H1. Shedding light on the role of the brain in polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is a highly prevalent endocrine disorder associated with elevated circulating androgens and anovulation frequently leading to infertility. High frequency luteinizing hormone (LH) secretion and impaired gonadal steroid hormone feedback to the brain are also evident in PCOS patients, suggesting that neuroendocrine dysfunction is likely to be central to PCOS pathology. In this talk I will describe our work investigating whether modifications in the synaptically connected neuronal network of GnRH neurons could account for this pathology. Using a pre-clinical mouse model that reflects the clinical neuroendocrine phenotype of PCOS, we have defined specific anatomical circuit abnormalities associated with the PCOS phenotype. Specifically, we have found evidence for disordered progesterone-sensitive GABAergic input to GnRH neurons, originating specifically within the arcuate nucleus. In this talk I will describe how opto- and chemo-genetic approaches to manipulate these and other arcuate nucleus populations is expanding our current understanding how GnRH/LH pulsatility is regulated and revealing potential PCOS treatment targets.

H2. Hypothalamic Leptin Sensitivity and Health Benefits of Time-Restricted Feeding are Dependent on the Time of Day in Male Mice

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Synchronization between biological clocks and metabolism is crucial for most species. Here, we examined the ability of leptin, important in the control of energy metabolism, to induce leptin signalling at the molecular as well as the behavioral level throughout the 24-hour day in mice fed either a control or a high-fat diet (HFD). Furthermore, we investigated the effects of time-restricted feeding (TRF, a limitation of HFD access to 6 hours each day) on energy metabolism during different periods throughout the 24-hour day. In control mice, molecular leptin sensitivity was highest at Zeitgeber time (ZT) 0 (lights on), declining during the light- and increasing during the dark phase. Surprisingly, leptin resistance in HFD mice was only present from the middle of the dark to the middle of the light period. Specifically when TRF occurred from ZT21-3 (when leptin resistance in HFD mice was most profound), it resulted in a disruption of the daily rhythms of locomotor activity and energy expenditure, and in increased plasma insulin levels compared with other TRF periods. These data provide evidence that leptin sensitivity is controlled by the circadian rhythm and that TRF periods may be most efficient when aligned with the leptin sensitive period.

H3. Intranasal Targeting Hypothalamic PTP1B and TCPTP Reinstates Leptin and Insulin Sensitivity and Promotes Weight Loss in Obesity

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The importance of hypothalamic leptin and insulin resistance in the development and maintenance of obesity remains unclear. The tyrosine phosphatases PTP1B and TCPTP attenuate leptin and insulin signalling and are elevated in the hypothalami of obese mice. We report that elevated PTP1B and TCPTP antagonise hypothalamic leptin and insulin signalling to repress feeding and promote white-adipose-tissue browning and contribute to the maintenance of obesity. Deletion of PTP1B and TCPTP in the hypothalami of obese mice enhanced enhances CNS leptin and insulin sensitivity, repressed represses feeding and increased increases browning, to decrease adiposity and improve glucose metabolism. The daily intranasal administration of a PTP1B inhibitor, plus the glucocorticoid antagonist RU486 that decreased decreases TCPTP expression, represses feeding, increases browning, promotes weight loss and improves glucose metabolism in obese mice.

Our findings causally link heightened hypothalamic PTP1B and TCPTP with the development of leptin and insulin resistance and the maintenance of obesity and define a viable pharmacological approach by which to reinstate hypothalamic leptin and insulin responsiveness to promote weight loss and combat obesity.

H4. Medial prefrontal cortex activity influences body weight loss in activity-based anorexia

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Anorexia nervosa (AN) has the highest mortality rate of any psychiatric disease, yet available treatments are largely ineffective, in part due to a lack of insight into the neurobiological drivers that underpin the condition. Functional neuroimaging in AN patients suggests that hyperactive cognitive neurocircuitry contributes to pathological body weight loss. Using pathway specific chemogenetics, we hypothesized that decreasing activity in neurons of the medial prefrontal cortex (PFC) with direct projections to ventral reward circuits would improve body weight maintenance in the activity-based anorexia (ABA) rat model and impact on cognitive flexibility in touchscreen operant paradigms.

Female Sprague-Dawley rats (n=33; 6 wks old) underwent bilateral stereotaxic injections of retrogradely-transporting Cre (AAV-pmSyn1-EBFP-Cre) into the nucleus accumbens (NAc) and coincident injections of either inhibiting (AAV-hSyn-DIO-hM4D(Gi)-mCherry), activating (AAV-hSyn-DIO-hM3D(Gq)-mCherry) or control (AAV-hSyn-DIO-mCherry) DREADD viruses into the PFC. During exposure to the ABA paradigm, which involves unhindered access to a running wheel and time-limited (90 min) access to food, all rats were administered clozapine-n-oxide (CNO) daily (0.3-3 mg/kg i.p.) at the onset of the dark phase. For assessment of cognitive performance and flexibility in an operant learning paradigm, a separate cohort of animals (n=24; 3-6 months old) underwent injections of DREADDs and CNO administration as described above.

Chemogenetic inhibition of PFC-NAc projection neurons prevented body weight loss during ABA ($\chi 2=8.77$, $p=0.013$) by increasing food anticipatory activity (FAA; $p=0.011$) and subsequent food intake ($F=4.14$, $p=0.025$). Conversely, activation of this pathway exacerbated hyperactivity induced by food restriction ($F=6.06$, $p=0.006$). There were no significant effects of prefrontal modulation on visual discrimination learning or cognitive flexibility.

Our data indicate that prefrontal circuits impact on food intake and running activity in ABA, but this does not correlate with behavioural measures of cognitive flexibility.

H5. Sexual dimorphism in the control of body weight and metabolic function in healthy men and women

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Women typically possess more adipose tissue than men, but they are relatively protected against developing cardiometabolic diseases, including cardiovascular disease and type 2 diabetes. Across the menopausal transition, women tend to gain weight and become susceptible to metabolic and cardiovascular diseases. Such weight gain and loss of cardiometabolic protection is due, at least in part, to declining levels of estrogen. Body weight is determined by energy intake and energy expenditure, wherein total energy expenditure is determined by basal metabolic rate, physical activity and adaptive thermogenesis. Adaptive thermogenesis refers to the dissipation of energy through cellular heat production and occurs in mitochondrial enriched tissues, such as brown adipose tissue (BAT). Numerous animal studies have demonstrated that estrogen acts within the brain to regulate both reproductive and metabolic functions. Regarding the latter, estrogen acts on neurons in the hypothalamus to reduce food intake and to increase energy expenditure. In particular, estrogen acts to increase thermogenesis. Despite this, little is known of how sex steroids modulate thermogenesis in healthy adults. This symposium presentation will explain sexual dimorphism in relation to thermogenesis and will examine the role of sex steroids in the regulation of the same in healthy young men and women. I will highlight a possible role for brown adipose tissue in conferring protection against cardiometabolic disease in women.

H6. Investigating the acute effects of prolactin upon hypothalamic prolactin-receptor expressing neurons

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The anterior pituitary hormone, prolactin, a fundamental regulator of lactation, plays a role in many other physiological processes including maternal behaviour, reproduction, immune response and even energy balance. Indeed, prolactin receptors (Prlr) are widely distributed throughout the brain, further attesting to its pleiotropic nature. Previous research has identified key areas upon which prolactin exerts transcriptional effects through the canonical JAK2/STAT5 pathway downstream of the Prlr. In some neurons such as the tuberoinfundibular dopamine neurons that control prolactin secretion, prolactin can also exert rapid actions to stimulate neuronal activity. In most areas where the Prlr is expressed, however, its acute modulation of electrical properties of Prlr-expressing neurons remains to be elucidated. To identify and probe the function of these Prlr cells, we utilised a transgenic mouse line in which Cre recombinase is specifically expressed in the coding region of the prolactin long form receptor gene (*Prlr*^{Cre}). This mouse line was crossed with a Cre-dependent calcium indicator (GCaMP6s) transgenic mouse, allowing us to visually monitor the electrical activity of Prlr-expressing neurons in *ex vivo* 200μm brain slice preparations. Here we survey hypothalamic regions implicated in prolactin's diverse physiological functions such as: the arcuate nucleus of the hypothalamus (ARC), the medial preoptic area (MPOA), the ventromedial nucleus of the hypothalamus (VMH) and the paraventricular nucleus of the hypothallus (PVN). We observe that in both males and virgin and lactating females, bath application of prolactin is able to induce electrical changes in a subset of Prlr-expressing cells that reside in the above-listed brain regions. The effects detected range from rapid or sustained increases in intracellular calcium to inhibitory effects, hinting at a heterogeneous nature of these Prlr-expressing populations. These results enhance our understanding of the neural circuits influenced by prolactin and provide a potential mechanism of prolactin's actions in the mouse brain.

H7. Ion channel mechanisms underlying sex differences in hypothalamic CRH neuron excitability

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Corticotropin-releasing hormone (CRH) neurons, located in the paraventricular nucleus of the hypothalamus, control stress evoked release of adrenal corticosteroids. Males and females have markedly different responses to acute stress and corticosteroid release profiles. It is currently unclear to what extent this is due to differences in CRH neuron intrinsic excitability.

Neuronal intrinsic excitability is heavily influenced by ion channel expression and function, which can in turn be regulated by estrogen. Specifically, estrogen modifies I_A and I_M channel currents and subunit expressions. I_A is a transient outward potassium (K^+) current driven by the Kv channel family. The M current (I_M) is a subthreshold, non-inactivating, slow delayed rectifier K^+ current (driven by KCNQ channels). Both I_A and I_M currents are important for CRH neuron function and an increase in either current corresponds to a decrease in neuronal excitability.

