1.0047**
Case C P.	**PS2.00167**
Caso Federico	**PS2.00130**
Cassatella Daniele	**PS1.00121**
Cassie Nikki	**PS2.0082**
Castel Julien	**BOP2M.001, PS1.0080**
Castellano Juan Manuel	**PS1.00134, PS1.00140**
Castillo-Campos Andrea	**PS2.00153**
Castro Gisele	**PS1.0099**
Castro María José	**PS2.0017**
Cates Hannah M.	**BOP2S.006**
Cathers Phillip	**PS2.0065**
Caughey Sarah D.	**PS2.0075**
Caulfield Jasmine	**PS1.00169, PS1.00177**
Caulfield Jasmine I.	**PS1.00181**

Cavanaugh Jon	**PS2.0054**
Cavigelli Sonia	**PS1.00169, PS1.00177**
Cavigelli Sonia A.	**PS1.00181**
Cázarez-Márquez Fernando	**PS1.00122**
Ceballos Esteban	**PS2.0068**
Cederberg Helms Hans C.	**PS1.00111**
Cengiz Pelin	**CS3R.001**
Cezar Luana C.	**PS2.0040**
Chachlaki Konstantina	**PS1.0055**
Cha David	**PS1.00109**
Chahal Navdeep	**PS1.00149**
Chaiton Jessica A.	**PS1.00151**
Chalmers Jennifer	**PS1.00100, PS1.0062, PS2.004**
Chalmers Jennifer A.	**PS1.0097**
Champagne Frances A.	**CS1S.001**
Chanana Vishal	**CS3R.001**
Chao Moses V.	**CS2S.002**
Charli Jean L.	**PS1.0089, PS2.00153**
Chartrel Nicolas	**BOP2M.005, PS1.0078**
Cheah Jeffrey	**PS1.00179**
Chee Melissa	**PS2.0035**
Chen Chun	**CS2S.004**
Chen Gary	**PS2.00162**
Chen Junfeng	**PS2.00188**
Chen Lunhao	**PS1.001**
Chen Xue Qun	**PS2.00181**
Cheong Rachel	**PS2.0012, PS2.0019**
Cherifi Saloua	**BOP2M.005**
Chi Qingsheng	**PS1.0095**
Chmiel Jessica R.	**PS2.0036**
Choe Han Kyoung	**PS2.00195**
Choi Dennis C.	**PS1.00163**
Choleris Elena	**CS1B.001, PS1.0015, PS1.00190, PS1.0027, PS1.006, PS2.0022**
Choudhury Agharul I.	**BOP1B.004**
Chowen Julie	**CS2M.001**
Cho Yoon Hee	**PS1.00104, PS2.0092**
Christensen Debora	**PS2.0073**
Christensen Jennifer	**PS2.0055**
Christou-Savina Sofia	**PS1.00101**
Chun Eileen K.	**PS2.0053**
Cigremis Yilmaz	**PS1.00115**
Cimino Irene	**PS1.00114, PS1.00121**
Cinar Resat	**PS1.00112**
Ciofi Philippe	**PS1.0055**
Cisneros-Larios Brenda	**PS1.00126**

Cisneros Miguel	**PS2.00153**
Clarke Iain J.	**BOP2M.003, PS1.0061**
Clarke Irenie	**PS1.0067**
Clarke Sophie	**PS1.00103**
Clarkson Jenny	**PS1.00136, PS1.00138**
Clasadonte Jerome	**PS1.00111, PS1.0064**
Clemenzi Matthew N.	**PS1.00100**
Cocco Cristina	**PS1.0084**
Coimbra Cândido C.	**BOP2R.002, PS2.00127**
Colak Cemil	**PS1.00115**
Coll Anthony P.	**PS1.00114, PS1.0057, PS1.0058**
Collden Gustav	**PS1.00102**
Colledge William H.	**BOP2R.001, PS2.00147**
Collins Andrila E.	**PS1.0015**
Collins Troy	**PS2.00135**
Comninos Alexander N.	**PS1.00103**
Conde Kristie	**PS2.00105**
Connolly George A.	**PS1.00139**
Connors John M.	**PS1.00125, PS1.00127, PS1.00142**
Constantinof Andrea	**PS2.00177**
Conway-Campbell Becky L.	**CS2S.001, PS1.0071**
Coolen Lique M.	**BOP1R.001, PS1.00135, PS1.00142**
Cork Simon C.	**PS1.0087**
Cornil Charlotte A.	**CS3R.002**
Corona Rebeca	**CS3R.002**
Corsini Silvia	**PS1.0080**
Cortés Carmen	**PS2.0024, PS2.0030, PS2.0031, PS2.0032**
Court Lucas	**CS3R.002**
Coutinho Eulalia	**PS1.00143, PS2.00120**
Coyle Chris	**PS2.00130**
Craig Tim	**PS1.00177**
Creeney Hannah	**PS1.0017**
Creighton Samantha	**PS1.00113**
Croizier Sophie	**PS2.0099**
Crozier Sophie	**BOP2M.002**
Cruz Gonzalo	**PS2.0020**
Cservenak Melinda	**PS2.00113, PS2.00126**
Cui Zhenzhong	**PS1.0063**
Cunningham J. Thomas	**BOP2S.001, PS2.00172, PS2.0021**
Cunningham Rebecca L.	**PS2.0029**
Curtis A M.	**PS2.00192**
Da Fonseca Caio Cesar N.	**PS2.0040**
Da Fonte Dillon F.	**PS2.00144**
Daimon Makoto	**PS2.00155, PS2.00156**
Dale Nicholas	**PS1.0060**
Dallmann Robert	**PS1.00195**

Dalvi Prasad S.	**PS1.0082, PS1.0097**
D'Angelo Heather M.	**PS2.0056**
Daniel Jill M.	**PS2.0039, PS2.0043**
D'Anna Kimberly	**PS2.0069**
Dantzer Ben	**BOP1S.001**
D'Aquila Andrea	**PS2.00101**
Dardente Hugues	**CS4T.001**
Darling Jeffrey S.	**PS2.0039**
Darnaudéry Muriel	**BOP2B.001**
Dartez Lauren	**PS2.0039**
Darwish Lina	**PS2.0089**
Dash Satya	**PS1.0096**
Da Silva Mayara B.	**PS2.0022**
David Anna-Julia	**PS2.0026**
David Caroline	**PS1.0045**
Davies Alison	**PS1.0057, PS1.0058**
Davies Jeff	**PS2.0014**
De Araujo Ivan E.	**CS3M.001**
D'Eath Rick B.	**PS2.0075**
De Backer Ivan	**PS1.00106**
De Bundel Dimitri	**PS1.0016, PS1.0053**
De Burgh Ross A.	**BOP2R.001, PS1.00133**
Decapo Madison	**PS2.0023**
Decarie-Spain Lea	**PS1.007**
De Castro Barbosa Thais	**PS1.00116**
Decatanzaro Denys	**PS2.00132**
Decourt Caroline	**PS1.00124, PS1.00139, PS2.00122**
De Fante Thaís	**PS1.0062**
Defazio Richard A.	**PS1.00118**
De Guzman Rose M.	**PS2.0068**
Delgado Nicol	**PS2.0020**
De Lima Ana Paula N.	**PS1.00183, PS2.0040**
Delli Virginia	**BOP2B.003**
De Lorme Kayla C.	**PS2.00182**
Demas Gregory E.	**PS1.0025**
De Miera Cristina	**BOP1R.002**
Deng Mengdie	**PS2.00157**
Denis Raphael	**PS1.0080**
De Rosa Maria Caterina	**PS1.00102**
De Roux Nicolas	**CS2R.004, PS1.00147**
Desban Laura	**PS1.0032, PS1.0035**
Desroziers Elodie	**PS1.00143, PS2.00120, PS2.0018**
Devaux Nadège	**PS1.0069**
De Vries Geert J.	**PS2.0046, PS2.0067**
Deyoe Jessica E.	**PS1.0025**
Dhillo Waljit S.	**CS3TR.002, PS1.00103, PS1.00106**

Diaz Bryan S.	**PS1.00163**
Diaz Nestor	**PS2.0016**
Dickson Suzanne L.	**PS1.003**
Dieguez Gonzalez Carlos	**PS1.00134**
Di Giorgio Noelia P.	**PS1.00149, PS2.00106, PS2.00115**
Dijkstra Dorieke	**PS2.0010**
Ding Xiaojing	**PS1.0043**
Djenoune Lydia	**PS1.0032, PS1.0035**
Dobolyi Arpad	**PS1.0013, PS2.00113, PS2.00126**
Doege Claudia A.	**PS1.00102**
Dokovna Lisa B.	**BOP2B.002**
Dong Fanglong	**PS2.00178**
Dong Lynn	**PS1.008**
Donovan Alexandra M.	**PS1.009**
Donovan Meghan L.	**PS1.0031, PS2.0053**
Dorantes-Nieto Ángeles	**PS2.0031**
Dorantes-Nieto María D.L.Á.	**PS2.0032**
Dorfman Mauricio D.	**BOP1M.001**
Douglass John D.	**BOP1M.001**
Drummond Lucas R.	**PS2.00127**
Duan Shumin	**PS1.001**
Duarte Ana I	**PS2.0084**
Duarte-Guterman Paula	**PS1.0047, PS2.00109, PS2.0057**
Duchesne Annie	**PS2.0025**
Duclot Florian	**PS1.0049**
Dudek Serena M.	**BOP1S.002**
Du Ji Zeng	**PS2.00181**
Dulac Catherine	**PL04.001**
Dulka Eden A.	**PS1.00129**
Duncan Jacqueline	**PS2.0010**
Duncan Katherine	**PS1.0033**
Duncan Peter	**PS2.00161**
Dunn Ian C.	**PS2.0075, PS2.0079**
Duong Phong	**PS2.0029**
Duquenne Manon	**PS1.0064, PS1.0079**
Duque-Wilckens Natalia	**PS1.00186**
Durant John	**PS2.0045**
Durate Rodrigo R.	**PS1.0017**
Duriez Philibert	**PS1.0078, PS1.0081**
Eagle Allison K.	**PS2.00180**
Eberini Ivano	**CS2R.002**
Ebling Francis J.	**CS4T.002, PS1.0084**
Eckel Lisa	**PS1.00108**
Eddy J. M.	**PS1.00180**
Edwards Alexander W.	**PS2.0035**
Egeciouglu Emil	**PS1.0081**

Eguibar José R.	**PS2.0024, PS2.0030, PS2.0031, PS2.0032**
Ehlert Ulrike	**PS2.00162**
Einstein Gillian	**BOP2B.006, PS1.0033, PS2.0025**
Elad Vissy M.	**PS1.006**
El Allali Khalid	**PS2.00125**
El Bousmaki Najlae	**PS2.00125**
Elgbeili Guillaume	**PS1.00191**
El Houda Mimouni Nour	**PS1.00121**
Elias Carol F.	**CS1T.001, PS1.00126, PS1.0059**
Ellacott Kate L.J.	**CS2M.002, PS1.00196**
El Mamoune Kahina	**PS1.00155**
El Mehdi Mouna	**BOP2M.005**
Eltahir Akif M.	**PS1.00171**
Emmerson Paul	**CS3TR.003**
Engledow Simon	**CS1S.003**
Eng Pei C.	**PS1.00103**
Enos Riley T.	**PS1.0019**
Epelbaum Jacques	**PS1.0081**
Ergang Peter	**PS2.00170, PS2.00171**
Ernszt Dávid	**PS1.00157**
Ervin Kelsy	**PS1.0027**
Escobar Carolina	**CS2T.001**
Esparza Lourdes A.	**PS2.00145**
Estay Camila	**PS2.0020**
Evans Maggie C.	**PS1.0072**
Evans Neil P.	**PS1.0021, PS2.00169, PS2.00192, YA3.002**
Fadahunsi Nicole	**PS1.0087**
Fageyinbo Samuel O.	**PS2.0097**
Fair Damien	**PS2.0023**
Falconi Atilio	**PS2.0017**
Fang Lisa	**PS1.0085**
Farmer George	**BOP2S.001, PS2.00172, PS2.0021**
Farooqi Sadaf	**BOP2M.002**
Fasnacht Rachael	**BOP1M.001**
Faure Paul A.	**PS2.00132**
Faure Philippe	**BOP2M.001**
Faykoo-Martinez Mariela	**PS2.00135**
Fazekas Emese	**PS2.00126**
Fehlert Ellen	**PS2.0087**
Felicio Luciano F.	**PS2.0040**
Feng Xixi	**BOP2S.002**
Ferland Guylaine	**PS2.0098**
Fernald Russell	**PS2.0065**
Fernandes Paola	**PS2.00127**
Fernando Anushka B..P.	**BOP1B.004**
Fernandois Daniela	**PS1.00140**

Ferrazzano Peter	**CS3R.001**
Ferreira Guillaume	**PS1.00197**
Ferreño Marcela	**PS2.0013**
Ferri Gian-Luca	**PS1.0084**
Fiacco Serena	**PS2.00162**
Finkenstädt Bärbel	**PS2.00190**
Fisette Alexandre	**PS1.00102, PS1.007**
Fishman Ruth	**PS2.00110**
Flamant Frédéric	**BOP1R.002**
Floresco Stan B.	**PS1.0019**
Flowers Matthew T.	**PS1.0038**
Flynn Benjamin P.	**PS1.0071**
Focke Caroline	**PS1.00174**
Folmerz Elin	**PS1.00116**
Fonken Laura K.	**PS2.0056**
Fontaine Romain	**PS2.00128**
Foppen Ewout	**PS1.0080**
Forlano Paul M.	**BOP1B.002**
Fornes Romina	**PS1.00116**
Fortin Jerome	**PS1.00148**
Foster William	**PS1.00159**
Fraigne Jimmy	**PS2.004**
Francescone Abigal R.	**PS1.0050, PS2.0047**
Frank Matthew G.	**PS2.0056**
Franssen Catherine L.	**PS2.00179, PS2.00180**
Franssen Delphine	**PS1.00134**
Frantz Kyle	**PS1.0046**
Freeman David A.	**PS2.0015**
French Jeffrey A.	**PS2.0054**
Frias Antonio	**PS2.0023**
Frick Karyn	**CS1B.002**
Friedman Jeffrey	**PL07.001**
Friesen Caitlin N.	**PS2.0028**
Fritsche Andreas	**PS1.0068, PS2.0087**
Froemke Robert C.	**PS1.0039**
Frost Gary	**PS1.0070**
Fudickar Adam	**PS1.00161**
Fudulu Daniel	**PS2.00186**
Fuller-Jackson John-Paul	**BOP2M.003**
Fulton Stephanie	**CS3M.002, PS1.007**
Fu Travis	**CS1T.003**
Gagne Collin	**PS2.0061, PS2.0063**
Gajewska Alina	**PS2.00148**
Gajewski Lukasz	**PS2.00175**
Galea Liisa A.M.	**CS1B.003, PS1.00151, PS1.0047, PS2.00109, PS2.0057**
Gallet Sarah	**PS1.00111**

Gangarossa Giuseppe	**BOP2M.001, PS1.0080**
Gao Di	**PS1.00110**
Gao Yan	**PS1.00110**
Gao Zhihua	**PS1.001**
Garabedian Michael	**CS2S.002**
Garbutt Lauren	**PS1.00195**
Garcia Caceres Cristina	**BOP2M.004, PS1.0088**
Garcia-Caceres Cristina	**PS1.00102, PS1.0080**
García-Cáceres Cristina	**BOP1M.002**
García Carlos	**PS2.0017**
Garcia Galiano David	**PS1.00126, PS1.00134**
Garcia James	**PS1.00162**
Gardiner James	**PS1.00106**
Gardini Elena	**PS2.00162**
Garthwaite John	**PS1.0055**
Gasman Stephane	**PS1.0079, PS2.00168**
Gaßner Barbara	**PS1.00164**
Gaston-Massuet Carles	**CS2R.002**
Gaudreau Pierrette	**PS2.0098**
Gay Joséphine	**PS1.00197**
Gaytan Luna Francisco	**BOP1R.003, PS1.00134, PS1.00140**
Gazetas James	**PS1.00191**
Gegenhuber Bruno	**PS2.0072**
Gelbenegger Therese	**PS1.00197**
Geniole Shawn N.	**PS1.0024, PS2.003, PS2.0033**
Georgescu Teodora	**PS2.0085**
Gérard Arlette	**BOP2B.003**
Gergely Cassandra K.	**PS1.004**
Gerutshang Achi	**PS2.00129**
Gervais Nicole	**BOP2B.006, PS1.0011, PS2.0025**
Giacobini Paolo	**PS1.00121, PS1.0055, PS1.0059, YA1.001**
Giglio Erin M.	**PS2.0044**
Giles Aaron G.	**PS1.00182**
Gill Andrew C.	**PS2.00154**
Gilon Chaim	**BOP2R.003**
Gjerstad Julia K.	**BOP2S.002**
Glegola Justyna A.	**BOP1B.004**
Gobinath Aarthi R.	**PS2.00109**
Godlewski Grzegorz	**PS1.00112**
Godó Soma	**PS1.00157**
Godsland Ian	**PS1.00103**
Go Kim	**PS1.0047, PS2.00109, PS2.0057**
Goldman David	**PS2.00134**
Gomez Romero Maria	**PS1.00103**
Gompel Anne	**PS1.00147**
Gonzalez-Abuin Noemi	**PS1.0070**

Goodman Robert	**PS1.00142**
Goodman Robert L.	**PS1.00125, PS1.00127, PS1.00135**
Gore Andrea C.	**PL10.001, PS1.0054**
Gorman Michael R.	**PS1.00119**
Goss Hannah M.	**CS1S.003**
Goss Matthew	**PS2.0011**
Gotlieb Neta	**PS2.00137**
Goularte Jeferson F.	**PS1.00123**
Goularte Jéferson F.	**PS2.0080**
Gouws Julia	**PS1.00166**
Grady Cheryl	**PS2.0025**
Grantham Kymberly	**PS1.0046**
Grattan David	**PS1.00117, PS1.00130, PS1.00131, PS1.00132, PS1.0020, PS1.0077, PS2.0088**
Gravelsins Laura	**PS1.0033, PS2.0025**
Greives Timothy	**PS1.00161**
Greville Lucas J.	**PS2.00132**
Gribble Fiona M.	**CS4M.001, PS1.0092**
Grieb Zachary	**PS2.00136**
Grinevich Valery	**PS1.00194**
Grisel Judy E.	**PS1.0036**
Gruber Tim	**BOP1M.002, BOP2M.004, PS1.0088**
Guerra Monserrat	**CS4R.004**
Gustafson Papillon	**PS1.00131**
Gutiérrez-Mariscal Mariana	**PS2.00153**
Haas Nicole A.	**PS1.0028**
Hacker Jennifer	**PS2.0038**
Haeberlé Anne-Marie	**PS1.0079**
Hager Gordon L.	**PS1.0071**
Hagey Travis	**PS2.0027**
Hahn Margaret	**PS1.0098**
Haleem Darakhshan J.	**PS2.0059**
Hamada Hirotaka	**PS2.00177**
Hamden Jordan E.	**PS1.0019**
Hampson Elizabeth	**BOP2B.006**
Hanalioglu Damla	**CS3R.001**
Handa Robert J.	**PS1.00173**
Hanics Janos	**PS2.00113**
Hanna Lydia	**PS1.00196**
Han Su Y.	**PS1.00136, PS1.00137, PS1.00138, PS2.0074**
Han Wenfei	**CS3M.001**
Han Ye Eon	**PS1.00104, PS2.0092**
Haq Naila A.	**PS1.00101**
Haraldsen Ira H.	**PS1.0021, PS2.00169**
Hardy Darran G.	**BOP1B.004**
Hardy Steven L.	**PS1.00127, PS1.00142**

Häring Hans-Ulrich	**PS1.0068, PS2.0087**
Harno Erika	**PS1.0057, PS1.0058**
Harter Campbell J.L.	**PS2.0011**
Hart Kirsten	**PS2.00135**
Harvey Alan R.	**PS2.0011**
Hashimoto Hirofumi	**PS2.0096**
Hausch Felix	**BOP2S.002**
Hazlerigg David	**PS2.00196**
Healy Sue D.	**PS2.00166**
Hecksher-Sørensen Jacob	**PS1.00111**
Heisler Lora K.	**PS1.0056, PS2.002, PS2.0085**
Heiss Christina	**CS4M.002**
Helbling Jean-Christophe	**PS1.00197**
Helfer Gisela	**PS1.0082**
Heni Martin	**PS1.0068**
Henningsen Jo B.	**PS2.0019**
Henriques Patricia C.	**PS1.00117**
Henry Belinda A.	**BOP2M.003, PS1.0061**
Heras Violeta	**BOP1R.003, PS1.00140**
Herbison Allan E.	**PL03.001, PS1.00136, PS1.00137, PS1.00138, PS1.00152**
Hernandez Morgan E.	**PS1.0054**
Heron Megan	**PS2.0063**
Herrera Moro Chao Daniela	**BOP1M.002, PS1.0080**
Herzog Erik	**PS2.00191**
Herzog Herbert	**CS3M.003, PS1.0067**
Hessler Sabine	**PS1.00143, PS1.00152**
He Wen	**PS1.00145**
Hicks Amirah-Iman	**PS1.0091**
Hicks Nelson Alexandria	**PS2.0042**
Hileman Stanley M.	**PS1.00125, PS1.00127, PS1.00142**
Hilliard Austin	**PS2.0065**
Hill Jennifer W.	**PS1.00150**
Hipolito Laisa T.M.	**BOP2R.002**
Hirasawa Michiru	**PS1.0085**
Hirata Keiji	**PS2.0096**
Hiura Lisa	**PS2.007**
Hnasko Thomas S.	**BOP2M.001**
Hoang Kim N.	**PS2.00182**
Ho Bryan	**PS2.00145**
Hodges Travis E.	**BOP2S.003, PS1.00171**
Hodgson Amy	**PS2.0051**
Hodne Kjetil	**PS2.00128**
Hoffman Jessica F.	**PS2.00134**
Hoffmann Hanne M.	**PL05.001, PS1.00119**
Hofmann Hans	**PS1.0012, PS2.0027, PS2.0028**
Hogg David W.	**PS2.00103**

Holihan Stephen	**PS2.0038**
Holland Sarah	**PS2.00120**
Holmes Elaine	**PS1.00103**
Holmes Megan C.	**CS4S.002**
Holmes Melissa	**PS2.00135, PS2.0071**
Holton Christopher	**PS1.00106**
Homma Koichi J.	**PS2.005**
Hornsby Amanda K.E.	**PS2.0014**
Horta Nayara A.C.	**PS2.00127**
Horvath Tamas L.	**BOP2M.004, PS1.0088**
Hoshi Aya	**PS1.00112**
Hosokawa Keisuke	**PS2.0086**
Hough Denise	**PS1.0021, PS2.00169, PS2.00192**
Houy Sébastien	**PS2.00168**
Howard Sarah	**PS1.0027**
Hrabovszky Erik	**PS1.003**
Hrabowszky Erik	**PS1.0055**
Hryhorczuk Cecile	**PS1.007**
Huang Qi	**PS2.00190**
Huang Yan	**PS1.00188**
Hübner Katharina	**PS1.00164**
Huffels Christiaan	**PS1.00194**
Hughes Jessica K.	**PS2.00140**
Hughes Stephen J.	**PS1.00103**
Hugon-Rodin Justine	**PS1.00147**
Huhman Kim L.	**PS1.00163**
Hume Catherine	**PS1.00194, PS1.003, PS2.0081**
Hu Ming	**PS1.00103**
Humphries Murray	**BOP1S.001**
Hunter Richard G.	**CS1S.002**
Hussain Sufyan	**PS1.00106**
Hussein Khalid	**CS2R.002**
Hyland Brian	**PS1.00130**
Hyland Lindsay	**PS2.0035**
Ibarra Juan M.	**PS2.0030**
Ignácio-Souza Letícia	**PS1.0062**
Imbernon Monica	**PS1.00111**
Imoesi Peter I.	**PS1.0056**
Inglis Megan A.	**PS1.00139, PS2.00122**
Innominato Pasquale F.	**PS2.00190**
Inquimbert Perrine	**PS2.00123**
Insel Nathan	**PS2.0026**
Inutsuka Ayumu	**CS3S.002, PS1.00165**
Iremonger Karl	**PS1.00166, PS1.00168, PS1.00174**
Irvine Elaine E.	**BOP1B.004**
Isaacs Lauren	**PS1.00113**

Isiordia Laura E.	**PS1.00180**
Ismail Nafissa	**PS1.00183**
Ito Etsuro	**PS2.00156**
Iwuamadi Elizabeth C.	**PS2.0097**
Izzi-Engbeaya Chioma	**PS1.00103, PS1.00106**
Jackson Meliame	**PS2.00121**
Jacob-Brassard Elizabeth	**PS1.007**
Jacob Dennis P.	**BOP2S.005**
Jacobskind Jason S.	**PS1.00185**
Jacob-Tomas Suleima	**PS1.0090**
Jalabert Cecilia	**PS1.0025**
Jama Kalson	**PS1.0021**
Jamieson Bradley	**BOP2R.005, PS2.00118**
Jastroch Martin	**BOP2M.004**
Jastrzebski Pawel	**PS1.0011**
Jeanneteau Freddy	**CS2S.002**
Jeffress Elizabeth C.	**PS1.00163**
Jensen Casper B.	**BOP2M.001**
Jethwa Preeti H.	**PS1.0084**
Jiang Lizhi	**PS1.00109**
Jiang Zhiying	**CS2S.004**
Jia Shuwei	**BOP1S.003**
Jimenez Roberto	**PS1.00180**
Ji Sihan	**PS1.00110**
Johnson Caroline	**PS2.00146**
Johnson Paul R.	**PS1.00103**
Johnston Fionnuala	**PS2.0014**
Jomard Anne	**PS1.00103**
Jones Jeff	**PS2.00191**
Jones Kathryn M.	**PS1.0031**
Jones Sherri Lee	**PS1.00191**
Jones Sophie	**PS1.00103**
Jordan Cynthia L.	**PS2.00183**
Joseph-Bravo Patricia	**PS1.0089, PS2.00153**
Jourdan Tony	**PS1.00112**
Juntti Scott	**PS1.0026, PS2.0065**
Justice Nicholas J.	**PS1.00185**
Kabbaj Mohamed	**PS1.0049**
Kachkovski Gueorgui V.	**PS1.0019**
Kagan Karl O.	**PS2.0087**
Kageyama Kazunori	**PS2.00155, PS2.00156**
Kaiser Ursula B.	**CS2R.003, PS2.00119**
Kakadellis Sarah	**BOP1B.001**
Kalinowski Leanna M.	**PS2.0047**
Kalsbeek Andries	**PS1.00122, PS2.00123**
Kanagasundaram Pruntha	**PS1.0098**

Kane Grace M.	**PS1.00136, PS1.00137**
Kang Chan Woo	**PS1.00104, PS2.0092**
Kang M. C.	**PS1.0099**
Kanpurwala Muhammad A.	**PS2.00174**
Kantamneni Sriharsha	**PS1.0082**
Kaplan Ariel	**PS2.00112**
Karnani Mahesh M.	**BOP1B.004**
Katsouri Loukia	**BOP1B.004**
Katsumata Harumi	**PS1.0042**
Katz Larry S.	**PS2.0036**
Kauffman Alexander S.	**CS1R.001, PS1.00149, PS2.00140, PS2.00145**
Kaur Gagandeep	**PS2.00178**
Kawamura Noriyuki	**PS1.00193**
Kearns Patrick	**PS2.00161**
Keay Kevin A.	**PS1.00182**
Keen Kim	**PS1.00162**
Keller David	**PS2.00126**
Kelley Lyla	**PS1.0094**
Kelly Aubrey M.	**BOP2B.005**
Kema Ido	**PS2.00186**
Kennedy Clare L.M.	**CS1S.003, PS2.00151, PS2.00152**
Kentner Amanda	**CS3B.003**
Keogh Julia	**BOP2M.002**
Kershaw Yvonne M.	**PS1.0071**
Ketterson Ellen	**PS1.00161**
Khant Aung Zin A.	**PS1.0077**
Khbouz Badr	**CS3R.002**
Kiefer-Schmidt Isabelle	**PS2.0087**
Kim Boil	**PS2.00195**
Kim Jean	**PS2.0092**
Kim Jihoon	**PS2.00195**
Kim Joon	**PS1.00168**
Kim Kyungjin	**PS2.00195**
Kim Sohyoung	**PS1.0071**
Kim Young-Bum	**PS1.0099**
King Suzanne	**PS1.00191**
Kinoshita Noriko	**PS2.00156**
Kirsten Thiago B.	**PS2.0040**
Klampfl Stefanie	**PS1.00164**
Klein Laura	**PS1.00177**
Klosen Paul	**PS1.00122**
Kober Caitlin	**PS2.0036**
Koekkoek Laura	**PS1.0080**
Kolbe Isa	**CS2T.002**
Komarzynski Sandra	**PS2.00190**
Kondo Daisuke	**PS1.0066**

Kondo Yasuhiko	**PS1.0010, PS1.0042, PS2.0037, PS2.0066**
Konji Sandra M.	**PS2.0041**
Konkle Anne T M.	**PS2.0041**
Koren Lee	**PS2.00110**
Korosi Aniko	**CS4S.003, PS1.00172**
Korpal Aaron K.	**PS2.0074**
Kostaki Alisa	**PS2.00177**
Kosten Therese A.	**PS2.00158**
Kowalchuk Chantel	**PS1.0098**
Krajewski-Hall Sally J.	**PS1.00154**
Krause Jesse S.	**PS1.00179**
Kraynak Marissa	**PS1.0038**
Kreisman Michael J.	**PS2.00116, PS2.00117**
Kriegsfeld Lance J.	**PS2.00137**
Krishnan Keerthi	**PS2.008**
Kroenke Chris	**PS2.0023**
Ku Cheol Ryong	**PS1.00104**
Kuehlmann Alex L.	**PS1.0038**
Kullmann Stephanie	**PS1.0068**
Kumagai Ryoko	**PS2.0037**
Kumar Shalini S.	**PS2.00141**
Kunos George	**PS1.00112**
Kurrasch Deborah M.	**BOP1M.004, PS1.0014**
Kusuhara Koichi	**PS1.0023**
Kyne Robert F.	**PS1.0050**
Labarthe Alexandra	**PS1.0081**
Labelle Morgan	**PS2.0041**
Lacasse Jesse M.	**PS2.0061, PS2.0063**
Lacreuse Agnes	**PS1.0041**
Ladyman Sharon R.	**PS1.00131, PS1.0020, PS1.0077**
La Fleur Susanne E.	**CS3M.004, PS1.00172**
Lalhou Najiba	**PS1.00147**
Lam Brian	**PS1.00114, PS2.0091**
Landaverde Amanda V.	**PS2.00182**
Landry Hannah K.	**PS2.0041**
Lane Jeffrey E.	**BOP1S.001**
Lange Gary M.	**PS2.0038**
Lanoix Joel	**PS2.00168**
Laplante David P.	**PS1.00191**
Lapointe Evelyne	**PS1.00148**
Laran-Chich Marie-Pierre	**PS1.00122**
Lardner Casey K.	**BOP2S.006**
Larkin Theresa A.	**PS2.00185**
Lass Geffen	**BOP2R.001, PS1.00133**
Lau Billy	**PS2.008**
Lauby Samantha	**PS1.00176**

Lawler Katherine	**BOP2M.002**
Layé Sophie	**BOP2B.001**
Layo Dana	**PS2.008**
Leach Zoe K.	**PS2.0022**
Lebeaupin Cynthia	**PS1.0069**
Ledbetter Rob	**PS2.00184**
Lee Eun Jig	**PS1.00104, PS2.0092**
Lee Jessica S.	**PS1.00119**
Lee Ji-Hyeon	**PS1.0063**
Lee Mi Kyung	**PS1.00104**
Lee Nicole S.	**PS2.0050**
Lee Se-Jin	**BOP2R.006**
Lee Soomin	**PS2.00195**
Lee Ying	**CS4M.002**
Legutko Beata	**BOP1M.002, BOP2M.004, PS1.00102, PS1.0088**
Lehman Michael N.	**BOP1R.001, PS1.00135, PS1.00142**
Leichner Emily	**PS2.0054**
Leko Andras H.	**PS2.00113**
Le May Marie V.	**PS1.003**
Lemus Moyra B.	**PS2.0080**
Lenfant Françoise	**CS3R.002**
Leng Gareth	**PS1.00194, PS1.003, PS2.0077, PS2.0078, PS2.0081**
León Silvia	**PS2.00129**
Leprince Jérome	**BOP2M.005**
Lerch Jason	**PS1.00105**
Lestage Alex	**PS2.0061**
Le Thuc Ophelia	**BOP1M.002, BOP2M.004, PS1.0078, PS1.0080, PS1.0088**
Le Thuc Ophélia	**PS1.0069, PS1.0074**
Le Tissier Paul	**PS2.0090**
Lettieri Antonella	**CS2R.002**
Levavi Sivan Berta	**BOP2R.003**
Lever Louise C.	**PS2.00180**
Levi David	**PS2.00160**
Lévi Francis A.	**PS2.00190**
Levine Jon E.	**CS3R.001, PS1.0038**
Levkowitz Gil	**CS1T.002**
Lewis` Jo E.	**CS4T.002, PS1.0084**
Lewis Matthew R.	**PS1.00103**
Leysen Valerie	**PS1.0055**
Liaw Reanna B.	**PS1.00149**
Libertun Carlos	**PS1.00149, PS2.00106, PS2.00115**
Lickliter Jason	**CS3TR.004**
Li De-Pei	**PS2.00158**
Lidhar Navdeep	**PS2.0026**
Li Dongdong	**BOP1M.002, PS1.0080**
Li Dongfeng	**PS2.001**