We hypothesize that different levels of CRH neuron excitability, driven by disparate ion channel function, underlies sexually dimorphic stress responses. Using whole cell *in vitro* electrophysiology we examined CRH neuron intrinsic excitability, I_A and I_M currents.

Examining frequency-current (F-I) curves revealed CRH neuron firing was significantly higher in males compared to estrous and diestrous females ($P=0.0007$ and $P<0.0001$ respectively). Interestingly proestrous females were much similar to males ($P=0.33$) than estrous ($P=0.0002$) or diestrous ($P=0.0029$) females.

H8. Inhibins and activins: From Reproduction to Metabolism

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Although first postulated to exist nearly 100 years ago, inhibins were only isolated in 1985 from bovine and porcine follicular fluid. Inhibin A and inhibin B (heterodimers of a common α- and differing β-subunits) act as part of a negative feedback loop to regulate synthesis of follicle stimulating hormone (FSH) by gonadotrope cells of the anterior pituitary. Inhibins modulate gonadotrope function by preventing activins (homodimers of β-subunits) from binding their receptors and activating SMAD 2/3 transcription factors. In the context of the pituitary, this mode of action ensures that inhibins control activin-induced FSH production. Over the past decade, my group has helped define the mechanisms underlying the synthesis, activation and receptor binding of inhibin and activin. Based on our understanding of these processes, we have generated highly-potent inhibin agonists and specific activin antagonists. My studies have also redefined the physiological roles of these "reproductive" molecules, with increased circulating levels of activin A promoting muscle wasting and cachexia, while loss of inhibin results in a complex metabolic phenotype. Using our detailed structural knowledge, we are now manipulating the inhibins and activins to study loss/gain of function, allowing us to identify new roles for these age old hormones.

H9. The neurobiology of homeostasis

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Our research investigates the neural mechanisms that govern hunger and thirst. Nearly a century ago, lesioning studies suggested that these fundamental drives originate from subcortical stuctures such as hypothalamus that are specialized for monitoring internal state. However the structure and dynamics of the underlying neural circuits has been poorly defined. I will discuss our work using calcium imaging to observe the natural activity of some of the key cell types that control eating and drinking. We have discovered that these homeostatic neurons receive sensory information from the outside world, which they use predict impending physiologic changes and adjust behavior preemptively. I will discuss our work investigating how these homeostatic circuits integrate external sensory cues with internal signals arising from the body in order to generate and shape goal-directed behaviors.

H10. MRAP2: an essential GPCR regulatory protein for the control of energy and glucose homeostasis

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Accessory proteins are a family of single transmembrane proteins that interact with and regulate GPCRs trafficking and signalling. The Melanocortin Receptor Accessory Protein 2 (MRAP2) is on such protein expressed in the central nervous system and the pancreas. Deletion of MRAP2 causes severe obesity, thus suggesting an important role of MRAP2 in the regulation of energy homeostasis. We have shown that MRAP2 interacts with the Melanocortin-4 Receptor (MC4R), the ghrelin receptor (GHSR1a), the prokineticin receptor (PKR1) and the orexin receptor (OXR1), all important GPCRs for controlling food intake, energy expenditure and glucose homeostasis. Whereas the signals downstream of a subset of those receptors (MC4R and GHSR1a) are potentiated by MRAP2, others (PKR1, OXR1) are inhibited. The pharmacology of GHSR1a is drastically altered by MRAP2. Ghrelin is a hormone secreted by the stomach during starvation periods. Whereas in hypothalamic Agouti related Protein (AGRP) expressing neurons, activation of the ghrelin receptor promotes food intake, in the endocrine pancreas ghrelin blocks insulin secretion. Using in-vitro assays we found that MRAP2 almost completely eliminate the high constitutive activity of GHSR1a but very significantly increases its $G\alpha_{q/11}$-dependent response to ghrelin. In fact, in the absence of MRAP2, the ghrelin response is minimal. In addition, MRAP2 strongly inhibits ghrelin-stimulated β-arrestin recruitment and β-arrestin-dependent signalling downstream of GHSR1a. In-vivo, we found that MRAP2 is expressed in AGRP neurons and in δ-cells of the endocrine pancreas. Deletion of MRAP2 results in a loss of both the hyperphagic response and the inhibition of insulin secretion triggered by ghrelin, thus confirming the requirement of MRAP2 for GHSR1a signalling in-vivo. Our studies so far demonstrate that MRAP2 is an important accessory protein for several GPCRs that regulate energy and glucose homeostasis and identify MRAP2 as an endogenous protein with the ability to bias GPCR signalling towards G-protein and away from β-arrestin.

H11. Leptin controls energy partitioning between fat and bone mass via a hypothalamic NPY relay

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Maintaining energy balance is important to ensure a healthy organism. However, energy partitioning, coordinating the distribution of sufficient energy to different organs and tissues is equally important, but far less is known about the control of this process. In obesity, increases in fat mass necessitates the production of additional bone mass to cope with the increase in bodyweight and processes need to be in place to communicate this new demand. However, it is now clear that this process is deregulated in obesity resulting in a detrimental effect on bone health with an increased risk of bone fractures and osteoporosis. Leptin and neuropeptide Y (NPY) are both critical to controlling fat as well as bone mass. Furthermore, they interact directly in the hypothalamus, an essential coordinating centre of energy homeostasis, although the exact mechanisms underlying their interaction are still unclear.

By specifically targeting the leptin receptor in NPY neurons, we show that chow-fed $Lepr^{lox/lox}$;$NPY^{Cre/+}$ mice exhibit significantly increased adiposity while bone mass is diminished, demonstrating a prominent role for leptin signaling in NPY neurons in the control of energy partitioning. Importantly, this occurred in the absence of changes in food intake or energy expenditure. Interestingly, this regulation in energy partitioning was reversed under conditions of positive energy balance induced by high fat diet (HFD) feeding. The obese phenotype of $Lepr^{lox/lox}$;$NPY^{Cre/+}$ mice was attenuated on HFD in both male and female mice. Furthermore, $Lepr^{lox/lox}$;$NPY^{Cre/+}$ mice were instead able to divert energy into the production of bone mass in response to the increase in bodyweight caused by HFD feeding, a change which was not evident in control mice.

Taken together, these results suggest that leptin signaling in NPY neurons is critical for coordinating energy partitioning between fat and bone mass especially under conditions of positive energy balance and this may have important therapeutic implications for both obesity and osteoporosis.

H12. Beneficial Effects of Leptin Antagonism on Glucose Homeostasis in DIO Mice

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Obesity is characterized by high circulating levels of leptin and a state of chronic low-grade inflammation. Recent studies have shown that pro-inflammatory signaling in the hypothalamus provokes a decrease of central leptin and insulin action associated with impaired glucose tolerance. Intriguingly, leptin not only regulates body weight and glucose homeostasis but also acts as a pro-inflammatory cytokine. Consequently, we hypothesized that hyperleptinemia may contribute to the manifestation of chronic low-grade inflammation leading to impairments in glucose tolerance. To test this hypothesis, we chronically administered different doses of a long-acting (pegylated) leptin antagonist (PESLAN) in mice fed a high-fat diet (HFD) to block excessive leptin action during diet-induced obesity (DIO). The initial use of a high dose of PESLAN further exacerbated the body weight gain induced by HFD and worsened glucose intolerance suggesting a substantial inhibition of leptin signaling and ongoing glucoregulatory action of the hormone during DIO. Using lower doses, chosen to block excessive leptin action while basic action is maintained, revealed an opposite effect and improved glucose tolerance significantly without affecting body weight. Immunohistochemical analysis of mouse brains treated with the low dose revealed a significant reduction in the number of both microglia and astrocytes in the arcuate nucleus of the hypothalamus. Our results suggest that excessive leptin action may increase pro-inflammatory signaling and thereby contributes to glucose intolerance during DIO. Lower doses of PESLAN partially but not fully restored glucose tolerance suggesting that removing hyperleptinemia is only one part of the puzzle to potentially treat DIO-induced glucose intolerance.

H13. Ghrelin at the crossroads between stress and reproduction

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An orexigenic gut-derived peptide ghrelin plays a multifaceted role in a number of physiological functions, including regulating stress responsivity and reproduction. It may therefore be pivotal in the integration of the hypothalamic-pituitary-adrenal (HPA) and –gonadal (HPG) axes. By using ghrelin-O-acyltransferase (GOAT) knockout (KO) mice that have no measurable levels of endogenous acyl ghrelin and chronically high levels of des-acyl ghrelin we have shown that an absence of acyl ghrelin does not prevent reproductive success, but that appropriate levels of acyl and des-acyl ghrelin may be necessary for optimal ovarian maturation. We then hypothesised that chronic stress would disrupt ovarian maturation and that this effect is mediated by a stress-induced increase in acyl ghrelin and activation of the growth hormone secretatogue receptor (GHSR). We subjected C57BL/6J female mice to 30 min of daily chronic predator stress for 4 weeks, used to model a posttraumatic stress disorder (PTSD), or no stress, and administered them daily with GHSR antagonist (D-Lys3-GHRP-6) or saline. Exposure to chronic predator stress reduced corticosterone and elevated acyl ghrelin levels. It did not affect the levels of circulating gonadotropins, but did deplete the primordial follicle reserve that was attenuated by GHSR antagonism. These findings suggest that chronic stress has subtle but potentially critical effects on female reproductive health and that at least some of these effects may be mediated by stress-induced acyl ghrelin.