Lieblich Stephanie E.	**PS1.00151, PS1.0047**
Lié Oriane	**PS2.0098**
Li Geng-Lin	**PS2.0049**
Lightman Stafford L.	**BOP1S.004, BOP2R.001, BOP2S.002, PS1.00160, PS1.00167, PS1.0071, PS2.00186**
Li Grace	**PS2.0062**
Li Grace R.	**BOP2B.002**
Limebeer Cheryl L.	**CS1B.001**
Li Ming	**PS1.0043**
Linden Hirschberg Angelica	**PS1.00116**
Lindo Ashley N.	**PS1.00125**
Lin Liping	**PS1.0050**
Linning-Duffy Katrina	**PS1.0048**
Lin Xian-Hua	**BOP2R.001, PS1.00133**
Liposits Zsolt	**PS1.003**
Lisci Carlo	**PS1.0084**
Li S Y.	**PS1.00160**
Li Tong	**BOP1S.003**
Little Joel T.	**BOP2S.001**
Liu Robert C.	**BOP2B.004**
Liu Yan	**PS1.00189, PS1.0031, PS2.0053**
Liu Yudan	**PS1.00110**
Liu Zhan	**PS1.00188**
Li Wing	**PS1.00146**
Li Xiao F.	**BOP2R.001, PS1.00133**
Li Xiaofeng	**PS1.00160**
Li Xiaonan	**PS1.0043**
Li Yanyu	**PS1.00167**
Locke Marius	**PS2.00101**
Lockstone Helen	**CS1S.003**
Loehfelm Aline	**PS1.0073**
Loganathan Neruja	**BOP2M.006, PS1.00100, PS1.0062, PS1.0075, PS2.00114**
Lomet Didier	**PS1.00124**
Lomniczi Alejandro	**CS1R.002, PS2.00104, PS2.00131**
Long Hong	**PS1.0094**
Long Michael	**PS1.0040**
Lonstein Joseph	**PS1.0048, PS2.00136, PS2.00138**
Lopes-Aguiar Cleiton	**PS1.00117**
Lopez Justin A.	**PS1.00125, PS1.00127, PS1.00142**
Lopez Perez Miguel	**PS1.00134, PS1.00140**
Lopez Rodriguez David	**BOP2B.003**
Lopez Sarah A.	**PS2.0050**
Louth Emma L.	**BOP2S.003**
Lovejoy David	**PS2.00101, PS2.00103**
Low Peter	**PS2.00126**
Lowry Christopher A.	**PS2.0056**

Lucassen Paul J.	**PS1.00172**
Luckman Simon M.	**BOP1M.003**
Ludwig Mike	**PS1.00194**
Lu Jian-Hua	**PS1.0044**
Luquet Serge	**BOP1M.002, BOP2M.001, PS1.0080**
Lu Ray	**PS1.00190**
Lustberg Daniel J.	**BOP1S.002**
Lutfy Kabir	**PS2.00178**
Lu Van B.	**PS1.0092**
Lux-Lantos Victoria	**PS1.00149, PS2.00106, PS2.00115**
Lynch Susan	**PS2.00179**
Lyons David J.	**PS2.002, PS2.0085**
Ma Bon De Sousa Ava	**PS1.0033**
Macari Soraia	**BOP2R.002**
Ma Cathy	**PS1.0019**
Macedo-Lima Matheus	**PS2.0064**
Macgregor Duncan	**PS2.0077, PS2.0078**
Machlab Karla	**PS1.0033**
Ma Chunqi	**PS1.0025**
Maclusky Neil	**PS1.00113, PS1.00190**
Madan Ajay	**CS3TR.004**
Maejima Sho	**PS2.0052, PS2.006, PS2.0066**
Maguire Caroline	**PS2.00129**
Mahmoud Rand	**PS1.00151, PS2.00109**
Maicas Royo Jorge	**PS2.0077, PS2.0078**
Maier Steven F.	**PS2.0056**
Main Cecil Dana	**PS1.0027**
Maliqueo Manuel	**PS1.00116**
Malone Samuel A.	**PS1.00121, PS1.0055**
Ma Marcella	**PS1.00114**
Manchishi Stephen M.	**PS2.00147**
Manfredsson Fredric	**PS2.00136**
Mannerås Holm Louise	**CS4M.002**
Manti Maria	**PS1.00116**
Marcellus Ashley L.	**PS1.0024**
Marchetti Piero	**PS1.00103**
Marciante Alexandria	**PS2.0021**
Marciniak Elzbieta	**PS2.00175**
Marcoux François-Pierre	**PS1.00191**
Markison Stacy	**CS3TR.004**
Marley Nicole	**PS1.0024**
Marnas Hugo	**PS1.0032, PS1.0035**
Martchenko Alexandre	**PS1.00100**
Marti Fabio	**BOP2M.001**
Martin Claire	**BOP1M.002, PS1.0080**
Martinez, Jr. Charles R.	**PS1.00180**

Martinez-Pinto Jonathan	**PS2.0020**
Martinoli Maria-Grazia	**PS2.0083**
Maruyama Takashi	**PS1.002, PS1.0023, PS2.00173, PS2.0094, PS2.0096**
Massaro Christina	**PS1.0011**
Mat Husin Haliza	**PS2.0087**
Matsuda Ken Ichi	**PS2.006**
Matta Richard	**CS1B.001, PS2.0022**
Matthews Stephen G.	**PS2.00177**
Maucotel Julie	**BOP2M.005**
Maurice Monique	**PS2.00190**
Maurnyi Csilla	**PS1.0055**
Mayer Heather S.	**PS2.0070**
Ma Zhi Qiang	**PS2.00181**
Mcadam Andrew G.	**BOP1S.001**
Mccaffery Peter J.	**PS1.0056**
Mccaffery Peter J.A.	**PS2.00189**
Mccann Katharine E.	**BOP1S.002, PS1.00163, PS1.0029**
Mcclafferty Heather	**PS2.00161**
Mcclure Heather H.	**PS1.00180**
Mccormick Cheryl M.	**BOP2S.003, PS1.00171, PS1.00183**
Mccosh Richard B.	**PS1.00125, PS1.00127, PS1.00142, PS2.00117**
Mccourt Andrew C	**PS2.0084**
Mcgowan Patrick	**PS1.00176**
Mcgowan Patrick O.	**PS1.00178**
Mcilwraith Emma K.	**BOP2M.006, PS1.0075, PS2.00114**
Mclaughlin Mark	**PS1.0021, PS2.00169, PS2.00192**
Mclouth Laryssa	**PS2.00182**
Mcmullen Nathaniel T.	**PS1.00154**
Mcnamee Clara	**PS1.0033**
Mcquillan Henry J.	**PS1.00136**
Meadows Jason D.	**PS1.00119**
Meaney Michael	**PL01.001**
Meddle Simone L.	**PS1.00179**
Medina Joanna	**PS2.0068**
Mehrotra Arjun	**PS2.0039**
Meijer Onno C.	**CS2S.003**
Melamed Philippa	**PS2.00112**
Mellon Pamela L.	**PL05.001, PS1.00119**
Ménard Caroline	**PS2.0098**
Mendell Ari	**PS1.00113**
Mendez-Hernandez Rebeca	**PS2.00194**
Mendoza-Mathison Lilian	**PS1.0070**
Menzies John	**PS1.003, PS2.0081**
Mequinion Mathieu	**PS1.0078**
Méquinion Mathieu	**PS2.0080**
Mercer Julian	**PS2.0010, PS2.0082**

Abbara Ali	**PS1.00103**
Abbas Zaheer	**PS1.0068**
Abbink Maralinde R.	**PS1.00172**
Abbott David H.	**PS1.0038**
Abe Chikara	**PS2.00173**
Abe Yuta	**PS2.0066**
Abizaid Alfonso	**CS1M.001, PS2.0035**
Ablow Measelle Eli	**PS1.00180**
Abolins-Abols Mikus	**PS1.00161**
Ábrahám István M.	**PS1.00157**
Abreu Ana P.	**PS2.00119**
Abuaish Sameera	**PS1.00178**
Achaâban Mohamed Rachid	**PS2.00125**
Acierno James	**PS1.00121**
Adams Gregg P.	**PS2.00139**
Adan Roger	**PS1.0086**
Adekunbi Daniel A.	**BOP2R.001, PS1.00160**
Adkins-Regan Elizabeth	**PS1.008**
Adriaensen Hans	**PS1.00155**
Ager-Wick Eirill	**PS2.00128**
Aggarwal Sanya	**PS1.00146**
Agrati Daniella	**PS2.0017**
Aguggia Julieta	**PS1.0034**
Aiani Lauren	**PS1.0046**
Ainani Hassan	**PS2.00125**
Aja Susan	**PS1.00109**
Akimoto Toshio	**PS1.0042**
Akinnibosun Olutope A.	**PS2.00163, PS2.00164**
Albers H E.	**PS1.0029, PS1.0046**
Alexander Georgia M.	**BOP1S.002**
Alexandre David	**PS1.0078**
Alfieri Alessio	**PS2.00167**
Allchorne Andrew	**PS1.00194**
Allen Tiffany-Jayne	**PS1.0057, PS1.0058**
Allet Cécile	**PS1.0059**
Almeida Osborne	**CS4S.001**
Al-Obaid H M.	**PS1.00160**
Alpar Alan	**PS2.00113**
Alquier Thierry	**PS1.007**
Alvarez-Curto Elisa	**BOP1B.004**
Alward Beau	**PS2.0065**
Al-Yasari Ali	**PS1.0093**
Amao Omowunmi S.	**PS2.0097**
Amborn Katharine M.	**CS3R.001**
Amin Anjali	**PS1.0070**
Ammari Rachida	**PS2.00193**

Amune Ana	**PS2.00172**
Anastassiadis Chloe	**PS1.00191**
Ancel Caroline	**PS2.00122**
Anderson-Buckingham Skylar	**PS1.00161**
Anderson Greg M.	**BOP2S.004, PS1.00139, PS1.0072, PS2.00122**
Anderson Richard	**PS2.0082**
Anderson Ross	**BOP2R.004, PS1.00144**
Andersson Leif	**PS2.00188**
Andoniadou Cynthia	**PS2.0090**
Andre' Valentina	**CS2R.002**
Andrews Zane B.	**CS1M.002, PS1.00123, PS2.0014, PS2.0080**
Angelopoulou Eleni	**PS2.00123**
Anouar Youssef	**BOP2M.005**
Aoki Naoya	**PS2.005**
Aquino Nayara S.S.	**PS1.00117**
Arabo Arnaud	**BOP2M.005**
Arango-Lievano Margarita	**CS2S.002**
Arase Koichi	**PS2.0096**
Araújo Flávia M.	**PS2.00127**
Araujo-Lopes Roberta	**PS1.00117, PS1.00130**
Arbaud Alexandre	**PS2.00190**
Arikawe Adesina P.	**PS2.00163, PS2.00164**
Armstrong Sabrina E..M.	**PS1.006**
Arnal Jean-François	**CS3R.002**
Asari Yuko	**PS2.00155**
Ashton Anna	**PS2.00189**
Asling Hayley A.	**PS1.006**
Atwell Jonathan	**PS1.00161**
Au April	**BOP2B.006**
Aucagne Vincent	**PS1.00124**
Audinat Etienne	**PS1.0069**
August Avery	**PS1.00177**
Augustine Rachael A.	**PS2.00121, PS2.00141**
Augustin Hellmut	**CS2R.002**
Avan Paul	**PS1.0055**
Avendaño Marisol	**BOP1R.003, PS1.00140**
Aylwin Carlos F.	**CS1R.002, PS2.00131**
Azam Amber B.	**PS1.0037**
Bachelor Martha	**PS2.00172**
Bäckhed Fredrik	**CS4M.002**
Baek Songjoon	**PS1.0071**
Bagci Esra	**PS1.0080**
Bagley Jennifer	**PS2.0023**
Bailbé Danielle	**BOP2B.001**
Bailey Craig D..C.	**BOP2S.003**
Bains Jaideep	**CS3S.001**

Baker Cydni N.	**PS2.00137**
Baker Matthew R.	**PS2.00149, PS2.00150**
Baker-Sullivan Elizabeth A.	**PS2.0025**
Bake Tina	**PS1.003**
Bakker Julie	**YA3.001**
Balabanov Ivaylo E.	**PS2.006**
Balapattabi Kirthikaa	**BOP2S.001**
Baldo Barbara	**PS2.0019**
Bale Tracy	**CS1M.003**
Banerjee Jineta	**BOP1M.001**
Bano Riffat	**PS1.00141**
Barabás Klaudia	**PS1.00157**
Baran Marta	**PS2.00148**
Baran Nicole M.	**PS2.0048**
Barantin Laurent	**PS1.00155**
Barbosa Alan S.	**PS2.00127**
Barcelos Lucíola D.S.	**PS2.00127**
Bardet Pierre-Luc	**PS1.0032, PS1.0035**
Barrett Catherine E.	**CS3B.004**
Barrett Perry	**CS4T.002, PS2.00130, PS2.0082**
Barroso Romero Alexia	**BOP1R.003, PS1.00134**
Bassareo Valentina	**PS2.0083**
Bassett Anne	**PS1.0096**
Bassett Paul	**PS1.00103**
Bass Noah	**CS1B.001**
Basso Julia C.	**YA3.003**
Batool Farhat	**PS2.0059**
Bauer Carolyn M.	**PS1.00161**
Bauer Ilena A.M.	**PS2.0087**
Baumgartner Nina E.	**PS2.0043**
Bayerl Doris	**PS1.00164**
Bayne Mitchell	**PS1.0083**
Beach Katherine	**PS1.00159**
Beach Linda Q.	**PS1.00163**
Beales P L.	**PS1.00101**
Beau Jacques	**PS2.00190**
Beaulieu Jimmy	**PS2.0083**
Beaumatin Nicolas	**PS2.00190**
Bech Paul	**PS1.00103**
Becker Susan E.	**PS2.0036**
Bedenbaugh Michelle N.	**PS1.00125, PS1.00127, PS1.00142**
Been Laura	**PS1.00159**
Beery Annaliese K.	**CS2B.001, PS2.0050, PS2.0055**
Behuliak Michal	**PS2.00170, PS2.00171**
Belal Marziyeh	**PS2.00100**
Bellingham Michelle	**PS1.0021, PS2.00169, PS2.00192, YA3.002**

Belsham Denise D.	**BOP2M.006, PS1.00100, PS1.0062, PS1.0075, PS1.0076, PS1.0096, PS1.0097, PS1.0098, PS2.00114, PS2.004**
Beltramo Massimiliano	**PS1.00124**
Benani Alexandre	**PS1.0081**
Benavidez Michelle	**PS2.00124**
Benketira Sarah	**PS2.0063**
Benrick Anna	**PS1.00116**
Bergström Ulrika	**PS1.003**
Berland Chloé	**BOP2M.001**
Berland Marco A.	**CS4R.004**
Bernard Daniel J.	**BOP2R.006, PS1.00148, PS2.00107**
Bernardi Maria M.	**PS2.0040**
Berreby Else-Yona	**PS1.0081**
Berrhamoune Hind	**BOP2M.005**
Berrio Alejandro	**BOP1B.003**
Berruien Nasrin	**PS2.0090**
Bettler Bernhard	**PS2.00106**
Betz Stephen	**CS3TR.004**
Beymer Matthew	**BOP1R.002, PS2.00196**
Bian Jiang Hui	**PS2.00181**
Biello S M.	**PS2.00192**
Biga Peggy	**PS2.00101**
Bird Brian M.	**PS1.0024, PS2.003, PS2.0033**
Bishop Olivia	**PS2.0038**
Bishop Valerie	**PS1.00179**
Bitar Mahmoud	**PS2.0058**
Bizzozzero Hiriart Marianne	**PS1.00149, PS2.00106, PS2.00115**
Björkqvist Maria	**PS2.0084**
Blacher Silvia	**BOP2B.003**
Black Natasha	**PS2.0057**
Blackshaw Seth	**PS1.00109**
Blaustein Jeffrey D.	**AW7.001**
Blondeau Nicolas	**PS1.0074**
Bochukova Elena G.	**BOP2M.002**
Boehm Ulrich	**BOP2R.006**
Boerboom Derek	**PS1.00148**
Bohlooly-Y Mohammad	**PS1.0081**
Bolborea Matei	**PS1.00195, PS1.0060, PS2.00190**
Booth April	**PS1.00156**
Borland Johnathan M.	**PS1.0046**
Bosch Oliver J.	**PS1.00164**
Bossong Frank	**PS2.00178**
Boswell Timothy	**PS2.0075**
Bo Ting-Bei	**PS1.0095**
Bouchahda Mohamed	**PS2.00190**
Boulos Vanessa	**PS2.0063**

Bouret Sebastien	**BOP2M.002, PS2.0099**
Bourguignon Jean-Pierre	**BOP2B.003**
Bourguignon Nadia S.	**PS2.00106**
Bourne Rebecca	**PS1.00177**
Bourque Charles	**PS2.00160**
Boutin Jean A.	**CS3TR.001**
Boutin Stan	**BOP1S.001**
Bouwer Greg	**BOP2R.005, PS2.00118**
Bovee Sonny	**PS2.0076**
Boyd Hannah	**PS2.0064**
Bradburn Steven	**PS2.0042**
Brake Wayne G.	**PS1.0011, PS2.0061, PS2.0063**
Brandenburger Matthias	**CS2T.002**
Brandon Nicholas J.	**PS1.0017**
Brau Frédéric	**PS1.0069**
Breda Gabriele	**PS2.00190**
Bredewold Remco	**PS1.005**
Breedlove S. M.	**PS2.00183**
Breen Kellie M.	**PS2.00116, PS2.00117**
Breuer Joseph A.	**PS1.00119**
Brinie Matthew T.	**PS1.0071**
Broberger Christian	**BOP1B.001, PS2.00193**
Brocklehurst Sarah	**PS2.0075**
Brodin Birger	**PS1.00111**
Bronstein Robert	**PS2.0072**
Brown Colin H.	**PS1.00153, PS2.00121, PS2.00141, PS2.0074**
Brown Gillian	**PS2.0051**
Brown Rosie S.E.	**AW5.001, PS1.00131, PS1.00132**
Brubaker Patricia L.	**PS1.00100**
Brucker Sara	**PS2.0087**
Brugge Doug	**PS2.0045**
Brûlé Emilie	**PS2.00107**
Brummelte Susanne	**CS3B.001, PS2.0060**
Brunaud Laurent	**PS2.00168**
Brunton Paula J.	**PS1.00164, PS2.00154, PS2.00167**
Buckinx An	**PS1.0053**
Buckley Stephen	**PS1.00111**
Bugliani Marco	**PS1.00103**
Buijs Ruud M.	**PS2.00194**
Bunn Stephen	**PS1.00130**
Burdakov Denis	**BOP1B.004**
Burke Mary	**BOP2M.001**
Burkhard Tracy	**PS2.0044**
Burn David	**PS2.0014**
Butler Michael	**PS1.00108**
Cador Martine	**BOP2M.001**

Caglayan Ahmet Burak	**PS1.00115**
Caillé-Garnier Stéphanie	**BOP2M.001**
Cairns Mark	**PS2.00161**
Calisi Rebecca M.	**PS2.00143**
Camacho Francisco	**PS2.0016**
Campbell Rebecca E.	**CS2R.001, PS1.00143, PS1.00152, PS2.00118, PS2.00120, PS2.0018**
Campbell-Yeo Marsha	**CS3B.002**
Campideli-Santana Ana C.	**BOP2R.002, PS1.00117**
Cansell Céline	**PS1.0069, PS1.0074**
Cao Bei-Bei	**PS1.00188, PS1.0044**
Cao Ye	**PS1.0087**
Capuron Lucile	**BOP2B.001**
Cara Alexandra	**PS1.0059**
Carcea Ioana	**PS1.0039**
Cardoso Thais S.R.	**PS2.00127**
Cariboni Anna	**CS2R.002**
Caron Emilie	**PS1.0064**
Carrasco Rodrigo A.	**PS2.00139**
Carrat Gaelle	**PS1.00103, PS1.00106**
Carré Justin M.	**PS1.0024, PS2.003, PS2.0033**
Carroll Quinn E.	**PS1.0050, PS2.0047**
Carroll Rona S.	**PS2.00119**
Carson Paige	**PS1.00159**
Carstens Kelly E.	**BOP1S.002**
Carter Jasmine	**PS2.0089**
Carter Kirsten M.	**PS1.0020**
Carter Sara N.	**PS2.0015**
Carter Sylvia D.	**PS2.00151**
Caruso Michael	**PS1.00177**
Casals Nuria	**PS1.00140**
Casanueva Laura	**PS1.0047**
Case C P.	**PS2.00167**
Caso Federico	**PS2.00130**
Cassatella Daniele	**PS1.00121**
Cassie Nikki	**PS2.0082**
Castel Julien	**BOP2M.001, PS1.0080**
Castellano Juan Manuel	**PS1.00134, PS1.00140**
Castillo-Campos Andrea	**PS2.00153**
Castro Gisele	**PS1.0099**
Castro María José	**PS2.0017**
Cates Hannah M.	**BOP2S.006**
Cathers Phillip	**PS2.0065**
Caughey Sarah D.	**PS2.0075**
Caulfield Jasmine	**PS1.00169, PS1.00177**
Caulfield Jasmine I.	**PS1.00181**

Cavanaugh Jon	**PS2.0054**
Cavigelli Sonia	**PS1.00169, PS1.00177**
Cavigelli Sonia A.	**PS1.00181**
Cázarez-Márquez Fernando	**PS1.00122**
Ceballos Esteban	**PS2.0068**
Cederberg Helms Hans C.	**PS1.00111**
Cengiz Pelin	**CS3R.001**
Cezar Luana C.	**PS2.0040**
Chachlaki Konstantina	**PS1.0055**
Cha David	**PS1.00109**
Chahal Navdeep	**PS1.00149**
Chaiton Jessica A.	**PS1.00151**
Chalmers Jennifer	**PS1.00100, PS1.0062, PS2.004**
Chalmers Jennifer A.	**PS1.0097**
Champagne Frances A.	**CS1S.001**
Chanana Vishal	**CS3R.001**
Chao Moses V.	**CS2S.002**
Charli Jean L.	**PS1.0089, PS2.00153**
Chartrel Nicolas	**BOP2M.005, PS1.0078**
Cheah Jeffrey	**PS1.00179**
Chee Melissa	**PS2.0035**
Chen Chun	**CS2S.004**
Chen Gary	**PS2.00162**
Chen Junfeng	**PS2.00188**
Chen Lunhao	**PS1.001**
Chen Xue Qun	**PS2.00181**
Cheong Rachel	**PS2.0012, PS2.0019**
Cherifi Saloua	**BOP2M.005**
Chi Qingsheng	**PS1.0095**
Chmiel Jessica R.	**PS2.0036**
Choe Han Kyoung	**PS2.00195**
Choi Dennis C.	**PS1.00163**
Choleris Elena	**CS1B.001, PS1.0015, PS1.00190, PS1.0027, PS1.006, PS2.0022**
Choudhury Agharul I.	**BOP1B.004**
Chowen Julie	**CS2M.001**
Cho Yoon Hee	**PS1.00104, PS2.0092**
Christensen Debora	**PS2.0073**
Christensen Jennifer	**PS2.0055**
Christou-Savina Sofia	**PS1.00101**
Chun Eileen K.	**PS2.0053**
Cigremis Yilmaz	**PS1.00115**
Cimino Irene	**PS1.00114, PS1.00121**
Cinar Resat	**PS1.00112**
Ciofi Philippe	**PS1.0055**
Cisneros-Larios Brenda	**PS1.00126**

Cisneros Miguel	**PS2.00153**
Clarke Iain J.	**BOP2M.003, PS1.0061**
Clarke Irenie	**PS1.0067**
Clarke Sophie	**PS1.00103**
Clarkson Jenny	**PS1.00136, PS1.00138**
Clasadonte Jerome	**PS1.00111, PS1.0064**
Clemenzi Matthew N.	**PS1.00100**
Cocco Cristina	**PS1.0084**
Coimbra Cândido C.	**BOP2R.002, PS2.00127**
Colak Cemil	**PS1.00115**
Coll Anthony P.	**PS1.00114, PS1.0057, PS1.0058**
Collden Gustav	**PS1.00102**
Colledge William H.	**BOP2R.001, PS2.00147**
Collins Andrila E.	**PS1.0015**
Collins Troy	**PS2.00135**
Comninos Alexander N.	**PS1.00103**
Conde Kristie	**PS2.00105**
Connolly George A.	**PS1.00139**
Connors John M.	**PS1.00125, PS1.00127, PS1.00142**
Constantinof Andrea	**PS2.00177**
Conway-Campbell Becky L.	**CS2S.001, PS1.0071**
Coolen Lique M.	**BOP1R.001, PS1.00135, PS1.00142**
Cork Simon C.	**PS1.0087**
Cornil Charlotte A.	**CS3R.002**
Corona Rebeca	**CS3R.002**
Corsini Silvia	**PS1.0080**
Cortés Carmen	**PS2.0024, PS2.0030, PS2.0031, PS2.0032**
Court Lucas	**CS3R.002**
Coutinho Eulalia	**PS1.00143, PS2.00120**
Coyle Chris	**PS2.00130**
Craig Tim	**PS1.00177**
Creeney Hannah	**PS1.0017**
Creighton Samantha	**PS1.00113**
Croizier Sophie	**PS2.0099**
Crozier Sophie	**BOP2M.002**
Cruz Gonzalo	**PS2.0020**
Cservenak Melinda	**PS2.00113, PS2.00126**
Cui Zhenzhong	**PS1.0063**
Cunningham J. Thomas	**BOP2S.001, PS2.00172, PS2.0021**
Cunningham Rebecca L.	**PS2.0029**
Curtis A M.	**PS2.00192**
Da Fonseca Caio Cesar N.	**PS2.0040**
Da Fonte Dillon F.	**PS2.00144**
Daimon Makoto	**PS2.00155, PS2.00156**
Dale Nicholas	**PS1.0060**
Dallmann Robert	**PS1.00195**

Dalvi Prasad S.	**PS1.0082, PS1.0097**
D'Angelo Heather M.	**PS2.0056**
Daniel Jill M.	**PS2.0039, PS2.0043**
D'Anna Kimberly	**PS2.0069**
Dantzer Ben	**BOP1S.001**
D'Aquila Andrea	**PS2.00101**
Dardente Hugues	**CS4T.001**
Darling Jeffrey S.	**PS2.0039**
Darnaudéry Muriel	**BOP2B.001**
Dartez Lauren	**PS2.0039**
Darwish Lina	**PS2.0089**
Dash Satya	**PS1.0096**
Da Silva Mayara B.	**PS2.0022**
David Anna-Julia	**PS2.0026**
David Caroline	**PS1.0045**
Davies Alison	**PS1.0057, PS1.0058**
Davies Jeff	**PS2.0014**
De Araujo Ivan E.	**CS3M.001**
D'Eath Rick B.	**PS2.0075**
De Backer Ivan	**PS1.00106**
De Bundel Dimitri	**PS1.0016, PS1.0053**
De Burgh Ross A.	**BOP2R.001, PS1.00133**
Decapo Madison	**PS2.0023**
Decarie-Spain Lea	**PS1.007**
De Castro Barbosa Thais	**PS1.00116**
Decatanzaro Denys	**PS2.00132**
Decourt Caroline	**PS1.00124, PS1.00139, PS2.00122**
De Fante Thaís	**PS1.0062**
Defazio Richard A.	**PS1.00118**
De Guzman Rose M.	**PS2.0068**
Delgado Nicol	**PS2.0020**
De Lima Ana Paula N.	**PS1.00183, PS2.0040**
Delli Virginia	**BOP2B.003**
De Lorme Kayla C.	**PS2.00182**
Demas Gregory E.	**PS1.0025**
De Miera Cristina	**BOP1R.002**
Deng Mengdie	**PS2.00157**
Denis Raphael	**PS1.0080**
De Rosa Maria Caterina	**PS1.00102**
De Roux Nicolas	**CS2R.004, PS1.00147**
Desban Laura	**PS1.0032, PS1.0035**
Desroziers Elodie	**PS1.00143, PS2.00120, PS2.0018**
Devaux Nadège	**PS1.0069**
De Vries Geert J.	**PS2.0046, PS2.0067**
Deyoe Jessica E.	**PS1.0025**
Dhillo Waljit S.	**CS3TR.002, PS1.00103, PS1.00106**

Diaz Bryan S.	**PS1.00163**
Diaz Nestor	**PS2.0016**
Dickson Suzanne L.	**PS1.003**
Dieguez Gonzalez Carlos	**PS1.00134**
Di Giorgio Noelia P.	**PS1.00149, PS2.00106, PS2.00115**
Dijkstra Dorieke	**PS2.0010**
Ding Xiaojing	**PS1.0043**
Djenoune Lydia	**PS1.0032, PS1.0035**
Dobolyi Arpad	**PS1.0013, PS2.00113, PS2.00126**
Doege Claudia A.	**PS1.00102**
Dokovna Lisa B.	**BOP2B.002**
Dong Fanglong	**PS2.00178**
Dong Lynn	**PS1.008**
Donovan Alexandra M.	**PS1.009**
Donovan Meghan L.	**PS1.0031, PS2.0053**
Dorantes-Nieto Ángeles	**PS2.0031**
Dorantes-Nieto María D.L.Á.	**PS2.0032**
Dorfman Mauricio D.	**BOP1M.001**
Douglass John D.	**BOP1M.001**
Drummond Lucas R.	**PS2.00127**
Duan Shumin	**PS1.001**
Duarte Ana I	**PS2.0084**
Duarte-Guterman Paula	**PS1.0047, PS2.00109, PS2.0057**
Duchesne Annie	**PS2.0025**
Duclot Florian	**PS1.0049**
Dudek Serena M.	**BOP1S.002**
Du Ji Zeng	**PS2.00181**
Dulac Catherine	**PL04.001**
Dulka Eden A.	**PS1.00129**
Duncan Jacqueline	**PS2.0010**
Duncan Katherine	**PS1.0033**
Duncan Peter	**PS2.00161**
Dunn Ian C.	**PS2.0075, PS2.0079**
Duong Phong	**PS2.0029**
Duquenne Manon	**PS1.0064, PS1.0079**
Duque-Wilckens Natalia	**PS1.00186**
Durant John	**PS2.0045**
Durate Rodrigo R.	**PS1.0017**
Duriez Philibert	**PS1.0078, PS1.0081**
Eagle Allison K.	**PS2.00180**
Eberini Ivano	**CS2R.002**
Ebling Francis J.	**CS4T.002, PS1.0084**
Eckel Lisa	**PS1.00108**
Eddy J. M.	**PS1.00180**
Edwards Alexander W.	**PS2.0035**
Egeciouglu Emil	**PS1.0081**

Eguibar José R.	**PS2.0024, PS2.0030, PS2.0031, PS2.0032**
Ehlert Ulrike	**PS2.00162**
Einstein Gillian	**BOP2B.006, PS1.0033, PS2.0025**
Elad Vissy M.	**PS1.006**
El Allali Khalid	**PS2.00125**
El Bousmaki Najlae	**PS2.00125**
Elgbeili Guillaume	**PS1.00191**
El Houda Mimouni Nour	**PS1.00121**
Elias Carol F.	**CS1T.001, PS1.00126, PS1.0059**
Ellacott Kate L.J.	**CS2M.002, PS1.00196**
El Mamoune Kahina	**PS1.00155**
El Mehdi Mouna	**BOP2M.005**
Eltahir Akif M.	**PS1.00171**
Emmerson Paul	**CS3TR.003**
Engledow Simon	**CS1S.003**
Eng Pei C.	**PS1.00103**
Enos Riley T.	**PS1.0019**
Epelbaum Jacques	**PS1.0081**
Ergang Peter	**PS2.00170, PS2.00171**
Ernszt Dávid	**PS1.00157**
Ervin Kelsy	**PS1.0027**
Escobar Carolina	**CS2T.001**
Esparza Lourdes A.	**PS2.00145**
Estay Camila	**PS2.0020**
Evans Maggie C.	**PS1.0072**
Evans Neil P.	**PS1.0021, PS2.00169, PS2.00192, YA3.002**
Fadahunsi Nicole	**PS1.0087**
Fageyinbo Samuel O.	**PS2.0097**
Fair Damien	**PS2.0023**
Falconi Atilio	**PS2.0017**
Fang Lisa	**PS1.0085**
Farmer George	**BOP2S.001, PS2.00172, PS2.0021**
Farooqi Sadaf	**BOP2M.002**
Fasnacht Rachael	**BOP1M.001**
Faure Paul A.	**PS2.00132**
Faure Philippe	**BOP2M.001**
Faykoo-Martinez Mariela	**PS2.00135**
Fazekas Emese	**PS2.00126**
Fehlert Ellen	**PS2.0087**
Felicio Luciano F.	**PS2.0040**
Feng Xixi	**BOP2S.002**
Ferland Guylaine	**PS2.0098**
Fernald Russell	**PS2.0065**
Fernandes Paola	**PS2.00127**
Fernando Anushka B..P.	**BOP1B.004**
Fernandois Daniela	**PS1.00140**

Ferrazzano Peter	**CS3R.001**
Ferreira Guillaume	**PS1.00197**
Ferreño Marcela	**PS2.0013**
Ferri Gian-Luca	**PS1.0084**
Fiacco Serena	**PS2.00162**
Finkenstädt Bärbel	**PS2.00190**
Fisette Alexandre	**PS1.00102, PS1.007**
Fishman Ruth	**PS2.00110**
Flamant Frédéric	**BOP1R.002**
Floresco Stan B.	**PS1.0019**
Flowers Matthew T.	**PS1.0038**
Flynn Benjamin P.	**PS1.0071**
Focke Caroline	**PS1.00174**
Folmerz Elin	**PS1.00116**
Fonken Laura K.	**PS2.0056**
Fontaine Romain	**PS2.00128**
Foppen Ewout	**PS1.0080**
Forlano Paul M.	**BOP1B.002**
Fornes Romina	**PS1.00116**
Fortin Jerome	**PS1.00148**
Foster William	**PS1.00159**
Fraigne Jimmy	**PS2.004**
Francescone Abigal R.	**PS1.0050, PS2.0047**
Frank Matthew G.	**PS2.0056**
Franssen Catherine L.	**PS2.00179, PS2.00180**
Franssen Delphine	**PS1.00134**
Frantz Kyle	**PS1.0046**
Freeman David A.	**PS2.0015**
French Jeffrey A.	**PS2.0054**
Frias Antonio	**PS2.0023**
Frick Karyn	**CS1B.002**
Friedman Jeffrey	**PL07.001**
Friesen Caitlin N.	**PS2.0028**
Fritsche Andreas	**PS1.0068, PS2.0087**
Froemke Robert C.	**PS1.0039**
Frost Gary	**PS1.0070**
Fudickar Adam	**PS1.00161**
Fudulu Daniel	**PS2.00186**
Fuller-Jackson John-Paul	**BOP2M.003**
Fulton Stephanie	**CS3M.002, PS1.007**
Fu Travis	**CS1T.003**
Gagne Collin	**PS2.0061, PS2.0063**
Gajewska Alina	**PS2.00148**
Gajewski Lukasz	**PS2.00175**
Galea Liisa A.M.	**CS1B.003, PS1.00151, PS1.0047, PS2.00109, PS2.0057**
Gallet Sarah	**PS1.00111**