H14. Small molecule agonists for class B G protein-coupled receptors: past, present and future

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Glucagon-like peptide-1 receptor (GLP-1R) belongs to the class B family of G-protein coupled receptors. The non-peptidic GLP-1R agonist Boc5 was shown to mimic a full spectrum of physiological actions of GLP-1. However, its druggability is hampered by poor oral bioavailability and difficulties in chemical synthesis. This led us to conduct structural biology studies of the receptor. Upon stabilization by negative allosteric modulators, two crystal structures of the human GLP-1R 7-transmembrane domain were determined in an inactive conformation, revealing a common binding pocket present in both GLP-1 and glucagon receptors. Molecular modeling and mutagenesis experiments indicate that agonist positive allosteric modulators (PAMs) target the same general region, but in a distinct sub-pocket which may facilitate the formation of an intracellular binding site that enhances G-protein coupling. The structure of human GLP-1R in complex with the G protein-biased peptide, exendin-P5, and Gs protein was also determined, offering insights into the structural basis of biased agonism. Allosteric modulation provides high selectivity, broad mimicry and less over-activation in terms of pharmacological properties. Based on the structural information, new efforts are being made to discover PAMs targeting the GLP-1R, with an ultimate goal of developing novel small molecule therapeutics to treat metabolic disorders.

H15. Characterising the GnRH pulse generator in female mice

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A subpopulation of kisspeptin neurons located in the arcuate nucleus (ARN) operate as the GnRH pulse generator, responsible for generating the pulsatile release of luteinising hormone, critical for fertility. The activity of this population of neurons can be monitored in real-time for long periods using kisspeptin neuron-selective GCaMP6 fiber photometry. Using this approach, we aimed to determine whether ARN kisspeptin neurons exhibit synchronisation episodes (SEs) prior to each LH pulse, as has been found for males and characterise the patterns of pulse generator activity that occur across the estrous cycle and in relation to the LH surge.

We found that ARN kisspeptin neurons exhibit brief (~50 seconds) periods of synchronised activity that precede pulses of LH in intact female mice. The dynamics and frequency of these SEs are stable at approximately one event every 40 minutes throughout metestrus, diestrus, and proestrus, but slow considerably on estrus to occur approximately once every 10 hours. Evaluation of ARN kisspeptin neuron activity across the light-dark transition, including the time of onset of the proestrus LH surge, revealed no changes in SE frequency. Longer 24-hour recordings across proestrus into estrus demonstrated that an abrupt decrease in SEs occurred ~ 4 to 5 hours after the onset of the LH surge to reach the low frequency of SEs observed on estrus. The frequency of SEs was stable across the 24-hour period from metestrus to diestrus. Administration of progesterone to diestrus mice resulted in the abrupt slowing of SEs. These observations show that the GnRH pulse generator exhibits an unvarying pattern of activity from metestrus through to the late evening of proestrus, at which time it slows dramatically, likely in response to postovulation progesterone secretion. The GnRH pulse generator maintains a constant frequency of activity across the time of the LH surge, demonstrating that it is not involved directly in surge generation.

H16. Chemogenetic activation of arcuate GABA neurons lead to reproductive dysfunction in female: implication for polycystic ovary syndrome.

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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 (PCOS), 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 used 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. Acute stimulation of ARN GABA fibers adjacent to GnRH neurons by local CNO delivery resulted in a significant and long-lasting increase in LH secretion in male and female mice. Chronic activation of ARN GABA neurons by CNO delivery in the drinking water impaired estrous cyclicity, decreased corpora lutea number, increased circulating testosterone and resulted in a trend toward increased LH pulse frequency similar to the PCOS condition. Altogether, these results support the hypothesis that ARN GABA neurons are a functional component of the GnRH neuronal network in both males and females and suggest that elevated activity in this circuit can drive reproductive dysfunction similar to the most common infertility disorder PCOS.

H17. Kisspeptin regulation of brown adipose tissue

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Kisspeptin neurons are found in the hypothalamus and are critical for fertility through stimulation of gonadotropin-releasing hormone (GnRH) neurons. In addition to key roles in puberty onset, kisspeptin neurons govern underlying mechanism for sex steroid positive- and negative-feedback, and it is now commonly accepted – at least in rodents – that the ARC kisspeptin neurons act as the GnRH pulse generator. Moreover, kisspeptin neurons are now recognized as a central pathway responsible for conveying key homeostatic information to GnRH neurons to modulate fertility. Thus, in states of severely altered energy balance (either negative or positive) fertility is compromised, as is kisspeptin gene (Kiss1) expression in the arcuate nucleus. Furthermore, in addition to being expressed in GnRH neurons, the kisspeptin receptor (Kiss1r) is also expressed in other areas of the brain, as well as in the periphery, suggesting kisspeptin may have additional functions outside of governing reproductive status. Evidence is building for a direct role for kisspeptin in regulating energy balance and metabolism. Interestingly, kisspeptin neurons located in the arcuate nucleus are anatomically linked to anorexigenic POMC neurons and orexigenic NPY neurons. Thus, kisspeptin may have a role in energy balance and our observations indicated that Kiss1r knockout mice displayed late onset obesity and reduced energy expenditure. Moreover, recent data suggest that this obesity may be primarily due to altered uncoupling protein-1 (UCP1) mRNA expression in brown adipose tissue (BAT). Kisspeptin receptor is expressed in BAT, but its role there does not appear to be consistent with the obesity in Kiss1r knockout mice. Overall, in addition to regulating reproduction, kisspeptin signaling may also be an important regulator of metabolism and adiposity but the precise mechanistic pathways are yet to be determined.

H18. Switch to motherhood: a glimpse into prolactin secretion

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Altered physiological states require neuronal adaptation. In late pregnancy and lactation, a sub-population of the mouse hypothalamic tuberoinfundibular dopaminergic (TIDA) neurons alters their behavior to synthesize and release met-enkephalin rather than dopamine. These neurons normally release dopamine to inhibit prolactin secretion and are activated by prolactin in a short-loop feedback manner. In lactation, dopamine synthesis is suppressed in an opioid-dependent (naloxone-reversible) manner, meaning that prolactin secretion is disinhibited. Conditional deletion of the prolactin receptor in neurons reveals that this change in phenotype appears to be driven by prolactin itself, apparently through an alteration in intracellular signaling downstream of the prolactin receptor that favors enkephalin production instead of dopamine. Thus, prolactin effectively facilitates its own secretion, which is essential for lactation and maternal behavior. These studies provide evidence of a physiologically important, reversible alteration in the behavior of a specific population of hypothalamic neurons in the adult brain.

H19. The hypothalamus and neurodegenerative disease: Looking from the bench to the bedside

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Neurodegenerative diseases are commonly characterized by progressive deterioration of brain structures of motor or cognitive control. However, preclinical and clinal evidence now show that non-motor and non-cognitive areas of the brain could also be impacted. There is evidence of dysfunction of central pathways of energy homeostasis, with the resulting metabolic dysfunction contributing to disease pathophysiology. Our studies seek to improve our understanding of the cause and consequence of metabolic dysfunction in neurodegenerative disease.

Although motor neurone disease (MND) is defined by the irreversible death of motor neurons in the brain and spinal cord, it is now accepted that metabolic derangements at the *systemic* and *cellular* level contribute to disease pathophysiology [1]. Weight loss in MND is associated with shorter survival[2,3], and several reports in MND suggest that a state of hypermetabolism (an above normal increase in resting energy expenditure) is a primary cause for weight loss[2]. Conducting the only study to directly assess the impact of hypermetabolism on body weight in patients with MND, we found that hypermetabolism does not universally lead to weight loss[4]. Rather, hypermetabolism independent of weight loss is associated with faster disease progression and earlier death. Building on this observation, we found that weight loss in MND is attributed to loss of appetite. One third of patients present with loss of appetite at study enrolment, and loss of appetite worsens as disease progresses. From these results, it is suggested that loss of appetite *early* in disease could contribute to weight loss and faster disease progression. Critically, loss of appetite in patients is not universally associated with the onset and progression of bulbar symptoms, and thus factors other than disability could contribute to loss of appetite and weight loss in MND.

I will discuss recent observations from our preclinical and clinical studies that clarify the cause for hypermetabolism and loss of appetite in MND. These studies include investigation into the breakdown of central and peripheral processes of energy homeostasis. Given the role of the hypothalamus as a regulator of metabolism and food intake, I will include a brief overview of emerging thoughts on the role of the hypothalamus as a disease modifier.

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H20. The lateral habenula mediates the thermoregulatory response to psychological stress

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The lateral habenula (LHb), a phylogenetically old nucleus in the epithalamus, has attracted much attention for its role in the coordination of behavioural strategy in response to adverse environmental situations. This nucleus is activated by aversive stimuli, and then proceeds to modify motor behaviour. However, in the behavioural response, autonomic physiological conditions must also be adjusted accordingly.
Physiological responses to aversive situations include an increase in body temperature - which is often referred to as “emotional hyperthermia”. The LHb may also play an important role in the control of emotional hyperthermia. Here, we have addressed this hypothesis. We have shown that pharmacological activation of neurones in the LHb stimulates heat production in brown adipose tissue (BAT), along with strong vasoconstriction in thermoregulatory cutaneous beds. These responses, in turn, lead to an increase in body temperature (1). We also show that Inhibition of LHb neurons attenuates emotional hyperthermia (2). Following these findings, we have studied the neural pathways projecting from the LHb, that mediate these responses. One of the major target nuclei that receives direct projection from the LHb is the ventral tegmental area (VTA). This region is the origin of the mesolimbic dopamine system. A body of evidence shows that VTA neurons are inhibited by the LHb, and suggests that this inhibitory action plays an important role in mediating LHb-dependent responses to aversive stimuli. In accordance with this view, our resent study shows that inhibition of the VTA increases BAT thermogenesis, and that LHb-elicited BAT thermogenesis is abolished by the blockade of GABAergic transmission in the VTA (3). Importantly, activation of dopamine D2-like receptors attenuates emotional hyperthermia in response to intruder stress. These lines of evidence suggest that the LHb, via its projection to the VTA dopamine system, mediates emotional hyperthermia.