Gangarossa Giuseppe	**BOP2M.001, PS1.0080**
Gao Di	**PS1.00110**
Gao Yan	**PS1.00110**
Gao Zhihua	**PS1.001**
Garabedian Michael	**CS2S.002**
Garbutt Lauren	**PS1.00195**
Garcia Caceres Cristina	**BOP2M.004, PS1.0088**
Garcia-Caceres Cristina	**PS1.00102, PS1.0080**
García-Cáceres Cristina	**BOP1M.002**
García Carlos	**PS2.0017**
Garcia Galiano David	**PS1.00126, PS1.00134**
Garcia James	**PS1.00162**
Gardiner James	**PS1.00106**
Gardini Elena	**PS2.00162**
Garthwaite John	**PS1.0055**
Gasman Stephane	**PS1.0079, PS2.00168**
Gaßner Barbara	**PS1.00164**
Gaston-Massuet Carles	**CS2R.002**
Gaudreau Pierrette	**PS2.0098**
Gay Joséphine	**PS1.00197**
Gaytan Luna Francisco	**BOP1R.003, PS1.00134, PS1.00140**
Gazetas James	**PS1.00191**
Gegenhuber Bruno	**PS2.0072**
Gelbenegger Therese	**PS1.00197**
Geniole Shawn N.	**PS1.0024, PS2.003, PS2.0033**
Georgescu Teodora	**PS2.0085**
Gérard Arlette	**BOP2B.003**
Gergely Cassandra K.	**PS1.004**
Gerutshang Achi	**PS2.00129**
Gervais Nicole	**BOP2B.006, PS1.0011, PS2.0025**
Giacobini Paolo	**PS1.00121, PS1.0055, PS1.0059, YA1.001**
Giglio Erin M.	**PS2.0044**
Giles Aaron G.	**PS1.00182**
Gill Andrew C.	**PS2.00154**
Gilon Chaim	**BOP2R.003**
Gjerstad Julia K.	**BOP2S.002**
Glegola Justyna A.	**BOP1B.004**
Gobinath Aarthi R.	**PS2.00109**
Godlewski Grzegorz	**PS1.00112**
Godó Soma	**PS1.00157**
Godsland Ian	**PS1.00103**
Go Kim	**PS1.0047, PS2.00109, PS2.0057**
Goldman David	**PS2.00134**
Gomez Romero Maria	**PS1.00103**
Gompel Anne	**PS1.00147**
Gonzalez-Abuin Noemi	**PS1.0070**

Goodman Robert	**PS1.00142**
Goodman Robert L.	**PS1.00125, PS1.00127, PS1.00135**
Gore Andrea C.	**PL10.001, PS1.0054**
Gorman Michael R.	**PS1.00119**
Goss Hannah M.	**CS1S.003**
Goss Matthew	**PS2.0011**
Gotlieb Neta	**PS2.00137**
Goularte Jeferson F.	**PS1.00123**
Goularte Jéferson F.	**PS2.0080**
Gouws Julia	**PS1.00166**
Grady Cheryl	**PS2.0025**
Grantham Kymberly	**PS1.0046**
Grattan David	**PS1.00117, PS1.00130, PS1.00131, PS1.00132, PS1.0020, PS1.0077, PS2.0088**
Gravelsins Laura	**PS1.0033, PS2.0025**
Greives Timothy	**PS1.00161**
Greville Lucas J.	**PS2.00132**
Gribble Fiona M.	**CS4M.001, PS1.0092**
Grieb Zachary	**PS2.00136**
Grinevich Valery	**PS1.00194**
Grisel Judy E.	**PS1.0036**
Gruber Tim	**BOP1M.002, BOP2M.004, PS1.0088**
Guerra Monserrat	**CS4R.004**
Gustafson Papillon	**PS1.00131**
Gutiérrez-Mariscal Mariana	**PS2.00153**
Haas Nicole A.	**PS1.0028**
Hacker Jennifer	**PS2.0038**
Haeberlé Anne-Marie	**PS1.0079**
Hager Gordon L.	**PS1.0071**
Hagey Travis	**PS2.0027**
Hahn Margaret	**PS1.0098**
Haleem Darakhshan J.	**PS2.0059**
Hamada Hirotaka	**PS2.00177**
Hamden Jordan E.	**PS1.0019**
Hampson Elizabeth	**BOP2B.006**
Hanalioglu Damla	**CS3R.001**
Handa Robert J.	**PS1.00173**
Hanics Janos	**PS2.00113**
Hanna Lydia	**PS1.00196**
Han Su Y.	**PS1.00136, PS1.00137, PS1.00138, PS2.0074**
Han Wenfei	**CS3M.001**
Han Ye Eon	**PS1.00104, PS2.0092**
Haq Naila A.	**PS1.00101**
Haraldsen Ira H.	**PS1.0021, PS2.00169**
Hardy Darran G.	**BOP1B.004**
Hardy Steven L.	**PS1.00127, PS1.00142**

Häring Hans-Ulrich	**PS1.0068, PS2.0087**
Harno Erika	**PS1.0057, PS1.0058**
Harter Campbell J.L.	**PS2.0011**
Hart Kirsten	**PS2.00135**
Harvey Alan R.	**PS2.0011**
Hashimoto Hirofumi	**PS2.0096**
Hausch Felix	**BOP2S.002**
Hazlerigg David	**PS2.00196**
Healy Sue D.	**PS2.00166**
Hecksher-Sørensen Jacob	**PS1.00111**
Heisler Lora K.	**PS1.0056, PS2.002, PS2.0085**
Heiss Christina	**CS4M.002**
Helbling Jean-Christophe	**PS1.00197**
Helfer Gisela	**PS1.0082**
Heni Martin	**PS1.0068**
Henningsen Jo B.	**PS2.0019**
Henriques Patricia C.	**PS1.00117**
Henry Belinda A.	**BOP2M.003, PS1.0061**
Heras Violeta	**BOP1R.003, PS1.00140**
Herbison Allan E.	**PL03.001, PS1.00136, PS1.00137, PS1.00138, PS1.00152**
Hernandez Morgan E.	**PS1.0054**
Heron Megan	**PS2.0063**
Herrera Moro Chao Daniela	**BOP1M.002, PS1.0080**
Herzog Erik	**PS2.00191**
Herzog Herbert	**CS3M.003, PS1.0067**
Hessler Sabine	**PS1.00143, PS1.00152**
He Wen	**PS1.00145**
Hicks Amirah-Iman	**PS1.0091**
Hicks Nelson Alexandria	**PS2.0042**
Hileman Stanley M.	**PS1.00125, PS1.00127, PS1.00142**
Hilliard Austin	**PS2.0065**
Hill Jennifer W.	**PS1.00150**
Hipolito Laisa T.M.	**BOP2R.002**
Hirasawa Michiru	**PS1.0085**
Hirata Keiji	**PS2.0096**
Hiura Lisa	**PS2.007**
Hnasko Thomas S.	**BOP2M.001**
Hoang Kim N.	**PS2.00182**
Ho Bryan	**PS2.00145**
Hodges Travis E.	**BOP2S.003, PS1.00171**
Hodgson Amy	**PS2.0051**
Hodne Kjetil	**PS2.00128**
Hoffman Jessica F.	**PS2.00134**
Hoffmann Hanne M.	**PL05.001, PS1.00119**
Hofmann Hans	**PS1.0012, PS2.0027, PS2.0028**
Hogg David W.	**PS2.00103**

Holihan Stephen	**PS2.0038**
Holland Sarah	**PS2.00120**
Holmes Elaine	**PS1.00103**
Holmes Megan C.	**CS4S.002**
Holmes Melissa	**PS2.00135, PS2.0071**
Holton Christopher	**PS1.00106**
Homma Koichi J.	**PS2.005**
Hornsby Amanda K.E.	**PS2.0014**
Horta Nayara A.C.	**PS2.00127**
Horvath Tamas L.	**BOP2M.004, PS1.0088**
Hoshi Aya	**PS1.00112**
Hosokawa Keisuke	**PS2.0086**
Hough Denise	**PS1.0021, PS2.00169, PS2.00192**
Houy Sébastien	**PS2.00168**
Howard Sarah	**PS1.0027**
Hrabovszky Erik	**PS1.003**
Hrabowszky Erik	**PS1.0055**
Hryhorczuk Cecile	**PS1.007**
Huang Qi	**PS2.00190**
Huang Yan	**PS1.00188**
Hübner Katharina	**PS1.00164**
Huffels Christiaan	**PS1.00194**
Hughes Jessica K.	**PS2.00140**
Hughes Stephen J.	**PS1.00103**
Hugon-Rodin Justine	**PS1.00147**
Huhman Kim L.	**PS1.00163**
Hume Catherine	**PS1.00194, PS1.003, PS2.0081**
Hu Ming	**PS1.00103**
Humphries Murray	**BOP1S.001**
Hunter Richard G.	**CS1S.002**
Hussain Sufyan	**PS1.00106**
Hussein Khalid	**CS2R.002**
Hyland Brian	**PS1.00130**
Hyland Lindsay	**PS2.0035**
Ibarra Juan M.	**PS2.0030**
Ignácio-Souza Letícia	**PS1.0062**
Imbernon Monica	**PS1.00111**
Imoesi Peter I.	**PS1.0056**
Inglis Megan A.	**PS1.00139, PS2.00122**
Innominato Pasquale F.	**PS2.00190**
Inquimbert Perrine	**PS2.00123**
Insel Nathan	**PS2.0026**
Inutsuka Ayumu	**CS3S.002, PS1.00165**
Iremonger Karl	**PS1.00166, PS1.00168, PS1.00174**
Irvine Elaine E.	**BOP1B.004**
Isaacs Lauren	**PS1.00113**

Isiordia Laura E.	**PS1.00180**
Ismail Nafissa	**PS1.00183**
Ito Etsuro	**PS2.00156**
Iwuamadi Elizabeth C.	**PS2.0097**
Izzi-Engbeaya Chioma	**PS1.00103, PS1.00106**
Jackson Meliame	**PS2.00121**
Jacob-Brassard Elizabeth	**PS1.007**
Jacob Dennis P.	**BOP2S.005**
Jacobskind Jason S.	**PS1.00185**
Jacob-Tomas Suleima	**PS1.0090**
Jalabert Cecilia	**PS1.0025**
Jama Kalson	**PS1.0021**
Jamieson Bradley	**BOP2R.005, PS2.00118**
Jastroch Martin	**BOP2M.004**
Jastrzebski Pawel	**PS1.0011**
Jeanneteau Freddy	**CS2S.002**
Jeffress Elizabeth C.	**PS1.00163**
Jensen Casper B.	**BOP2M.001**
Jethwa Preeti H.	**PS1.0084**
Jiang Lizhi	**PS1.00109**
Jiang Zhiying	**CS2S.004**
Jia Shuwei	**BOP1S.003**
Jimenez Roberto	**PS1.00180**
Ji Sihan	**PS1.00110**
Johnson Caroline	**PS2.00146**
Johnson Paul R.	**PS1.00103**
Johnston Fionnuala	**PS2.0014**
Jomard Anne	**PS1.00103**
Jones Jeff	**PS2.00191**
Jones Kathryn M.	**PS1.0031**
Jones Sherri Lee	**PS1.00191**
Jones Sophie	**PS1.00103**
Jordan Cynthia L.	**PS2.00183**
Joseph-Bravo Patricia	**PS1.0089, PS2.00153**
Jourdan Tony	**PS1.00112**
Juntti Scott	**PS1.0026, PS2.0065**
Justice Nicholas J.	**PS1.00185**
Kabbaj Mohamed	**PS1.0049**
Kachkovski Gueorgui V.	**PS1.0019**
Kagan Karl O.	**PS2.0087**
Kageyama Kazunori	**PS2.00155, PS2.00156**
Kaiser Ursula B.	**CS2R.003, PS2.00119**
Kakadellis Sarah	**BOP1B.001**
Kalinowski Leanna M.	**PS2.0047**
Kalsbeek Andries	**PS1.00122, PS2.00123**
Kanagasundaram Pruntha	**PS1.0098**

Kane Grace M.	**PS1.00136, PS1.00137**
Kang Chan Woo	**PS1.00104, PS2.0092**
Kang M. C.	**PS1.0099**
Kanpurwala Muhammad A.	**PS2.00174**
Kantamneni Sriharsha	**PS1.0082**
Kaplan Ariel	**PS2.00112**
Karnani Mahesh M.	**BOP1B.004**
Katsouri Loukia	**BOP1B.004**
Katsumata Harumi	**PS1.0042**
Katz Larry S.	**PS2.0036**
Kauffman Alexander S.	**CS1R.001, PS1.00149, PS2.00140, PS2.00145**
Kaur Gagandeep	**PS2.00178**
Kawamura Noriyuki	**PS1.00193**
Kearns Patrick	**PS2.00161**
Keay Kevin A.	**PS1.00182**
Keen Kim	**PS1.00162**
Keller David	**PS2.00126**
Kelley Lyla	**PS1.0094**
Kelly Aubrey M.	**BOP2B.005**
Kema Ido	**PS2.00186**
Kennedy Clare L.M.	**CS1S.003, PS2.00151, PS2.00152**
Kentner Amanda	**CS3B.003**
Keogh Julia	**BOP2M.002**
Kershaw Yvonne M.	**PS1.0071**
Ketterson Ellen	**PS1.00161**
Khant Aung Zin A.	**PS1.0077**
Khbouz Badr	**CS3R.002**
Kiefer-Schmidt Isabelle	**PS2.0087**
Kim Boil	**PS2.00195**
Kim Jean	**PS2.0092**
Kim Jihoon	**PS2.00195**
Kim Joon	**PS1.00168**
Kim Kyungjin	**PS2.00195**
Kim Sohyoung	**PS1.0071**
Kim Young-Bum	**PS1.0099**
King Suzanne	**PS1.00191**
Kinoshita Noriko	**PS2.00156**
Kirsten Thiago B.	**PS2.0040**
Klampfl Stefanie	**PS1.00164**
Klein Laura	**PS1.00177**
Klosen Paul	**PS1.00122**
Kober Caitlin	**PS2.0036**
Koekkoek Laura	**PS1.0080**
Kolbe Isa	**CS2T.002**
Komarzynski Sandra	**PS2.00190**
Kondo Daisuke	**PS1.0066**

Kondo Yasuhiko	**PS1.0010, PS1.0042, PS2.0037, PS2.0066**
Konji Sandra M.	**PS2.0041**
Konkle Anne T M.	**PS2.0041**
Koren Lee	**PS2.00110**
Korosi Aniko	**CS4S.003, PS1.00172**
Korpal Aaron K.	**PS2.0074**
Kostaki Alisa	**PS2.00177**
Kosten Therese A.	**PS2.00158**
Kowalchuk Chantel	**PS1.0098**
Krajewski-Hall Sally J.	**PS1.00154**
Krause Jesse S.	**PS1.00179**
Kraynak Marissa	**PS1.0038**
Kreisman Michael J.	**PS2.00116, PS2.00117**
Kriegsfeld Lance J.	**PS2.00137**
Krishnan Keerthi	**PS2.008**
Kroenke Chris	**PS2.0023**
Ku Cheol Ryong	**PS1.00104**
Kuehlmann Alex L.	**PS1.0038**
Kullmann Stephanie	**PS1.0068**
Kumagai Ryoko	**PS2.0037**
Kumar Shalini S.	**PS2.00141**
Kunos George	**PS1.00112**
Kurrasch Deborah M.	**BOP1M.004, PS1.0014**
Kusuhara Koichi	**PS1.0023**
Kyne Robert F.	**PS1.0050**
Labarthe Alexandra	**PS1.0081**
Labelle Morgan	**PS2.0041**
Lacasse Jesse M.	**PS2.0061, PS2.0063**
Lacreuse Agnes	**PS1.0041**
Ladyman Sharon R.	**PS1.00131, PS1.0020, PS1.0077**
La Fleur Susanne E.	**CS3M.004, PS1.00172**
Lalhou Najiba	**PS1.00147**
Lam Brian	**PS1.00114, PS2.0091**
Landaverde Amanda V.	**PS2.00182**
Landry Hannah K.	**PS2.0041**
Lane Jeffrey E.	**BOP1S.001**
Lange Gary M.	**PS2.0038**
Lanoix Joel	**PS2.00168**
Laplante David P.	**PS1.00191**
Lapointe Evelyne	**PS1.00148**
Laran-Chich Marie-Pierre	**PS1.00122**
Lardner Casey K.	**BOP2S.006**
Larkin Theresa A.	**PS2.00185**
Lass Geffen	**BOP2R.001, PS1.00133**
Lau Billy	**PS2.008**
Lauby Samantha	**PS1.00176**