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H21. The central actions of hormones in the control of blood glucose

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Obesity and type 2 diabetes, develop so often concurrently and hence there are key links between weight gain and glucose homeostasis. In mice we are looking how key peripheral hormones, including leptin regulate glucose tolerance. Leptin is a hormone secreted from fat cells, significantly elevated as body fat is gained. Leptin is a key long-term regulator of body weight regulation and rodents and humans lacking leptin or leptin receptors become obese but also type II diabetes. Leptin has been suggested to play a role in glucose regulation, but due to its substantial effects on food intake and body weight, determining the mechanism of leptin on glucose control has been challenging. Using new technology, we demonstrate that leptin has a significant role in reducing plasma blood glucose concentration even in obesity in mice. The actions of leptin are through the brain and in particular the neurons that express leptin receptors in Dorsomedial hypothalamic region of the brain. Knocking out leptin receptors in only the DMH of genetic obese mice, basal blood glucose levels increase and response to a glucose bolus is also worse, demonstrating the importance of this receptors in obesity. The DMH leptin receptors importantly control the activity of brown adipose tissue, and concurrent with worsening glucose tolerance, knockout of leptin receptors in the DMH also decreases BAT thermogenesis. Using a chemogenic setup in DIO mice implanted with continuous blood glucose probes and BAT probes, we demonstrate that activating the leptin receptors in the DMH drives a increase in BAT temp and this is followed by a decrease in peripheral plasma blood glucose concentration. This research demonstrates an ability to utilizing the DMH lepR to cause improved glucose tolerance even in obesity.

H22. Exercise interventions in obesity – impact on brain, microbiome and beyond

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As exercise can impact several obesity-related metabolic changes, we are interested in investigating the impact of exercise in the context of obesogenic diets. More recent work explores the effects of diet and exercise on the gut microbiome. Adult female rats were exposed to regular chow or cafeteria diet, comprising chow, water, and various foods with 10% sucrose solution, then half were allocated to treadmill exercise for four weeks. Short-term exercise reduced fat mass and plasma leptin, and increased WAT leptin receptor gene expression. WAT uncoupling protein 1 expression was upregulated in exercised rats fed cafeteria diet. In the hypothalamus the lower levels of NPY and higher levels of glucose transporter 1 induced by cafeteria diet were both normalised by exercise. Short-term exercise induced subtle changes in microbiome composition in chow-fed rats but did not overcome the profound microbiome changes induced by cafeteria diet.

In transgenerational work we have demonstrated that maternal obesity is also associated with several changes in hypothalamic appetite regulators in offspring, as well as increased expression of inflammatory markers, including SOC3, TNF, IL-1b and IL-6 in the arcuate nucleus. In the PVN, Y1 receptor, melanocortin 4 receptor, and TNF mRNA were elevated. Some of these changes were impacted by exercise in the offspring.

I will also discuss the nexus between brain changes in gene expression, behaviour and gut microbiome composition, which we found was impacted by exercise in the mother.

Summary of Abstracts for the HNNA Poster Session

No.	Title	Presenter	Institutions
H23	Mechanosensitivity of TRPV channels; implications for vasopressin neuron activity	E. Brown	BHRC, University of Otago
H24	TRPV channels reset the threshold for vasopressin neuron activation in pregnant rats	A.J. Seymour	BHRC, University of Otago
H25	Local kisspeptin regulation of oxytocin neuron activity in late pregnancy	M. Abbasi	BHRC, University of Otago
H26	Prolactin action on kisspeptin neurons is required for maintaining lactational infertility.	E.C.R. Hackwell	CNE, University of Otago
H27	Characterisation of plasma prolactin levels during proestrus in mice	H.R. Phillipps	CNE, University of Otago
H28	Identifying a novel role for prolactin in the transition to paternal care	K.O. Smiley	CNE, University of Otago
H29	Suprachiasmatic Vasopressin Neurons Alter the Activity of Preoptic Kisspeptin Neurons in Female Mice	B.B. Jamieson	CNE, University of Otago
H30	Regulation of calcium in the GnRH neuron dendrons near the median eminence	X. Liu	CNE, University of Otago
H31	In vivo population activity of RP3V kisspeptin neurons across the mouse estrous cycle.	J. Clarkson	CNE, University of Otago
H32	Identifying the Role of RFRP Neurons in Stress Induced Anovulation	A. Mamgain	CNE, University of Otago
H33	Characterisation of metabolic and reproductive dysfunction in two PCOS mouse models	R.I. Kerbus	CNE, University of Otago
H34	Androgen receptor expression across the estrous cycle in the hypothalamus and new methods for its targeted deletion.	C. Coyle	CNE, University of Otago
H35	Examining alterations to arcuate nucleus NPY neurons and their neural projections in a mouse model of polycystic ovary syndrome	C. J. Marshall	CNE, University of Otago
H36	Prenatal androgen excess impairs sexual behavior in adult female mice: perspective on sexual dysfunction in PCOS	E. Desroziers	CNE, University of Otago
H37	Investigating changes in androgen and progesterone receptor expression in the aetiology of PNA-induced polycystic ovary syndrome	Y. Watanabe	CNE, University of Otago
H38	Prefrontal glutamate projections to the lateral hypothalamus regulate food	Z.B. Andrews	Monash University

	intake and behaviour.		
H39	Loss of ghrelin receptor-positive neurons in the mouse dentate gyrus reduces food intake, neurogenesis and locomotor activity	S. H. Lockie	Monash University
H40	The effects of leptin mutations and diet-induced weight gain on glucose homeostasis in the zebrafish	K. Kamstra	CNE, University of Otago
H41	The parabrachial nucleus regulates initial intake of ethanol, sucrose and other solutions.	P.J. Ryan	Florey, Melbourne
H42	Region-specific deletion of β-catenin leads to impaired glucose tolerance and increased bodyweight	M.Z. Rizwan	CNE, University of Otago
H43	Peripartum prolactin and growth hormone concentrations in diet-induced obese mice	Z. Khant Aung	CNE University of Otago
H44	Distribution of O-GlcNAc in glucose sensing areas of the rat brain	J.C. Murrell	BHRC, University of Otago
H45	Do mice with gestational glucose intolerance have increased O-linked glycosylation in the brain?	R.A. Augustine	BHRC, University of Otago
H46	Central mechanisms underlying cardiac dysfunction in type 2 diabetes	S. Sethi	Department of Physiology, University of Otago
H47	17-α Estradiol ameliorates age-associated sarcopenia and improves late life physical function in male mice but not in females or castrated males	M. Garratt	Department of Anatomy, University of Otago
H48	Neuroimmune interaction in the ageing brain	S. Malik	RMIT, Melbourne
H49	Analysis of Ca2+ Imaging in Rat Adrenal Medullary Slices	W. Aye	CNE, University of Otago
H50	A lipolytic model for the effects of oxytocin on 3T3-L1 differentiated adipocytes	B. Boumelhem	University of Sydney

Poster H23

Mechanosensitivity of TRPV channels; implications for vasopressin neuron activity

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Vasopressin is synthesised by magnocellular neurons in the hypothalamic paraventricular and supraoptic nuclei, and is secreted from the posterior pituitary gland in response to increased plasma osmolality to maintain body fluid balance by promoting renal water retention. Vasopressin neurons are depolarised by mechanosensitive transient receptor potential vanilloid (TRPV) channels that are inactivated by membrane stretch caused by reduced osmolality. Most TRPV channels express more than one type of TRPV subunit, such as ΔN-TRPV1, TRPV2 and 4. However, the subunit composition of the TRPV channels expressed by vasopressin neurons is unknown, as is the potential contribution of any changes in composition to vasopressin neuron function. Here, we test the hypothesis that a change in TRPV channel composition alters the mechanosensitivity of ΔN-TRPV1-containing channels. To test TRPV channel function, ΔN-TRPV1, TRPV2 and TRPV4 channels were expressed in *Xenopus* oocytes and transmembrane current was recorded via two-electrode-voltage-clamp (TEVC). However, TRPV channels in *Xenopus* oocytes do not appear to be functional because there was no change in membrane current during application of TRPV antagonists and agonists, independent of injected RNA amount (1-20ng) and incubation time (1-4 days). This was most likely due to *Xenopus* oocytes lacking specific cytoskeletal proteins, such as beta-tubulin which is crucial for gating of TRPV channels. HEK-293 cells express the required cytoskeletal proteins for TRPV trafficking, membrane tethering and gating. Therefore, ΔN-TRPV1, TRPV2 and TRPV4 channels co-expressed with a fluorescent protein are now being expressed exogenously in HEK-293 cells as homomeric and heteromeric channels. Whole-cell patch clamp recordings will determine the functionality of the different TRPV channel subunit compositions.

Poster H24

TRPV channels reset the threshold for vasopressin neuron activation in pregnant rats

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During pregnancy, the homeostatic set-point for body fluid osmolality is reduced to allow blood volume expansion to cope with the cardiovascular demands placed on the mother by the developing offspring. This reduction in the set-point is caused by a reduction in the osmotic threshold for release of the anti-diuretic hormone, vasopressin, but the mechanisms that reduce the threshold are unknown. Vasopressin neurons are directly osmosensitive via mechanosensitive transient receptor potential vanilloid-1 (TRPV1) channels that are opened by cell shrinkage in hyperosmotic conditions. Therefore, we hypothesised that vasopressin neurons are active at lower osmolality during pregnancy because of increased TRPV1 activation.

To determine whether TRPV1 is involved in resetting the osmotic threshold for vasopressin secretion in pregnancy, single-unit extracellular electrophysiological recordings were made from vasopressin neurons in urethane-anaesthetised non-pregnant and late-pregnant rats. As expected, plasma osmolality was lower in late-pregnant rats compared to non-pregnant rats. Nevertheless, the baseline firing rate of vasopressin neurons was similar in non-pregnant and late-pregnant rats and IV hypertonic saline infusion over 1 h increased the firing rate of vasopressin neurons to a similar extent in non-pregnant and late-pregnant rats, confirming that vasopressin neuron activity is reset to a lower osmotic threshold in pregnancy. Microdialysis administration of the TRPV channel blocker, ruthenium red, over 1 h reduced vasopressin neuron firing rate to a similar extent in non-pregnant and late-pregnant rats, indicating that TRPV channels are active at a lower osmolality in late-pregnant rats than in non-pregnant rats. These effects of ruthenium red were specific to vasopressin neurons because the firing rate of neighbouring oxytocin neurons (that principally regulate uterine contraction during birth) was not affected by ruthenium red in non-pregnant or late-pregnant rats.

Taken together, these results suggest that increased TRPV channel activation contributes to the reduction in the osmotic threshold for vasopressin release during pregnancy.

Poster H25

Local kisspeptin regulation of oxytocin neuron activity in late pregnancy

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Oxytocin is secreted from the posterior pituitary gland by oxytocin neurons to trigger uterine contractions during birth. We have recently shown that the oxytocin neuron firing rate increases in response to intracerebroventricular (ICV) kisspeptin only in late-pregnant rats. Furthermore, the kisspeptin projection from the periventricular nucleus of the hypothalamus (PeVN) to the perinuclear zone (PNZ) surrounding the supraoptic nucleus (SON) increases in late pregnancy. To determine whether the stimulatory effects of central kisspeptin in late pregnancy result from a local action in the SON, *in vivo* extracellular single unit recordings were made from SON neurons in urethane-anaesthetised non-pregnant and late-pregnant rats during microdialysis application of kisspeptin (100 μM in the dialysate) into the SON. Intra-SON kisspeptin consistently increased the firing rate of oxytocin neurons in late-pregnant rats but not in non-pregnant rats. Two-way repeated measures ANOVA revealed a significant interaction between reproductive status and time ($F_{(6,88)}$ = 10.31, $p < 0.001$); post hoc all pairwise Holm-Sidak tests revealed a significant increase in firing rate in late-pregnant rats at 30 ($p < 0.05$), 40, 50 and 60 minutes (all $p < 0.001$ versus pre-kisspeptin), to higher firing rates than in time-matched recordings from non-pregnant rats at 50 and 60 minutes ($p < 0.05$). Hence, it appears that kisspeptin excites oxytocin neurons at the end of pregnancy by a local action to stimulate peripheral oxytocin release and so might contribute to oxytocin neuron activation for parturition.

Poster H26

Prolactin action on kisspeptin neurons is required for maintaining lactational infertility.

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In mammals, lactation is associated with a period of infertility, in order for a mother's metabolic resources to be directed towards caring for her newborn, rather than supporting another pregnancy. This lactational infertility is characterised by a reduction in the secretion of luteinizing hormone (LH) and a consequent cessation of ovulation. Lactation is accompanied by chronically elevated levels of the anterior pituitary hormone prolactin. Despite elevated prolactin being a well-recognised cause of infertility, the specific role prolactin plays in lactational infertility is currently unclear. To determine whether prolactin is involved in lactational infertility, we used two mouse models with specific deletions of the prolactin receptor (Prlr), in either forebrain neurons ($Prlr^{lox/lox}$/CKC-Cre mice) or specifically in kisspeptin neurons (Kiss1-Cre/$Prlr^{lox/lox}$), neurons which are key for fertility. While no control animals experienced an estrus until after weaning of pups (>20 days of lactation), both neuron-specific Prlr-KO and kisspeptin-specific Prlr-KO mice did not exhibit the normal period of lactational infertility, returning to estrus within 6-10 and 6-17 days of lactation respectively. In neuron-specific Prlr-KO mice, immunohistochemical analysis of kisspeptin cell number and fibre density in the rostral periventricular region of the third ventricle and arcuate nucleus showed a significant attenuation of the normal lactation-induced suppression of kisspeptin immunoreactivity. LH pulsatility was examined in virgin and lactating mice by collecting serial blood samples and while neuron-specific Prlr-KO mice did not show the normal lactation-associated reduction of LH pulsatility, kisspeptin-specific Prlr-KO mice showed suppressed LH secretion, similar to lactating control mice. These data provide evidence that high prolactin levels acting specifically on kisspeptin neurons in the brain during lactation is essential for the suppression of estrous cyclicity during lactation, however, it would appear that suppression of LH pulsatility may also involve prolactin acting through a mechanism not involving kisspeptin neurons.

Poster H27

Characterisation of plasma prolactin levels during proestrus in mice

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Prolactin secretion patterns are species specific, variable and influenced by multiple factors including circadian and hormonal cues, stress, physiological state and reproductive strategy. Secretion is regulated via an atypical short loop negative feedback mechanism (1). Prolactin acts directly on tuberoinfundibular dopaminergic neurons increasing their firing rate and dopamine output (1). Dopamine acts on lactotroph cells in the anterior pituitary gland leading to tonic inhibition of prolactin secretion (2, 3). In nonpregnant females, circulating prolactin levels are low; however, during the afternoon of proestrus an estradiol-induced prolactin surge coinciding with the preovulatory LH surge has been recorded in many (but not all) species. In mice, there have been conflicting reports relating to the occurrence and timing of this surge. To gain insight into the characteristics of circulating prolactin levels during proestrus we have used trunk blood collection and repeated tail blood sampling in C57BL/6 mice (N=33) to profile prolactin secretion in individual mice. For this study we have measured prolactin levels during diestrus and proestrus using an ultra-sensitive prolactin ELISA. To establish the relationship between circulating prolactin levels and the timing of the LH surge we have simultaneously measured LH levels in the same samples via ELISA. Firstly, we found high variability in both prolactin and LH levels during proestrus. Circulating prolactin levels were significantly increased on the morning of proestrus (44.88 ? 9.63 ng/ml) compared with average levels in diestrus (17.98 ± 0.98 ng/ml) and reached peak levels (78.88 ± 8.97 ng/ml) coinciding with the LH surge in the evening. The levels gradually decreased through the dark phase, however remained significantly increased on the morning of estrus (47.42 ± 10.81 ng/ml). Thus, in C57BL/6 mice circulating prolactin levels in proestrus do not follow a classical surge pattern but show prolonged elevation which is not tightly linked to the light/dark cycle.

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Poster H28

Identifying a novel role for prolactin in the transition to paternal care

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Parental care is essential for healthy offspring development, and in some species (including humans), involves both male and female parents. However, in comparison to maternal care, the neuroendocrine regulation of *paternal* care remains poorly understood. There is a well-established role for the hormone prolactin in promoting mammalian maternal behavior through its actions on central prolactin receptors (PRLR). Although male mice have a similar central PRLR distribution as females, it is unknown whether prolactin plays a role in paternal behavior. To test this, we used mice with a conditional deletion of PRLR in forebrain neurons ($Prlr^{lox/lox}$/CamKIIa-Cre), and showed that paternal behaviors (retrieving pups and crouching over them) were either eliminated or greatly reduced in knockout animals, compared to controls. Next, to identify which prolactin-responsive neurons were active during paternal care, we examined c-fos (a marker for recent neural activation) immunolabelling in the brains of father mice expressing a fluorescent reporter in PRLR-expressing neurons (PRLR-IRES-Cre/tdTomato). Fathers were separated from their pups for 24 hours on day 3 post-partum and then either exposed or not exposed to pups for 30 minutes in their home cage. We found that fathers exposed to pups showed increased c-fos expression in PRLR-expressing cells in the medial preoptic area (MPOA), a critical site for the regulation of maternal behavior. Lastly, we performed a conditional deletion of PRLR specifically from the MPOA of adult male mice using an adeno-associated virus driving Cre expression in $Prlr^{lox/lox}$ males and showed that a MPOA-specific PRLR knockout also resulted in the elimination of pup retrieval behavior, compared to controls which normally retrieved pups. Together, these results are the first demonstration that prolactin action in the brain, particularly the MPOA, is critical for mammalian paternal behaviors. Additionally, these data suggest that there are likely similar neuroendocrine mechanisms which underlie both maternal and paternal behaviors.

Poster H29

Suprachiasmatic Vasopressin Neurons Alter the Activity of Preoptic Kisspeptin Neurons in Female Mice

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For rodents, the appropriate timing of the surge of gonadotropin secretion, triggering ovulation, is critical to their reproductive success. The biological clock in the suprachiasmatic nucleus (SCN) is thought to time the hormone secretion, by activating circuitry that controls gonadotropin secretion. We have used an anatomical and a function approach to investigate the innervation of preoptic area (POA) kisspeptin neurons, that control the preovulatory surge, by a group of SCN neurons that produce vasopressin (AVP).