Lawler Katherine	**BOP2M.002**
Layé Sophie	**BOP2B.001**
Layo Dana	**PS2.008**
Leach Zoe K.	**PS2.0022**
Lebeaupin Cynthia	**PS1.0069**
Ledbetter Rob	**PS2.00184**
Lee Eun Jig	**PS1.00104, PS2.0092**
Lee Jessica S.	**PS1.00119**
Lee Ji-Hyeon	**PS1.0063**
Lee Mi Kyung	**PS1.00104**
Lee Nicole S.	**PS2.0050**
Lee Se-Jin	**BOP2R.006**
Lee Soomin	**PS2.00195**
Lee Ying	**CS4M.002**
Legutko Beata	**BOP1M.002, BOP2M.004, PS1.00102, PS1.0088**
Lehman Michael N.	**BOP1R.001, PS1.00135, PS1.00142**
Leichner Emily	**PS2.0054**
Leko Andras H.	**PS2.00113**
Le May Marie V.	**PS1.003**
Lemus Moyra B.	**PS2.0080**
Lenfant Françoise	**CS3R.002**
Leng Gareth	**PS1.00194, PS1.003, PS2.0077, PS2.0078, PS2.0081**
León Silvia	**PS2.00129**
Leprince Jérome	**BOP2M.005**
Lerch Jason	**PS1.00105**
Lestage Alex	**PS2.0061**
Le Thuc Ophelia	**BOP1M.002, BOP2M.004, PS1.0078, PS1.0080, PS1.0088**
Le Thuc Ophélia	**PS1.0069, PS1.0074**
Le Tissier Paul	**PS2.0090**
Lettieri Antonella	**CS2R.002**
Levavi Sivan Berta	**BOP2R.003**
Lever Louise C.	**PS2.00180**
Levi David	**PS2.00160**
Lévi Francis A.	**PS2.00190**
Levine Jon E.	**CS3R.001, PS1.0038**
Levkowitz Gil	**CS1T.002**
Lewis` Jo E.	**CS4T.002, PS1.0084**
Lewis Matthew R.	**PS1.00103**
Leysen Valerie	**PS1.0055**
Liaw Reanna B.	**PS1.00149**
Libertun Carlos	**PS1.00149, PS2.00106, PS2.00115**
Lickliter Jason	**CS3TR.004**
Li De-Pei	**PS2.00158**
Lidhar Navdeep	**PS2.0026**
Li Dongdong	**BOP1M.002, PS1.0080**
Li Dongfeng	**PS2.001**

Lieblich Stephanie E.	**PS1.00151, PS1.0047**
Lié Oriane	**PS2.0098**
Li Geng-Lin	**PS2.0049**
Lightman Stafford L.	**BOP1S.004, BOP2R.001, BOP2S.002, PS1.00160, PS1.00167, PS1.0071, PS2.00186**
Li Grace	**PS2.0062**
Li Grace R.	**BOP2B.002**
Limebeer Cheryl L.	**CS1B.001**
Li Ming	**PS1.0043**
Linden Hirschberg Angelica	**PS1.00116**
Lindo Ashley N.	**PS1.00125**
Lin Liping	**PS1.0050**
Linning-Duffy Katrina	**PS1.0048**
Lin Xian-Hua	**BOP2R.001, PS1.00133**
Liposits Zsolt	**PS1.003**
Lisci Carlo	**PS1.0084**
Li S Y.	**PS1.00160**
Li Tong	**BOP1S.003**
Little Joel T.	**BOP2S.001**
Liu Robert C.	**BOP2B.004**
Liu Yan	**PS1.00189, PS1.0031, PS2.0053**
Liu Yudan	**PS1.00110**
Liu Zhan	**PS1.00188**
Li Wing	**PS1.00146**
Li Xiao F.	**BOP2R.001, PS1.00133**
Li Xiaofeng	**PS1.00160**
Li Xiaonan	**PS1.0043**
Li Yanyu	**PS1.00167**
Locke Marius	**PS2.00101**
Lockstone Helen	**CS1S.003**
Loehfelm Aline	**PS1.0073**
Loganathan Neruja	**BOP2M.006, PS1.00100, PS1.0062, PS1.0075, PS2.00114**
Lomet Didier	**PS1.00124**
Lomniczi Alejandro	**CS1R.002, PS2.00104, PS2.00131**
Long Hong	**PS1.0094**
Long Michael	**PS1.0040**
Lonstein Joseph	**PS1.0048, PS2.00136, PS2.00138**
Lopes-Aguiar Cleiton	**PS1.00117**
Lopez Justin A.	**PS1.00125, PS1.00127, PS1.00142**
Lopez Perez Miguel	**PS1.00134, PS1.00140**
Lopez Rodriguez David	**BOP2B.003**
Lopez Sarah A.	**PS2.0050**
Louth Emma L.	**BOP2S.003**
Lovejoy David	**PS2.00101, PS2.00103**
Low Peter	**PS2.00126**
Lowry Christopher A.	**PS2.0056**

Merkle Florian	**PS2.00119**
Mernone Laura	**PS2.00162**
Messina Andrea	**CS4R.001, PS1.00121, PS1.0055**
Micevych Paul E.	**CS3R.003, PS2.00146**
Mifsud Karen R.	**CS1S.003, PS2.00151, PS2.00152, PS2.00159**
Mikulecka Anna	**PS2.00171**
Milanski Marciane	**CS1M.004, PS1.0062**
Milesi Sebastien	**PS1.00122**
Millar Robert	**BOP2R.004, PS1.00144, PS1.00146**
Millet Marion	**PS1.0079**
Milligan Graeme	**BOP1B.004**
Minami Shiro	**PS1.0042**
Minielly Nicole C.	**PS2.0057**
Minnie Vanessa	**PS1.00186**
Mintz Eric M.	**CS2B.002**
Miranda Dos Santos Filipa	**PS1.00154**
Mir Franco R.	**PS2.00176**
Misztal Tomasz	**PS2.00175**
Mitchell Jane	**PS2.0089**
Miyamoto Josiane É.	**PS1.0062**
Mizrahi Naama	**BOP2R.003**
Mlotkowska Patrycja	**PS2.00175**
M Navarro Víctor	**PS2.00129**
Mo Chunheng	**PS2.00188**
Modi Morni A.	**PS2.0043**
Moeller Jacob	**PS2.00137**
Moenter Suzanne M.	**BOP1R.004, PS1.00118, PS1.00128, PS1.00129, PS2.00108**
Mohr Margaret A.	**AW6.001**
Moisan Marie-Pierre	**PS1.00197**
Moisiadis Vasilis G.	**PS2.00177**
Mondino Alejandra	**PS2.0017**
Monks D. A.	**PS1.0037, PS2.0058**
Moog Sophie	**PS2.00168**
Moon Hyewon	**PS2.00195**
Moore Aleisha M.	**BOP1R.001**
Mora-Bolaños Alfonso	**PS2.0024**
Morel Chloe	**PS1.0080**
Moreno Amielle	**BOP2B.004**
Morgan Peter J.	**AW3.001, PS1.0056**
Morishita Makoto	**PS1.00175**
Morishita Masahiro	**PS1.00120, PS2.0052**
Morita Hironobu	**PS2.00173**
Mori Takuma	**PS2.0076**
Morrell Joan I.	**YA3.003**
Mortessagne Pierre	**PS1.00197**

Mosser Coralie-Anne	**PS1.0069**
Moss Stephen J.	**PS1.0017**
Motojima Yasuhito	**PS1.0023, PS2.00173, PS2.0094, PS2.0096**
Motomura Ai	**PS2.00182**
Movassat Jamileh	**BOP2B.001**
Moyse Emmanuel	**PS2.0098**
Muglia Lisa	**PS1.00192**
Muglia Louis J.	**PS1.00192**
Mukherjee Jayanta	**PS1.0017**
Mukherjee Sayani	**PS1.0093**
Mukhtar Nasir	**PS2.0075**
Mumby Dave G.	**PS1.0011**
Munley Kathleen M.	**PS1.0025**
Murata Kazuyoshi	**PS2.0034**
Murgatroyd Christopher	**PS1.00187, PS2.0042**
Murphy David	**PS1.0071**
Murphy E A.	**PS1.0019**
Murphy Kevin	**PS1.0070, PS1.0087**
Murphy Michelle	**PS2.0010**
Murray Joanne	**PS2.0090**
Murugan Sengottuvelan	**PS1.0093**
Muylle Katoo	**PS1.0016**
Myers Martin G.	**BOP1R.004**
Myers Martin Jr. G.	**PS1.00126**
Mykytyn Kirk	**CS4R.002**
Nabi Ghulam	**PS1.00141**
Nahon Jean-Louis	**PS1.0069, PS1.0074**
Nakamura Kazuaki	**PS1.0010**
Nakamura Yuko	**BOP2M.001**
Nakashima Shizuka	**PS2.0066**
Nakazato Masamitsu	**PL08.001**
Naninck Eva F.G.	**PS1.00172**
Nanou Sophia	**PS1.0082**
Narayanaswamy Shakunthala	**PS1.00103**
Narkaj Klotilda	**PS1.0037**
Nasanbuyan Naranbat	**CS3S.002**
Naule Lydie	**PS2.00119**
Nedelec Emmanuelle	**PS1.0081**
Negm Ahmed	**PS1.0074**
Nelson Randy J.	**CS2B.003**
Nemeth Alexandra	**PS2.0045**
Nentwig Todd B.	**PS1.0036**
Nephew Ben	**PS1.00187, PS2.0042, PS2.0045**
Nesan Dinu	**PS1.0014**
Nestler Eric J.	**BOP2S.006, PS1.00159**
Newton Claire L.	**BOP2R.004, PS1.00144**

Nguyen-Tu Marie-Sophie	**PS1.00106**
Nicholson Jeremy K.	**PS1.00103**
Nikonova Larissa	**PS1.00108**
Nishijima Tsuguo	**PS2.0086**
Nishimura Haruki	**PS1.0023, PS2.00173, PS2.0094**
Nishimura Kazuaki	**PS1.0023, PS2.00173, PS2.0094**
Noël Agnès	**BOP2B.003**
Noel Jacques	**PS1.0074**
Nogueira Javier	**PS2.0013**
Northcutt Katie	**PS1.0018**
Norton Mariana	**PS1.00106, PS1.0087**
Norvelle Alisa	**PS1.00163, PS1.0029**
Nugent Bridget	**CS1M.003**
O'Byrne Kevin T.	**BOP2R.001, PS1.00133, PS1.00160**
O'Driscoll Maeve	**PS1.0070**
Ogawa Sonoko	**PS2.0052, PS2.0066**
O'Hara Laura	**PS2.00111**
Ohashi Yoshiaki	**PS1.00112, PS1.00193**
Ohba Koji	**PS2.0086**
Oh Joo Heon	**PS1.00104**
Oh Paul I.	**PS2.0089**
Oh Yoon-Mi	**PS2.0076**
Ojeda Sergio	**CS4R.004**
Okabe Shota	**CS3S.002**
Okada Yosuke	**PS2.0094**
Okobi Daniel	**PS1.0040**
Olah Szilvia	**PS2.00126**
Olarte-Sánchez Cristian	**PS1.0056, PS2.002, PS2.0085**
O'Laughlin Kylie	**PS1.0046**
Oldfield Brian J.	**PS2.00102**
Oleari Roberto	**CS2R.002**
Olofsson Louise	**CS4M.002**
Olsen Rosanna	**PS2.0025**
Olusanya Adedunni W.	**PS2.00163, PS2.00164**
Onaka Tatsushi	**CS3S.002, PS1.00165**
Ongaro Luisina	**BOP2R.006**
Onieva Rocio	**BOP1R.003**
Ophir Alexander G.	**BOP2B.005, PS1.0030, PS2.007**
O'Rahilly Stephen	**PS1.00114**
Orduña Vladimir	**PS2.002**
Orikasa Chitose	**PS1.0042**
Ortiz Triana L.	**PS1.0024, PS2.003, PS2.0033**
Orts Del Immagine Adeline	**PS1.0032**
Ory Stéphane	**PS1.0079, PS2.00168**
Osei-Hyiaman Douglas	**PS1.00112, PS1.00193**
Oster Henrik	**CS2T.002**

Ouassat Mohamed	**PS2.00125**
Pacha Jiri	**PS2.00170, PS2.00171**
Pais Ramona	**PS1.0092**
Paiva Pessoa Maria R.	**BOP1B.004**
Paletta Pietro	**PS1.0015**
Pál József	**PS1.00157**
Palmert Mark	**PS1.00105**
Palmiter Richard	**PL02.001**
Panchenko Polina	**CS1S.003**
Panda Satchidananda	**CS2T.003**
Pandolfi Erica C.	**PL05.001**
Papadakis Georgios	**PS1.00121, PS1.0055**
Papadopoulou Deborah	**PS1.00103**
Papazoglou Ioannis	**PS1.0063**
Paramithiotis Eustache	**PS2.00168**
Paredes Guerrero Raul	**PS2.0016**
Parent Anne-Simone	**BOP2B.003**
Parker Linda A.	**CS1B.001**
Park Jin Ho	**PS1.0045**
Park Se Hee	**PS1.00104**
Parra Ruby A.	**PS1.00149, PS2.00140**
Partrick Katherine A.	**PS1.00163**
Patel Nisha	**BOP2M.002**
Patel Raj	**PS2.0011**
Patel Smita	**PS2.0061**
Paterson Andrew	**PS1.0096**
Paul Matthew J.	**PS1.0050, PS2.0047**
Pauluschke-Fröhlich Jan	**PS2.0087**
Pawelczyk Caroline	**PS1.0011**
Pearce Jake T..M.	**PS1.00103**
Pedersen Alyssa	**PS1.00184**
Pedroni Silvia M..A.	**BOP1B.004**
Peever John	**PS2.004**
Pena Catherine J.	**BOP2S.006**
Peng Yu-Ping	**PS1.00188, PS1.00189, PS1.0044**
Penney Jenna	**PS1.00190**
Peragine Diana	**PS2.00135**
Perdices-Lopez Cecilia	**BOP1R.003**
Pereira Mariana	**PS2.00142**
Perelmuter Jonathan	**BOP1B.002**
Perez Elizabeth	**PS1.0047**
Perez Jonathan H.	**PS1.00179**
Peronace Vanessa	**PS2.0061**
Peter M C Subhash	**BOP2S.005**
Petersen Asa	**PS2.0012, PS2.0019, YA3.004**
Peters Nicole V.	**PS2.0067**

Merkle Florian	**PS2.00119**
Mernone Laura	**PS2.00162**
Messina Andrea	**CS4R.001, PS1.00121, PS1.0055**
Micevych Paul E.	**CS3R.003, PS2.00146**
Mifsud Karen R.	**CS1S.003, PS2.00151, PS2.00152, PS2.00159**
Mikulecka Anna	**PS2.00171**
Milanski Marciane	**CS1M.004, PS1.0062**
Milesi Sebastien	**PS1.00122**
Millar Robert	**BOP2R.004, PS1.00144, PS1.00146**
Millet Marion	**PS1.0079**
Milligan Graeme	**BOP1B.004**
Minami Shiro	**PS1.0042**
Minielly Nicole C.	**PS2.0057**
Minnie Vanessa	**PS1.00186**
Mintz Eric M.	**CS2B.002**
Miranda Dos Santos Filipa	**PS1.00154**
Mir Franco R.	**PS2.00176**
Misztal Tomasz	**PS2.00175**
Mitchell Jane	**PS2.0089**
Miyamoto Josiane É.	**PS1.0062**
Mizrahi Naama	**BOP2R.003**
Mlotkowska Patrycja	**PS2.00175**
M Navarro Víctor	**PS2.00129**
Mo Chunheng	**PS2.00188**
Modi Morni A.	**PS2.0043**
Moeller Jacob	**PS2.00137**
Moenter Suzanne M.	**BOP1R.004, PS1.00118, PS1.00128, PS1.00129, PS2.00108**
Mohr Margaret A.	**AW6.001**
Moisan Marie-Pierre	**PS1.00197**
Moisiadis Vasilis G.	**PS2.00177**
Mondino Alejandra	**PS2.0017**
Monks D. A.	**PS1.0037, PS2.0058**
Moog Sophie	**PS2.00168**
Moon Hyewon	**PS2.00195**
Moore Aleisha M.	**BOP1R.001**
Mora-Bolaños Alfonso	**PS2.0024**
Morel Chloe	**PS1.0080**
Moreno Amielle	**BOP2B.004**
Morgan Peter J.	**AW3.001, PS1.0056**
Morishita Makoto	**PS1.00175**
Morishita Masahiro	**PS1.00120, PS2.0052**
Morita Hironobu	**PS2.00173**
Mori Takuma	**PS2.0076**
Morrell Joan I.	**YA3.003**
Mortessagne Pierre	**PS1.00197**