AVP-ires2-cre mice (that express cre reombinase (cre) in AVP neurons) were injected at the SCN with a viral vector carrying a cre-dependent fluorescent reporter, mCherry. This revealed innervation of the POA by SCN AVP neurons, closely apposing 44.6 ± 6.6% (n = 6 mice) of POA kisspeptin neurons, and potentially establishing putative synaptic inputs. *AVP-ires2-cre* mice expressing the green fluorescent protein (GFP) in kisspeptin neurons were injected in the SCN with a viral vector carrying a cre-dependent channelrhodopsin, and brain slices were taken for patch-clamp electrophysiological recordings. 92% of POA GFP-expressing kisspeptin neurons (n = 26, 9 mice) did not display fast postsynaptic currents in response to brief blue-light stimulation of AVP axons. High-frequency blue-light stimulation (20 Hz, 60 s) did, however, increase the firing rate of 59% of kisspeptin neurons recorded (n = 17, 9 mice; $p < 0.05$ compared to control).

This work suggests that although AVP neurons from the SCN project to the POA, they may not communicate with POA kisspeptin neurons via fast amino acid neurotransmission, but rather through the release of neuropeptide. This reveals a mechanism through which the biological clock may control the POA kisspeptin neurons and stimulate the onset of the preovulatory surge release of gonadotropins.

Poster H30

Regulation of calcium in the GnRH neuron dendrons near the median eminence

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The gonadotrophin-releasing hormone (GnRH) cell bodies are scattered throughout the basal forebrain but project and release GnRH at the median eminence to control fertility in all mammals. Their projections have characteristics of both dendrites to receive synaptic inputs and axons, and therefore they have been termed "dendrons". To provide functional evidence that those dendrons near the median eminence receive different synaptic inputs, we used confocal microscopy in combination of GCaMP calcium and acute brain slice from GnRH-cre transgenic mice to investigate whether the classical and non-classical (neuropeptides) neurotransmitters modulate GnRH neuron dendron calcium concentrations. Studies were undertaken in acute brain slices prepared from adult male and female GnRH-cre mice sterotaxically injected with a cre-dependent AAV (adeno-associated virus) expressing GCaMP6s. We made following findings: 1) glutamate had no effect on calcium level in GnRH neuron dendrons in either males or females. 2) GABA decreased GnRH neuron dendron calcium via $GABA_B$ receptors in males and females, but increased it via $GABA_A$ receptors. 3) kisspeptin caused a long-lasting elevation in GnRH neuron dendron calcium in both males and females. 4) NKB (neurokinin B) and dynorphin had no effect on GnRH neuron dendron calcium concentrations. 5) GABA and baclofen ($GABA_B$ receptor agonist) depressed the kisspeptin-induced long-lasting calcium elevation in almost all GnRH neuron dendrons in both males and females but dynorphin had no such effect. The results show that kisspeptin and GABA exert potent modulatory actions upon the calcium concentrations and likely excitability of the GnRH neuron Dendron in both male and female mice. Unexpectedly we find that GABA can excite or inhibit Dendron activity depending on which GABA receptor is activated.

Poster H31

In vivo population activity of RP3V kisspeptin neurons across the mouse estrous cycle.

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Kisspeptin signalling 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 genetically encoded calcium indicators (GCaMP) combined with 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 designer receptor exclusively activated by designer drugs (hM3Dq) 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 clozapine N-oxide activation of hM3Dq signalling. GCaMP activity was recorded for up to 20-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.

Poster H32

Identifying the Role of RFRP Neurons in Stress Induced Anovulation

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The hypothalamus-pituitary-gonadal (HPG) axis regulates the reproductive system, and thus fertility in women1. A pre-ovulatory surge of luteinising hormone (LH) secretion from the anterior pituitary gland is vital for ovulation to occur, and this function may be disrupted by increased glucocorticoid levels as a result of stress[1]. The mechanism through which glucocorticoids inhibit hormone secretion from HPG axis remains unknown. The activity of RFamide-related peptide (RFRP) neurons has been shown to increase concurrently with increased plasma glucocorticoid levels[2]. Furthermore, central delivery of the neuropeptide RFRP-3 has been shown to block LH surges in female mice[3]. The wider aim of this project investigates whether RFRP neurons acts as an intermediary between the reproductive and stress axes. To achieve this, we first needed to establish a model of stress-induced reproductive suppression.

The estrous cycle of female C57BL/6J mice were monitored by vaginal cytology. Mice in proestrus were blood sampled in the morning for basal LH level (which averaged 0.8ng/μl) and again one hour before lights off to measure peak LH surge. A robust preovulatory LH surge (>6ng/ml; average of 18.8ng/ml) was observed in 10 out of 15 animals. In mice treated with rodent glucocorticoid corticosterone (3x1mg/mg injection dose at 3 hour time intervals; n=7), no surges were observed, but this was also the case in a cohort of vehicle-treated mice (n=7) subjected to a more intensive blood sampling regime. These results suggest that a more reliable surge model is required. Follow-up experiments are using an ovariectomised, estrogen-induced surge model. Ultimately, DREADDS (designer receptors exclusively activated by designer drugs) expressed in RFRP neurons will be used to silence these neurons during corticosterone exposure, testing for their role in stress-induced surge suppression.

Findings of this experiment could establish a novel role of RFRP-3 in meditating the interaction between stress and reproductive axis suppression.

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Poster H33

Characterisation of metabolic and reproductive dysfunction in two PCOS mouse models

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Polycystic ovarian syndrome (PCOS), is a leading cause of infertility in the world. Along with the hallmark reproductive consequences (polycystic ovaries, hyperandrogenism, oligo- or anovulation), obesity, hyperinsulinemia and insulin resistance have been shown to occur in about 50% of women with the syndrome. Hyperinsulinemia appears to be a key factor in driving ovarian theca cells to over-secrete androgens. Therefore, we hypothesise this may be important in driving or exacerbating the reproductive deficits seen in PCOS women.

In order to investigate the role of hyperinsulinemia in thecal androgen production as a cause of PCOS, we need an animal model which presents both the reproductive and metabolic deficits; specifically insulin resistance and hyperinsulinemia. The model should also have endogenous androgen hypersecretion (rather than exogenous androgen treatment). A current PCOS model being used in our Centre is the prenatally androgenised (PNA) mouse model[1] which presents many of the reproductive symptoms that occur in PCOS women but lacks the metabolic consequences. Therefore, this study aims to characterise two models, peri-pubertal implantation of a letrozole (an aromatase inhibitor) capsule (4.5mg/pellet 90 day release)[2] and the PNA model subjected to a high-fat diet from conception, to determine which reliably presents both reproductive and metabolic consequences. This will be done by analysing lutenizing hormone pulsatility, monitoring estrous cycles (via vaginal cytology), glucose and insulin tolerance, and measuring bodyweight. We will also be investigating the morphological and inflammatory phenotypes in these models within the brain, fat mass and ovaries.

The results of this study will determine which model is best suited for further experimentation and analysis of the impact of hyperinsulinemia on thecal androgen production in PCOS and determine whether alleviating these symptoms could ease both reproductive and metabolic consequences of PCOS.

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Poster H34

Androgen receptor expression across the estrous cycle in the hypothalamus and new methods for its targeted deletion.

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The role of androgen receptor (AR) mediated actions in the hypothalamus have increasingly been implicated in the pathogenesis of PCOS. A common observation in PCOS is a resistance to ovarian estradiol and progesterone negative feedback to the neuronal network that regulates fertility[1,2]. This negative feedback can be restored by the administration of the AR antagonist flutamide in woman with PCOS and preclinical PCOS models, highlighting that AR signalling must play a role in PCOS pathology[3,4]. In order to dissect out the importance of AR signalling in mediating PCOS pathology, we aim to knock-out the AR specifically from the arcuate nucleus (ARN), a discrete hypothalamic nucleus involved in relaying the steroid hormone milieu to GnRH neurons, the central regulators of fertility.

Initially this study will determine the expression of AR in the hypothalamus over the estrous cycle. Perfusion fixed brains from female AR^{flox} mice (n=9) were collected at different stages of the estrous cycle and stained for AR using immunohistochemistry to determine endogenous AR expression. These studies will be used to determine the estrous cycle stage at which AR expression is greatest.

In addition, to validate the targeted knock-out of AR in the ARN, male AR^{flox} mice (n=10), which have a robust expression of AR, were used. An AAV viral vector with a *Cre* recombinase transgene and m-Cherry tag was sterotaxically delivered bilaterally to the ARN. Wherever *Cre* is expressed, exon-2 of the floxed AR is excised, causing loss of AR expression and the expression of m-Cherry. After 4 weeks to allow for successful transfection, brains were perfused fixed and stained for AR using immunofluorescence and assessed for m-Cherry expression. This study will establish our ability to knock out AR specifically in the ARN and both studies combined will inform future experiments in a preclinical model of PCOS in female mice.

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Poster H35

Examining alterations to arcuate nucleus NPY neurons and their neural projections in a mouse model of polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS), the most common form of anovulatory infertility, is associated with a breakdown in signalling within the hormone sensitive neural network that regulates gonadotropin-releasing hormone (GnRH) neurons, ultimately increasing GnRH secretion. Circuitry between GABAergic neurons in the arcuate nucleus (ARN) and GnRH neurons is remodelled in a preclinical mouse model of PCOS, implicating their role in this disorder[1]. One-third of ARN GABA neurons co-express neuropeptide Y (NPY)[2], a known regulator of GnRH neurons. This project examined whether NPY-expressing ARN GABA neurons are similarly altered in a PCOS-like state.

To determine whether NPY^{ARN}-to-GnRH neuron innervation is altered, a PCOS-like phenotype was generated in mice expressing green fluorescent protein (GFP) in agouti-related peptide neurons (always co-expressed in NPY^{ARN} neurons) by administration of dihydrotestosterone (PNA) or a vehicle (VEH) control in late gestataion[3]. Immunohistochemistry (IHC) against GnRH and GFP was carried out to assess the density of putative synaptic contact to GnRH neurons made by NPY^{ARN} fibres. This revealed robust NPY^{ARN}-to-GnRH neuron innervation that was not different between VEH (n=5) and PNA (n=8) mice. Sensitivity to steroid hormones was also assessed by IHC detection of progesterone receptor (PR), estrogen receptor alpha (ERa) and androgen receptor (AR) within NPY^{ARN} neurons. While PR and ERa were virtually absent from NPY^{ARN} neurons, the proportion expressing AR was significantly greater in PNA mice compared with VEH controls (n=4/group, $p<0.05$). Additionally, gene transcription changes in hypothalamic regions were investigated using a Nanostring™ assay in VEH/PNA mice (n=12/group). NPY-Y_1 receptor mRNA expression was significantly decreased in the rostral periventricular region of the 3rd ventricle, a key region in regulation of fertility ($p < 0.05$). Overall these results suggest heightened androgen sensitivity in NPY^{ARN} neurons, and reduced NPY signalling in an important hypothalamic region regulating fertility within PNA mice which may contribute to their infertile phenotype.

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Poster H36

Prenatal androgen excess impairs sexual behavior in adult female mice: perspective on sexual dysfunction in PCOS

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Polycystic ovary syndrome (PCOS) is the most common anovulatory infertility disorder, affecting 1 in 10 women of reproductive age worldwide. PCOS is characterised by high circulating androgen levels, oligo- or anovulation, and polycystic ovaries. Recent epidemiological studies indicate that PCOS patients also experience sexual dysfunction, such as decreased sexual desire, increased sexual dissatisfaction and gender dysphoria. Very little is currently understood about the development of female sexual behaviour in androgen excess states such as PCOS. Prenatally androgenized (PNA) animal model of PCOS exhibit an adult hyperandrogenism, impaired sensitivity to progesterone signalling in the brain and alterations in the neuronal network regulating reproductive function. Here, we 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 in lordosis quotient compared to VEH females ($p<0.01$). These data suggest that increased androgen receptor mediated signalling during the prenatal period impairs sexual differentiation of the female brain and behaviour in addition to other PCOS features. To date, using cfos expression as an indicator of neuronal activation, no significant differences have been found in the different brain regions known to be involved in the control of sex behaviour. We are currently investigating the effect of prenatal androgen on neuronal Nitric Oxide Synthase neurons which are critical for lordosis behaviour.

Poster H37

Investigating changes in androgen and progesterone receptor expression in the aetiology of PNA-induced polycystic ovary syndrome

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Neural circuitry is largely influenced by hormonal exposure at developmental period and it links to the functional changes of neuroendocrine activity later in life. Polycystic ovary syndrome (PCOS) is a female endocrine disorder that is associated with androgen excess. It is hypothesised that high levels of this typically male hormone in specific windows of development may cause the changes in gene expression of progesterone receptor (PR) in arcuate nucleus, which may drive impaired negative feedback of progesterone in the gonadal axis in PCOS[1]. However, the impact of androgen excess on PR expression, and whether the androgen excess is involved in the modification of neural circuitry in developing brain, is not known.

To address these knowledge gaps, we first investigated the effect of androgen on the expression of steroid hormone receptors in the arcuate nucleus at different developmental times using prenatal androgen exposed (PNA) female mice. Expression of PR and androgen receptor (AR) mRNA in PNA mice did not show a significant difference compared to vehicle controls at postnatal day 25, pre-pubertal stage. The mRNA expression levels at the other ages that are important for the formation of the fertility circuitry, including puberty and adulthood, still remain to be investigated, but are ongoing.

Second, we tested the direct action of androgen on γ-aminobutyric acid (GABA) neuronal development. GABA neurons in the arcuate nucleus are known to exhibit clear morphological and gene expression changes following PNA treatment[2]. Using VGaT-ires-Cre/tdTomato mice, we investigated the effect of androgen on GABA neuron morphology and axonal and dendritic development. Further studies will antagonise AR in these cultured neurons to confirm the direct effect of androgen on GABA neuron development.

Together, these experiments will lead to clearer understanding of the impact of androgen actions in the developing female brain, and both initiation and maintenance of PCOS.

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Poster H38

Prefrontal glutamate projections to the lateral hypothalamus regulate food intake and behaviour.

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Food choices are influenced by both metabolic need and hedonic motivation. The lateral hypothalamus (LH) is uniquely positioned to receive information related to both internal metabolic status and external cues. How the LHA balances homeostatic and hedonic inputs to coordinate feeding and motivated behaviour has not yet been fully elucidated. The cortical regions of the brain including the medial pre-frontal cortex (mPFC) understood to be involved in decision-making processes surrounding food and food reward. Anatomical tracing studies demonstrate monosynaptic glutamate projections from the mPFC to the LHA in Vglut1 cre mice. All animal experiments were conducted in line with Monash Animal Ethics requirements. This study aims to investigate whether these mPFC-LHA glutamatergic circuits are functionally relevant to feeding and motivated behaviours. Injection of retrograde AAV2 expressing cre-dependent ChR2 in the LHA labelled glutamate neurons in the pre- and intralimbic regions of the PFC and photoactivation of this pathway, using a novel wireless optogenetic system, increased food intake. Real-time place preference indicated that mice preferred to avoid the zone with photostimulation suggesting this pathway also induces aversion. Studies are focusing on the role of this pathway in motivation behaviours, reward and context-dependent feeding. These results suggest PFC glutamate neurons projecting to the LHA play an important role in feeding behaviour and aversive behavioural responses.

Poster H39

Loss of ghrelin receptor-positive neurons in the mouse dentate gyrus reduces food intake, neurogenesis and locomotor activity

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Episodic memory is also known as 'what, where, when' memory. In free-living animals, including humans, this kind of memory is critical for food security – remembering what food was found where, and when it was available. One key area for generating this kind of memory is the dentate gyrus (DG) in the hippocampus, an area densely populated with ghrelin receptors (GHSRs). Considering ghrelin's role in appetite regulation, we wondered whether loss of GHSR neurons from the DG would affect memory for food availability. We injected a cre-dependent AAV driving caspase expression to ablate GHSR neurons in the DG (DGGHSR- and DGWT mice, respectively). Mice were restricted to 2x two hour feeding windows within the dark phase in an automated feeding cage system that continuously measured food intake, activity and brown adipose tissue thermogenesis. DGGHSR- mice showed reduced daily food intake compared to DGWT mice. Food anticipatory activity was reduced leading up to the second meal, but not the first, which together with impaired performance on the novel object recognition task indicates memory deficit. DG GHSRs may facilitate memory formation under conditions of caloric insufficiency, because mice lacking GHSRs on DG neurons show deficits in hippocampal-dependent memory tasks, only under conditions of calorie restriction (CR). The same mice showed a reduced re-feeding response following 8 weeks of CR, but did not differ in response to an acute fast. This finding was supported by significantly fewer doublecortin and BRDU-positive cells in the DG four months after caspase-induced cell death, indicating decreased neurogenesis. Together, this suggests loss of GHSR neurons in the DG play a role in memory, and may be important for recall of details of food availability, or arousal, as in their absence mice 'forget to eat' once the immediate pressure of fasting is removed.

Poster H40

The effects of leptin mutations and diet-induced weight gain on glucose homeostasis in the zebrafish

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Leptin is classically thought to be an adipostatic signal communicating information from the adipose tissue to the brain, thereby regulating energy intake and expenditure. Recently, it has been demonstrated that in the zebrafish (*Danio rerio*), contrary to humans and other mammals, leptin does not regulate adipostasis but glucose homeostasis. From an evolutionary perspective, this suggests that a role for leptin in regulation of glucose homeostasis is conserved across vertebrates, whereas its role as an adipostatic factor is likely to be a secondary role acquired by mammals over the course of evolution. Moreover, due to a fish-specific third whole genome duplication event, zebrafish have two leptins: leptin-a and leptin-b. The specific functions of these paralogues are yet to be fully elucidated. In the current study, we utilized the CRISPR/Cas9 system to generate knockout mutant zebrafish for both leptin-a and leptin-b, and for the leptin receptor. 6-month old male mutant fish and wild type control fish were then exposed to either an 8-week long overfeeding regime, consisting of 6 daily feeds, or a standard diet of 1 feed per day. One feed consisted of 20 mg/fish of ZM-400© fish pellets. Food intake was monitored during and after every feed. Body weights were measured weekly. Glucose tolerance tests were performed to assess changes in glucose homeostasis at the beginning and the end of the 8-week period. Collectively, these data provide new insight into the interplay between leptin and glucose homeostasis.

Poster H41

The parabrachial nucleus regulates initial intake of ethanol, sucrose and other solutions.

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Oxytocin receptor-expressing neurons in the parabrachial nucleus ($Oxtr^{PBN}$) suppress water intake, but not food or highly caloric liquids such as Ensure[1], suggesting $Oxtr^{PBN}$ neurons may differentiate solutions based on their caloric content and/or palatability; however, the effect of these neurons on intake of other solutions has not been investigated. In this study, we examined whether $Oxtr^{PBN}$ neurons regulated consumption of caloric solutions (ethanol, sucrose, Ensure), and non-caloric solutions (saccharin, salt). $Oxtr^{Cre}$ mice were injected with the Cre-dependent stimulatory DREADD, hM_3Dq, into the parabrachial nucleus, then tested in a two-bottle choice of water vs another solution (Ensure, ethanol, sucrose, saccharin or salt) at different concentrations. Mice had access to fluids for 4 h/day. When $Oxtr^{PBN}$ neurons were activated by injecting the designer drug, CNO (3 mg/kg ip), we observed a significant decrease in rapid (15-minute) intake of all solutions ($P<0.05$). There was also significantly decreased intake of low palatable, non-caloric solutions at 2 hours in a concentration-related manner ($P<0.05$), but not of highly caloric and/or palatable solutions, suggesting the major effect of $Oxtr^{PBN}$ neurons is on initial rapid fluid consumption. We also assessed expression of Fos in different subdivisions of the parabrachial nucleus after intake of different solutions and observed increased Fos in the dorsolateral PBN following intake of all fluids, in the external lateral PBN following intake of caloric solutions, and in the central lateral PBN following intake of sweet-tasting fluids, suggesting differential activation of PBN subdivisions depending upon fluid properties. This study reveals a key but complex role of the parabrachial nucleus in regulating fluid intake.