Mosser Coralie-Anne	**PS1.0069**
Moss Stephen J.	**PS1.0017**
Motojima Yasuhito	**PS1.0023, PS2.00173, PS2.0094, PS2.0096**
Motomura Ai	**PS2.00182**
Movassat Jamileh	**BOP2B.001**
Moyse Emmanuel	**PS2.0098**
Muglia Lisa	**PS1.00192**
Muglia Louis J.	**PS1.00192**
Mukherjee Jayanta	**PS1.0017**
Mukherjee Sayani	**PS1.0093**
Mukhtar Nasir	**PS2.0075**
Mumby Dave G.	**PS1.0011**
Munley Kathleen M.	**PS1.0025**
Murata Kazuyoshi	**PS2.0034**
Murgatroyd Christopher	**PS1.00187, PS2.0042**
Murphy David	**PS1.0071**
Murphy E A.	**PS1.0019**
Murphy Kevin	**PS1.0070, PS1.0087**
Murphy Michelle	**PS2.0010**
Murray Joanne	**PS2.0090**
Murugan Sengottuvelan	**PS1.0093**
Muylle Katoo	**PS1.0016**
Myers Martin G.	**BOP1R.004**
Myers Martin Jr. G.	**PS1.00126**
Mykytyn Kirk	**CS4R.002**
Nabi Ghulam	**PS1.00141**
Nahon Jean-Louis	**PS1.0069, PS1.0074**
Nakamura Kazuaki	**PS1.0010**
Nakamura Yuko	**BOP2M.001**
Nakashima Shizuka	**PS2.0066**
Nakazato Masamitsu	**PL08.001**
Naninck Eva F.G.	**PS1.00172**
Nanou Sophia	**PS1.0082**
Narayanaswamy Shakunthala	**PS1.00103**
Narkaj Klotilda	**PS1.0037**
Nasanbuyan Naranbat	**CS3S.002**
Naule Lydie	**PS2.00119**
Nedelec Emmanuelle	**PS1.0081**
Negm Ahmed	**PS1.0074**
Nelson Randy J.	**CS2B.003**
Nemeth Alexandra	**PS2.0045**
Nentwig Todd B.	**PS1.0036**
Nephew Ben	**PS1.00187, PS2.0042, PS2.0045**
Nesan Dinu	**PS1.0014**
Nestler Eric J.	**BOP2S.006, PS1.00159**
Newton Claire L.	**BOP2R.004, PS1.00144**

Nguyen-Tu Marie-Sophie	**PS1.00106**
Nicholson Jeremy K.	**PS1.00103**
Nikonova Larissa	**PS1.00108**
Nishijima Tsuguo	**PS2.0086**
Nishimura Haruki	**PS1.0023, PS2.00173, PS2.0094**
Nishimura Kazuaki	**PS1.0023, PS2.00173, PS2.0094**
Noël Agnès	**BOP2B.003**
Noel Jacques	**PS1.0074**
Nogueira Javier	**PS2.0013**
Northcutt Katie	**PS1.0018**
Norton Mariana	**PS1.00106, PS1.0087**
Norvelle Alisa	**PS1.00163, PS1.0029**
Nugent Bridget	**CS1M.003**
O'Byrne Kevin T.	**BOP2R.001, PS1.00133, PS1.00160**
O'Driscoll Maeve	**PS1.0070**
Ogawa Sonoko	**PS2.0052, PS2.0066**
O'Hara Laura	**PS2.00111**
Ohashi Yoshiaki	**PS1.00112, PS1.00193**
Ohba Koji	**PS2.0086**
Oh Joo Heon	**PS1.00104**
Oh Paul I.	**PS2.0089**
Oh Yoon-Mi	**PS2.0076**
Ojeda Sergio	**CS4R.004**
Okabe Shota	**CS3S.002**
Okada Yosuke	**PS2.0094**
Okobi Daniel	**PS1.0040**
Olah Szilvia	**PS2.00126**
Olarte-Sánchez Cristian	**PS1.0056, PS2.002, PS2.0085**
O'Laughlin Kylie	**PS1.0046**
Oldfield Brian J.	**PS2.00102**
Oleari Roberto	**CS2R.002**
Olofsson Louise	**CS4M.002**
Olsen Rosanna	**PS2.0025**
Olusanya Adedunni W.	**PS2.00163, PS2.00164**
Onaka Tatsushi	**CS3S.002, PS1.00165**
Ongaro Luisina	**BOP2R.006**
Onieva Rocio	**BOP1R.003**
Ophir Alexander G.	**BOP2B.005, PS1.0030, PS2.007**
O'Rahilly Stephen	**PS1.00114**
Orduña Vladimir	**PS2.002**
Orikasa Chitose	**PS1.0042**
Ortiz Triana L.	**PS1.0024, PS2.003, PS2.0033**
Orts Del Immagine Adeline	**PS1.0032**
Ory Stéphane	**PS1.0079, PS2.00168**
Osei-Hyiaman Douglas	**PS1.00112, PS1.00193**
Oster Henrik	**CS2T.002**

Ouassat Mohamed	**PS2.00125**
Pacha Jiri	**PS2.00170, PS2.00171**
Pais Ramona	**PS1.0092**
Paiva Pessoa Maria R.	**BOP1B.004**
Paletta Pietro	**PS1.0015**
Pál József	**PS1.00157**
Palmert Mark	**PS1.00105**
Palmiter Richard	**PL02.001**
Panchenko Polina	**CS1S.003**
Panda Satchidananda	**CS2T.003**
Pandolfi Erica C.	**PL05.001**
Papadakis Georgios	**PS1.00121, PS1.0055**
Papadopoulou Deborah	**PS1.00103**
Papazoglou Ioannis	**PS1.0063**
Paramithiotis Eustache	**PS2.00168**
Paredes Guerrero Raul	**PS2.0016**
Parent Anne-Simone	**BOP2B.003**
Parker Linda A.	**CS1B.001**
Park Jin Ho	**PS1.0045**
Park Se Hee	**PS1.00104**
Parra Ruby A.	**PS1.00149, PS2.00140**
Partrick Katherine A.	**PS1.00163**
Patel Nisha	**BOP2M.002**
Patel Raj	**PS2.0011**
Patel Smita	**PS2.0061**
Paterson Andrew	**PS1.0096**
Paul Matthew J.	**PS1.0050, PS2.0047**
Pauluschke-Fröhlich Jan	**PS2.0087**
Pawelczyk Caroline	**PS1.0011**
Pearce Jake T..M.	**PS1.00103**
Pedersen Alyssa	**PS1.00184**
Pedroni Silvia M..A.	**BOP1B.004**
Peever John	**PS2.004**
Pena Catherine J.	**BOP2S.006**
Peng Yu-Ping	**PS1.00188, PS1.00189, PS1.0044**
Penney Jenna	**PS1.00190**
Peragine Diana	**PS2.00135**
Perdices-Lopez Cecilia	**BOP1R.003**
Pereira Mariana	**PS2.00142**
Perelmuter Jonathan	**BOP1B.002**
Perez Elizabeth	**PS1.0047**
Perez Jonathan H.	**PS1.00179**
Peronace Vanessa	**PS2.0061**
Peter M C Subhash	**BOP2S.005**
Petersen Asa	**PS2.0012, PS2.0019, YA3.004**
Peters Nicole V.	**PS2.0067**

Peter Valsa S.	**BOP2S.005**
Petrulis Aras	**PS2.0046, PS2.0067**
Pham Cuong	**PS1.0080**
Phelps Steven M.	**BOP1B.003, PS1.0040, PS2.0044**
Phillipps Holly R.	**PS1.00132**
Phillips Allyssa	**PS2.0062**
Phillips Thomas J.	**PS2.00167**
Phumsatitpong Chayarndorn	**PS1.00128**
Phung Thanh	**PS2.0058**
Picot Marie	**BOP2M.005**
Piemonti Lorenzo	**PS1.00103**
Pierre Anouk	**PS1.0016**
Piet Richard	**BOP2R.005, PS2.00118**
Pigny Pascal	**PS1.00121**
Pilot Michel	**PS1.00160**
Pineda Rafael	**PS1.00140**
Pinilla Jurado Leonor	**BOP1R.003, PS1.00134, PS1.00140**
Pintwala Sara K.	**PS2.004**
Piro Mohammed	**PS2.00125**
Pitteloud Nelly	**PS1.00121, PS1.0055**
Pittet Florent	**PS2.0042**
Placzek Marysia	**CS1T.003**
Plagnol Vincent	**BOP2M.002**
Plaisier Fabrice	**PS1.00194, PS2.0081**
Plate Mathilda	**PS2.00122**
Platt Georgia	**PS1.0031**
Pnueli Lilach	**PS2.00112**
Poher Anne-Laure	**BOP2M.004**
Poletini * Maristela O.	**PS2.00127**
Pollock Tyler	**PS2.00132**
Pooley Apryl E.	**PS2.00183**
Porteous Robert W.	**PS1.00136**
Porter Danielle T.	**PS1.00135, PS1.00142**
Portha Bernard	**BOP2B.001**
Portillo Wendy	**PS2.0016**
Portovedo Mariana	**PS1.0062**
Poutanen Matti	**BOP1R.003**
Prada Patricia O.	**PS1.0099**
Prager-Khoutorsky Masha	**AW4.001, PS1.0090, PS1.0091, PS2.00160**
Prague Julia K.	**PS1.00103**
Preissl Hubert	**PS1.0068, PS2.0087**
Prendergast Andrew	**PS1.0032, PS1.0035**
Prescott Mel	**PS1.00143, PS1.00152, PS2.00120, PS2.0018**
Prévost Gaëtan	**BOP2M.005**
Prevot Vincent	**BOP1R.003, PL09.001, PS1.00111, PS1.0055, PS1.0064, PS1.0079**

Price Emily M.	**CS1S.003, PS2.00152**
Procyshyn Tanya L.	**PS1.0024, PS2.003**
Proietti Valentina M.	**PS2.0033**
Prounis George	**PS1.0030**
Pruessner Jens C.	**PS1.00191**
Pull Kassandra	**PS2.00182**
Qiu Lily	**PS1.00105**
Qiu Liyao	**PS1.001**
Qiu Wansu	**CS1B.003, PS1.0047, PS2.00109, PS2.0057**
Qiu Yi-Hua	**PS1.00188, PS1.00189**
Qi Xiaojuan	**PS1.00116**
Quan Feng	**PS1.0032**
Quan Feng B.	**PS1.0035**
Quaresma Paula G.F.	**PS1.0099**
Quarta Carmelo	**PS1.00102**
Quignon Clarisse	**BOP1R.002**
Radovick Sally	**CS4R.003**
Ragan Christina	**PS1.0052**
Ramakrishnan Selvakumar	**PS1.008**
Rame Marion	**PS2.00168**
Rame Nagib	**PS2.0030**
Ramgulam Anya	**PS1.0070**
Ramzan Firyal	**PS1.0037, PS2.0058**
Rance Naomi	**CS1R.003, PS1.00154**
Randall Andrew	**PS1.00196**
Rand Christy	**PS1.00132**
Rane Sushil G.	**PS1.0063**
Rao Alexandra	**BOP2M.003, PS1.0061**
Ratnasabapathy Risheka	**PS1.00103, PS1.00106**
Ratto Marcelo H.	**CS4R.004**
Rausch Rick	**PS1.00102**
Raval Pooja	**PS1.0017, PS1.0022**
Reed Felicia	**PS1.0067**
Regan Daniel	**PS2.009**
Reginato Andressa	**PS1.0062**
Regin Yannick	**PS1.0016**
Regula Brea	**PS1.0052**
Reichenbach Alexander	**PS2.0080**
Reid Angus M..A.	**PS1.00179, PS2.0075, PS2.0079**
Reid Ross	**PS2.00101**
Reilly Michael	**PS1.0054**
Reimann Frank	**PS1.0092**
Reiter Russel J.	**PL06.001**
Remage-Healey Luke	**PS2.0049, PS2.0064**
Remington Gary	**PS1.0098**
Renaud Justine	**PS2.0083**

Ren Clarissa	**PS1.0025**
Renner Eva	**PS2.00126**
Reppucci Christina J.	**PS1.004**
Reuben Rebekah	**BOP2B.006, PS2.0025**
Reul Johannes M.H.M.	**CS1S.003, PS2.00151, PS2.00152, PS2.00159**
Rhinehart Erin	**PS1.0036**
Richardson Errol	**PS1.00106**
Rievaj Juraj	**PS1.0092**
Rigney Nicole	**PS2.0046, PS2.0067**
Rimmington Debra	**PS1.00114**
Rincel Marion	**BOP2B.001**
Rivarola Angélica	**PS1.0034**
Rizwan Mohammed Z.	**PS2.0088**
Roa Rivas Juan	**BOP1R.003, PS1.00134, PS1.00140**
Roberge Chelsea L.	**PS2.0060**
Roberts Charlie	**PS2.0023**
Roberts Victoria	**PS2.0023**
Robert Vincent	**PS1.00124**
Robinson Brittney A.	**PS2.0033**
Robinson Jane E.	**PS1.0021, PS2.00169, PS2.00192**
Rodriguez Cortes Beatriz	**PS1.0065**
Rodriguez Rodriguez Adair Jonathan	**PS1.0089**
Roepke Troy A.	**PS2.00105, PS2.009**
Rogers Mark	**PS1.0071**
Romaní-Pérez Marina	**BOP2B.001**
Romano Nicola	**PS2.0090**
Rosas Daniela	**PS2.0020**
Rosenhauer Anna M.	**PS1.00163**
Rosinger Zachary J.	**PS1.00185**
Rosin Jessica M.	**BOP1M.004**
Ross Amy P.	**PS1.0029**
Roszkowicz-Ostrowska Katarzyna	**PS2.00175**
Roth Lise	**CS2R.002**
Rovere Carole	**PS1.0069, PS1.0074, PS1.0078**
Roy Anna	**PS1.00105**
Rubinow David R.	**PS2.00134**
Rudnizky Sergei	**PS2.00112**
Rudzinskas Sarah A.	**PS2.00134**
Rugerio Sandra L.	**PS2.0030**
Ruigrok Silvie R.	**PS1.00172**
Ruiz-Gayo Mariano	**PS1.00197**
Ruiz Pino Francisco	**BOP1R.003, PS1.00134**
Ruiz-Pino Francisco	**PS1.00140**
Ruiz-Rodriguez Jose Manuel	**BOP1R.003**
Russell Ashley L.	**PS1.00173**

Russ Jacalyn	**PS2.00150**
Rutter Guy A.	**PS1.00103, PS1.00106**
Ryan Erin	**PS1.009**
Ryu Soojin	**CS3S.003**
Sabatier Nancy	**PS1.003**
Sacchi Federico	**PS2.00144**
Saenz De Miera Cristina	**PS2.00196**
Saffar Malak	**PS2.0060**
Sagoshi Shoko	**PS2.0052**
Sailer Lindsay	**PS1.0049**
Saito Reiko	**PS1.0023, PS2.00173, PS2.0094, PS2.0096**
Sakai Takafumi	**PS1.0066**
Sakamoto Hirotaka	**PS1.00175, PS2.0034, PS2.0052**
Sakamoto Tatsuya	**PS1.00175, PS2.0034, PS2.0052**
Sakata Ichiro	**PS1.0066**
Sakurai Shigeru	**PS2.0086**
Salatino Silvia	**CS1S.003**
Saldanha Colin	**PS1.00184**
Salehi Ashkan	**PS1.00100, PS1.0076**
Samad Faiez	**PS1.0048**
Sanchez-Tapia Maria Jesús	**BOP1R.003**
Sandal Suleyman	**PS1.00115**
Sandoval Darleen	**CS4M.003**
Sands Caroline	**PS1.00103**
Santos Hudson	**PS1.00187**
Sardini Alessandro	**BOP1B.004**
Sarkar Dipak K.	**PS1.0093**
Sato Shigemitsu	**PS2.0086**
Saulsbery Angela I.	**PS2.0068**
Sawyer India L.	**BOP2S.004**
Scagliotti Valeria	**CS2R.002**
Scarpa Garrett B.	**PS2.0049**
Schang Gauthier	**BOP2R.006**
Schatz Kelcie C.	**PS1.0050**
Scheer Frank A.J.L.	**CS2T.004**
Scheffler Klaus	**PS1.0068**
Scheiman Jessie	**PS1.00192**
Schéle Erik	**PS1.003**
Schiavo Jennifer K.	**PS1.005**
Schleger Franziska	**PS2.0087**
Schmidt Kim L.	**PS1.0019**
Schmidt Peter J.	**PS2.00134**
Schoenberg Maia E.	**PS2.0043**
Schopf Kerri	**PS1.00169, PS1.00181**
Schramm Milena	**PS1.00164**
Schulz Kalynn M.	**PS2.00184**

Schwarz Jaclyn M.	**PS1.0028**
Schwenke Daryl O.	**PS2.0074**
Schwulst Alexis	**PS1.00144**
Scott Hannah	**PS2.00167**
Sefton Charlotte	**PS1.0057, PS1.0058**
Sellers Katherine J.	**PS1.0017**
Seminara Stephanie	**PS1.00162**
Serebin Molly	**CS3R.001**
Seymour Alexander J.	**PS1.00153**
Shahab Muhammad	**PS1.00141**
Shah Asad H.	**PS2.0059**
Shah Nadim J.	**PS1.0068**
Shah Nirao	**CS3R.004**
Sha Lei	**PS1.00110**
Shamas Shazia	**PS1.00141**
Shams Waqqas	**PS1.0011**
Shanas Uri	**PS2.00110**
Sharma Eshita	**CS1S.003**
Shaughnessy Emma K.	**BOP1S.002**
Shaver Madeleine	**PS1.00183**
Shcherbina Liliya	**PS2.0084**
Shenasa Mohammad Ali	**BOP2M.001**
Shepherd Peter	**PS2.0088**
Sheppard Paul A.S.	**PS1.006**
Shetty Kirti	**BOP2R.001, PS1.00133**
Shimizu Kie	**PS1.0010**
Shipston Mike	**CS3S.004, PS2.00161**
Shoop Rosemary	**PS1.0057**
Shota Takemi	**PS1.0066**
Shruti Sonal	**PS1.0055, PS1.0064**
Shum Andrew	**PS1.0022**
Si Jie	**PS1.00110**
Silva Juneo F.	**PS1.00117**
Silva Mauro	**PS1.00152, PS2.00120**
Silva Tarcilia A.	**BOP2R.002**
Simola Nicola	**PS2.0083**
Simonneaux Valérie	**BOP1R.002, PS1.00122, PS2.00123, PS2.00125, PS2.00196**
Simon Tatiana	**PS2.00191**
Simpson Victoria	**PS2.0036**
Simundic Amanda	**PS1.0011**
Singh Aditi	**PS1.0040**
Sing Kristen	**PS1.00146**
Sisneros Joseph A.	**BOP1B.002**
Sjögren Marie	**PS2.0084**
Skrapits Katalin	**PS1.0055**

Small Dana M.	**BOP2M.001**
Smiley Kristina O.	**AW6.002, PS1.008**
Smith Brandon	**PS1.00190**
Smith Caroline	**PS2.0090**
Smith Jeremy T.	**PS2.0011**
Smith Kevin	**PS1.00183**
Smith Lee B.	**PS2.00111**
Smith Mark A.	**BOP1B.004**
Smith Spencer	**PS1.0018**
Smith William L.	**PS2.0039**
Smit Joshua A.	**PS1.0015**
Smolders Ilse	**PS1.0016, PS1.0053**
Snodgrass J. J.	**PS1.00180**
Snyder Brina	**PS2.0029**
Sockalingam Sanjeev	**PS1.0096**
Sohrabji Farida	**CS1B.004**
Solbakk Anne-Kristin	**PS1.0021, PS2.00169**
Solomon-Lane Tessa	**PS1.0012, PS2.0027**
Soma Kiran K.	**PS1.0019, PS1.0025**
Sominsky Luba	**PS1.00123, PS1.00170**
Song Christopher I.	**PS2.00117**
Sonoda Satomi	**PS1.0023, PS2.00173, PS2.0094, PS2.0096**
Sørensen Jacob H.	**BOP2M.001**
Sotomayor-Zarate Ramon	**PS2.0020**
Soylu-Kucharz Rana	**PS2.0019**
Sparrock Lindsey S.	**PS2.00179, PS2.00180**
Spencer Karen A.	**PS2.00166**
Spencer Sarah J.	**PS1.00123, PS1.00170**
Spencer-Segal Joanna	**AW6.003**
Spiga Francesca	**BOP1S.004, BOP2S.002, PS1.00167**
Spinieli Richard L.	**PS1.00178**
Splinter Jared E.J.	**PS1.0047, PS2.0057**
Spring Shoshana	**PS1.00105**
Squires Erica C.	**PS1.00180**
Srivastava Deepak P.	**PS1.0017, PS1.0022**
Stagkourakis Stefanos	**BOP1B.001**
Stahel Priska	**PS1.0096**
Stanley Sarah	**PS1.0083**
Stanton Jo-Ann	**PS1.00132**
Starrett Joseph R.	**PS2.0049**
Stavreva Diana	**PS1.0071**
Steculorum Sophie M.	**AW2.001**
Stefanelli Gilda	**PS1.0037**
Stefanidis Aneta	**PS2.00102**
Steinman Michael	**PS1.00186**
Stener-Victorin Elisabet	**PS1.00116**

Stephens Shannon B..Z.	**PS1.00149**
Stevenson Tyler J.	**PS2.00130, PS2.00189**
Stobbe Katharina	**PS1.0069, PS1.0074**
Stolzenberg Danielle S.	**PS2.0070**
Storme Laurent	**PS1.0055**
Straub András	**PS1.00157**
Streelman J. T.	**PS2.0048**
Struthers Scott	**CS3TR.004**
Stumpfig Margaret	**PS1.0048**
Suárez Marta M.	**PS1.0034, PS2.00176**
Sugamori Kim S.	**PS2.0089**
Sugiyama Aya	**PS2.00155**
Sukhchuluun Gansukh	**PS1.0095**
Sullivan Elinor	**PS2.0023**
Sun Xue	**BOP2M.001**
Suwabe Akira	**PS2.0086**
Swardfager Walter	**PS2.0089**
Swift-Gallant Ashlyn	**AW1.001**
Szawka Raphael	**PS1.00130**
Szawka Raphael E.	**BOP2R.002, PS1.00117, PS2.00127**
Sze Ying	**PS2.00154, PS2.00167**
Tabak Joel	**PS2.00100**
Tabares Florencia P.	**PS2.00106, PS2.00115**
Tabet Dana	**PS2.0041**
Tadross John	**PS1.00114**
Tafoya Kathryn	**PS2.00124**
Tahir Sophia	**CS2R.002**
Tajima Kazuki	**PS2.0086**
Takahashi Kazuhiro	**PS2.0086**
Takakura Natsumi	**PS1.0066**
Takanami Keiko	**PS1.00175, PS2.0034**
Takayanagi Yuki	**CS3S.002**
Takehara-Nishiuchi Kaori	**PS2.0026**
Talbi Rajae	**PS2.00129**
Tamashiro Kellie L.	**CS1T.004**
Tanaka Kentaro	**PS1.0023, PS2.00173, PS2.0094**
Tanaka Masaki	**PS2.006, PS2.0076**
Tanaka Yoshiya	**PS2.0094**
Tanauli Tariq	**PS2.00174**
Tang Celion	**PS1.00146**
Tang Karena	**PS2.00178**
Tang Yu Ping	**PS1.0048**
Tan Tricia	**CS4M.004, PS1.00103**
Tao Cindy	**PS1.0037**
Tasker Jeffrey	**CS2S.004**
Taylor Christopher	**PS1.00186**

Taylor Tiegh	**PS1.00190**
Taziaux Mélanie	**CS3R.002**
Tchaderjian Megan	**PS1.0011**
Tekin Suat	**PS1.00115**
Tello Javier A.	**PS1.00146**
Templin Jay S.	**PS1.0045**
Tena-Sempere Manuel	**BOP1R.003, PS1.00134, PS1.00140, PS1.0055, PS2.00104**
Terasawa Ei	**PS1.00162**
Terry John	**BOP1S.004**
Thackray Varykina G.	**PS2.00145**
Thaker Vidhu V.	**PS1.00102**
Thakur Ayushi	**PS2.0026**
Thaler Joshua P.	**BOP1M.001, CS2M.003**
Thirouin Zahra S.	**PS2.00165**
Thomas Susan	**PS2.00185**
Thompson Brittany M.	**PS1.00163**
Thompson Jacqeline	**PS2.0023**
Thompson Lindsay	**PS1.0054**
Tian Katherine	**PS2.00117**
Tierney Mary	**BOP2B.006**
Tiessen Angela N.	**CS1B.001, PS2.0022**
Tillet Yves	**PS1.00155**
Tilson Kristy	**PS2.0060**
Tito Noemie	**PS2.0063**
Tobiansky Daniel J.	**PS1.0019**
Tolhurst Gwen	**PS1.0092**
Tolla Elisabetta	**PS2.00189**
Tolle Virginie	**PS1.0078, PS1.0081**
Tollkuhn Jessica	**PS2.0072**
Tolu Stefania	**BOP2M.001**
Tomlinson George	**PS1.0096**
Tomm Ryan J.	**PS1.0019**
Toor Ilapreet	**PS2.0071**
Toro Carlos A.	**CS1R.002, PS2.00104, PS2.00131**
Torres Encarnacion	**PS1.00140**
Torres Lisette Y.	**PS2.0070**
Torsoni Adriana S.	**PS1.0062**
Torsoni Márcio	**PS1.0062**
Torterolo Pablo	**PS2.0017**
Tostivint Hervé	**PS1.0035**
Toufaily Chirine	**PS1.00148**
Towers Matthew	**CS1T.003**
Trainor Brian C.	**PS1.00186**
Tran Amy	**PS1.00186**
Tran Andy	**BOP2M.006**

Treier Mathias	**PS1.00102**
Trower Mike	**CS3TR.005**
Trudeau Vance L.	**PS2.00144**
Trujillo Angélica	**PS2.0024, PS2.0031**
Trujillo Verónica	**PS2.00176**
Tsaneva-Atanasova Krasimira	**PS1.00133**
Tschop Mathias	**PS1.0080**
Tschöp Matthias	**BOP1M.002, BOP2M.001**
Tschöp Matthias H.	**BOP2M.004, CS3TR.006, PS1.00102, PS1.0088**
Tse Erika K.	**PS1.00100**
Tsujimura Atsushi	**PS2.0076**
Tsukahara Shinji	**PS1.00120, PS2.0052, PS2.006, PS2.0066**
Tucker Matthew J.	**BOP2B.004**
Tung Loraine	**PS1.00114**
Tups Alexander	**PS1.0073, PS2.0088**
Turano Alexandra	**PS1.0028**
Turecki Gustavo	**CS1S.004, PS2.00162**
Uchiyama Kei	**PS2.006**
Udenze Ifeoma C.	**PS2.00163, PS2.00164**
Udvari Edina	**PS2.00113**
Ueno Hiromichi	**PS1.0023, PS2.00173, PS2.0094, PS2.0096**
Ueta Yoichi	**PS1.002, PS1.0023, PS2.00173, PS2.0094, PS2.0096**
Uezono Yasuhito	**PS2.0096**
Ugarte Araceli	**PS2.0024, PS2.0031, PS2.0032**
Ullah Hamid	**PS1.00141**
Ulusakarya Ayhan	**PS2.00190**
Umpierrez Eleuterio	**PS2.0017**
Underwood Emily A.	**CS1B.001, PS2.0022**
Unger Kristen	**PS2.0068**
Ungless Mark A.	**BOP1B.004**
Upton Thomas	**PS2.00186**
Urbanski Henryk F.	**PS2.00133**
Uriarte Natalia	**PS2.0013**
Uribe Rosa	**PS1.0089**
Valencia Torres Lourdes	**PS2.002**
Valera-Marin Guillermo	**PS2.0016**
Vanacker Charlotte H.	**PS2.00108**
Van Den Bout Iman	**PS1.00144**
Van Faasen Martijn	**PS2.00186**
Varela Luis	**BOP2M.004**
Vavrinova Anna	**PS2.00170, PS2.00171**
Vazquez Maria J.	**BOP1R.003, PS1.00134, PS1.00140, PS2.00104**
Veenema Alexa H.	**PS1.004, PS1.005**
Velasco Inmaculada	**BOP1R.003, PS1.00140**
Vellone Daniella A.	**PS1.006**
Verlezza Silvanna	**PS1.0094**

Vertes Alex C.	**PS2.0022**
Viltart Odile	**PS1.0078, PS1.0081**
Virtue Sam	**PS1.00114**
Viskaitis Paulius	**BOP1B.004**
Vitale Erika	**PS2.00138**
Vivas Laura M.	**PS2.00176**
Vodicka Martin	**PS2.00170, PS2.00171**
Vogel Heike	**PS1.003**
Voliotis Margaritis	**PS1.00133**
Volk Katrina M.	**PS2.00140**
Vortman Yoni	**PS2.00110**
Wada Reiko	**PS1.0066**
Wagner Christine K.	**PS2.0062**
Walker Claire-Dominique	**PS1.0094**
Walker David J.	**PS2.00166**
Walker Deena M.	**BOP2S.006**
Walker Jamie	**BOP1S.004, PS2.00100**
Wallin Chela	**PS2.0060**
Wang Dehua	**PS1.00158, PS1.0095**
Wang Fan	**PS1.001**
Wang Lei	**PS2.0021**
Wang Luhong	**BOP1R.004**
Wang Ping	**BOP1S.003**
Wang Xiao-Dong	**CS4S.004**
Wang Ying	**PS2.00107**
Wang Yirun	**PS1.00110**
Wang Yu-Feng	**BOP1S.003**
Wang Zuoxin	**PS1.0031, PS1.0049, PS2.0053**
Warwick Alexa	**PS2.0027**
Wasserman Michael	**PS2.00124**
Watkins Linda R.	**PS2.0056**
Watson Neil V.	**PS1.0024, PS2.003, PS2.0033**
Weinberg Amy	**PS1.0054**
Weiss Grant	**CS2S.004**
Weiss Magdalene	**PS2.0087**
Welker Keith M.	**PS1.0024**
Wells Tim	**PS2.0014**
Welsh David K.	**PS1.00119**
Weltzien Finn-Arne	**PS2.00128**
Westwick Rebecca R.	**PS2.0044**
Wheeler Michael B.	**PS1.0097**
White Anne	**PS1.0057, PS1.0058**
Whitely Kirstin	**PS2.00179**
Whitten Connner	**PS2.0069**
Whylings Jack	**PS2.0046, PS2.0067**
Wierup Nils	**PS2.0084**

Williams Paul	**BOP1B.001**
Wilson Dana	**PS2.0082**
Wilson Diane E.	**PS1.0036**
Wilson Hayley	**PS1.00113**
Wilson Peter W.	**PS2.0075**
Windy Matthew	**PS2.0038**
Wingfield John C.	**PS1.00179**
Winokur Sarah B.	**PS2.00142**
Winters Boyer	**PS1.00113**
Withers Dominic J.	**BOP1B.004**
Wolfe Andrew	**BOP2S.006, PS1.00109**
Wong Ryan Y.	**PS2.00149, PS2.00150**
Wood Ruth I.	**BOP2B.002, PS1.009, PS2.0062**
Wood Shona H.	**CS4T.003**
Workman Joanna L.	**PS2.0068**
Wray Jonathan R.	**PS1.0057, PS1.0058**
Wright Hollis	**PS2.00131**
Wu Melody V.	**PS2.0072**
Wu Ruiyong	**PS1.0043**
Wu T. John	**PS1.00173**
Wyart Claire	**PS1.0032, PS1.0035**
Wyrosdic Joshua C.	**PS1.0045, PS2.00160**
Wyrwoll Caitlin S.	**CS4S.002**
Wyse Cathy A.	**PS2.00192**
Wyse-Jackson Alice	**BOP1M.001**
Xiao Wei	**PS1.001**
Xing Lei	**PS2.00144**
Xiong Hang	**PS1.0048**
Xu Bo	**PS2.00188**
Xu Cheng	**PS1.00121**
Xu Yanjun	**PS1.00102**
Yagi Hiroko	**PS2.00156**
Yamada Shunji	**PS2.006, PS2.0076**
Yamagata Satoshi	**PS2.00156**
Yamaguchi Shinji	**PS2.005**
Yamaguchi Shohei	**PS2.006, PS2.0066**
Yamamoto Yukiyo	**PS1.0023**
Yamashiro Yoshihiro	**PS2.0086**
Yam Kit Y.	**PS1.00172**
Yang Jennifer A.	**PS1.00149, PS2.00140**
Yang Jia Fang	**PS2.00181**
Yang Lisa	**PS1.00103**
Yan Lily	**CS2B.004, PS1.0048**
Yao Wei	**PS1.00158**
Yarnall Alison	**PS2.0014**
Yasrebi Ali	**PS2.009**

Yeo Giles S.H.	**PS1.00114**
Yeo Shel H.	**BOP2R.001, PS2.00147**
Yi Chun-Xia	**CS2M.004**
Yip Siew H.	**PS1.00130**
Yokosuka Makoto	**PS1.0010**
Yokoyama Sae	**PS1.00186**
Yoo Sooyeon	**PS1.00109**
York Jade	**PS1.00130**
Yoshida Masahide	**CS3S.002**
Yoshii Keizuke	**PS1.00147**
Yoshimura Mitsuhiro	**PS1.002, PS1.0023, PS2.00173, PS2.0094, PS2.0096**
Yoshimura Takashi	**CS4T.004**
Young Larry J.	**CS3B.004, PS2.0016**
Yuan Joseph	**PS2.00172**
Yue Ma	**PS1.00106**
Yu Xiao	**PS1.00107**
Zaelzer-Perez Cristian A.	**PS2.0095**
Zafer Dila	**CS3R.001**
Zanotto Tamires M.	**PS1.0099**
Zavala Eder	**BOP1S.004**
Zeisler Zach	**PS1.0018**
Zeltser Lori	**PS1.00102**
Zhang Bin	**BOP2S.006, PS1.001**
Zhang Lei	**PS1.0067**
Zhang Nan	**PS1.00110**
Zhang Xueying	**PS1.00158, PS1.0095**
Zhang Y	**PS2.00192**
Zhao Zidong	**BOP1S.004, BOP2S.002, PS1.00167**
Zheng Da-Jiang	**PS1.0040**
Zhou Jing-Jing	**PS2.00158**
Zhou Xiang	**PS1.00148**
Zhou Xianxiao	**BOP2S.006**
Zhu Yun Fei	**CS3TR.004**
Zicha Josef	**PS2.00170, PS2.00171**
Zielinska-Gorska Marlena	**PS2.00148**
Ziko Ilvana	**PS1.00170**
Zimmer Cedric	**PS2.00166**
Ziolkowski Justine	**PS2.00135**
Zizzari Philippe	**PS1.0081**
Zoubovsky Sandra	**PS1.00192**
Zovkic Iva B.	**PS1.0037**
Zubair Hira	**PS1.00141**
Zuloaga Damian G.	**PS1.00185**