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Poster H42

Region-specific deletion of β-catenin leads to impaired glucose tolerance and increased bodyweight

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β-catenin is a signalling molecule in the Wnt-signalling pathway, which has typically been associated with embryogenesis and tumorigenesis. In its active form, β-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. In the current study, we sought to determine the physiological role of β-catenin in the hypothalamus in regulating metabolism. Using transgenic mice in which the β-catenin gene is flanked by LoxP sites (floxed), we performed bilateral injections of AAV2-mCherry-iCre virus into the arcuate nucleus (ARC) to specifically delete the β-catenin gene in that region (β-cat ARC KO). We kept the mice on normal chow for 4 weeks, and then swapped them to high-fat diet for a further 6 weeks, while measuring daily body weight and metabolic analysis. Whilst we did not see any difference in body weight when the mice were on normal chow for 4 weeks post-injection, the β-cat ARC KO animals did show impaired glucose clearance, only in males ($p=0.02$). In addition, when these mice were exposed to high-fat diet, both males and females in the β-cat ARC KO group showed a significant increase in body weight after 6 weeks compared to the control animals ($p<0.0001$ and $p<0.05$, respectively), with no difference in glucose tolerance. We next evaluated measures of energy homeostasis and found that even though there was no difference in energy expenditure among the groups, the maximal oxygen consumption in both males and females of β-cat ARC KO animals were significantly higher than the control animals ($p<0.0001$). This preliminary study indicates that β-catenin may have a critical role in regulating glucose homeostasis, and deleting β-catenin specifically in the ARC exacerbates diet-induced obesity.

Poster H43

Peripartum prolactin and growth hormone concentrations in diet-induced obese mice

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Obesity during pregnancy represents a significant health issue, and can lead to increased risk of complications during pregnancy and impairments with breastfeeding. In rodent models, diet-induced obesity (DIO) leads to poor outcomes for offspring, with a high pup mortality rate. However, the mechanisms leading to these deficits in DIO mothers are poorly understood. Firstly, we show that DIO mice spend less time interacting with their pups following birth. We hypothesis that maternal obesity disrupts the normal changes in hormones that occur just prior to birth, which may be important for the development of maternal behaviour. Mice were fed either a control diet (n=11) or a high fat diet (HFD, n=9) for 6 weeks to generate DIO mice. From day 18 of pregnancy, blood samples (4 µl per sample) were collected from the tail every 3 hours until 24 hours after birth, with birth normally occurring on day 20 of pregnancy. Whole blood samples were then assessed for prolactin and GH concentrations using ultra-sensitive ELISAs. In both control and DIO mice, a surge of prolactin was detected in the 24-hour period before birth, and both timing of the surge and prolactin concentrations were similar between groups. but there were no differences in prolactin concentrations between the two treatment groups. GH increases during pregnancy, but our data is the first to show that in the 24 hours before birth, GH concentrations rapidly drop. Again, we did not observe any differences in GH concentrations between our DIO and control mice. Overall, our results describe the patterns of prolactin and GH secretion during late pregnancy in mice, and demonstrate that these hormones do not differ between DIO and control pregnant mice. Hence, changes in the secretion patterns of these hormones do not explain the impaired early maternal behaviour in DIO mice.

Poster H44

Distribution of O-GlcNAc in glucose sensing areas of the rat brain

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During pregnancy adaptations occur in the maternal brain and body to ensure optimal nutrition for both mother and fetus. Over nutrition in the mother can lead to gestational diabetes mellitus (GDM), which is increasing in direct proportion to type 2 diabetes mellitus (T2DM). GDM in mothers is a significant risk factor for development of cardiovascular disease and pre-eclampsia[1]. The protein modification O-linked N-acetylglucosamine (O-GlcNAc) has been shown to increase in hyperglycemic conditions and plays a role in the etiology of many diseases, including T2DM[2]. Research on the changes of O-GlcNAcylation during pregnancy is scarce. We aimed to investigate the expression of O-GlcNAc in glucose sensing areas of the brain, in pregnant and lactating rats compared with non-pregnant rats. Our hypothesis is that O-GlcNAc will be increased in glucose sensing areas of the brain in pregnant rats that have higher glucose levels, compared to non-pregnant rats.

Female Sprague Dawley rats were divided into 3 groups (Diestrus, 21 days pregnant and day 7 lactating), perfused with 4% paraformaldehyde, brains removed and coronally sliced for immunohistochemistry. Single-label chromogen staining for O-GlcNAc in the ventromedial hypothalamus (VMH), paraventricular nucleus (PVN) and supraoptic nucleus (SON) was carried out. Sections were photographed and analysed using FIJI to count the number of O-GlcNAc-positive cells in each area. There was no significant change between the number of O-GlcNAc-positive cells in pregnant and lactating rats compared with non-pregnant rats in the PVN, SON and VMH.

Double-label immunofluorescence for O-GlcNAc and ΔFosB, a marker of chronic activation, was also carried out. Analysis from this experiment is on-going and will be presented at the conference. The results from our study suggests that in a normal rat pregnancy the expression of O-GlcNAc does not differ from non-pregnant expression. Future research will investigate if expression changes in a rodent model of gestational diabetes.

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Poster H45

Do mice with gestational glucose intolerance have increased O-linked glycosylation in the brain?

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Maternal metabolism changes during pregnancy to support the developing fetus and to prepare for lactation. Among the many maternal changes that occur is an increase in the circulating glucose levels, which in many cases cannot be fully offset by increased insulin production, leading to hyperglycemia and even gestational diabetes. Glucose can affect cell function by O-linked N-acetylglucosamine (O-GlcNAc) modification of proteins, but it is unknown whether this process plays a physiological role in the central regulation of metabolism in pregnancy. . We carried out immunohistochemistry for O-GlcNAc in hypothalamic nuclei involved in energy balance and glucose metabolism to determine if there was any change in O-GlcNAc modifications in the brains of non-pregnant and pregnant, Prlr $^{lox/lox}$/Pdx-Cre$^{+/+}$, Prlr $^{lox/lox}$/Pdx-Cre$^{-/-}$ mice, of which the pregnant Cre$^{+/+}$ mice develop gestational diabetes. Sections were photographed using an Olympus microscope and were analyzed using FIJI image processing software to count the number of O-GlcNAc positive cells in regions of interest. Means ± SEM were compared between non-pregnant and pregnant Cre$^{+/+}$ and Cre$^{-/-}$ mice. In the paraventricular nucleus (PVN) there was a trend towards higher O-GlcNAc expression in pregnant mice with gestational diabetes. This observation was followed up with double-label immunohistochemistry for O-GlcNAc and oxytocin, to investigate if this neuronal population prominent in the PVN is targeted by modifications by O-GlyNAcylation. There was no significant difference between the percentage of double-label oxytocin neurons in the PVN co-expressing O-GlcNAc between the groups. This suggests that another neuronal population in the PVN may express increased O-GlcNAc in mice with gestational diabetes.

Poster H46

Central mechanisms underlying cardiac dysfunction in type 2 diabetes

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Cardiac autonomic dysfunction is a serious complication of type 2 diabetes mellitus (DM). Cardiac sympathetic nerve activity (CSNA) is increased in DM but the central mechanisms underlying increased CSNA remain unknown. The hypothalamic paraventricular nucleus (PVN) contains oxytocin, vasopressin and corticotrophin-releasing-hormone (CRH)-expressing neurones that activate pre-sympathetic spinal cord-projecting catecholaminergic neurones in the rostral ventrolateral medulla (RVLM), an integrative site for central sympathetic outflow. We hypothesised that increased activation of PVN oxytocin, vasopressin and/or CRH neurones drives the increased activation of RVLM catecholaminergic neurones in DM.

Dual-label immunohistochemistry was performed for ΔFosB (a marker of chronic neuronal activation) and tyrosine hydroxylase (TH, the rate-limiting enzyme in catecholamine synthesis) in the RVLM and for ΔFosB and either oxytocin, vasopressin or CRH in the PVN of 20-week-old male DM and non-diabetic (nDM) Zucker Diabetic Fatty rats. Intracerebroventricular colchicine treatment was administered to reduce axonal transport and thereby allow the visualisation of CRH-positive neurones.

More TH-positive RVLM neurones co-expressed ΔFosB in DM rats (9 ± 1, n = 10) than in nDM rats (3 ± 0, n = 8; $P < 0.001$). After colchicine treatment, more CRH-positive PVN neurones co-expressed ΔFosB in DM rats (74 ± 3, n = 8) than in nDM rats (59 ± 5, n = 7; $P < 0.05$). Numbers of neurones co-expressing ΔFosB and either OT or VP in the PVN were not different between DM and nDM rats.

In conclusion, increased CSNA in DM is associated with increased activation of TH-positive RVLM neurones and CRH-positive PVN neurones, indicating increased neuronal activation of specific central sympathoregulatory regions in DM. Further studies will use retrograde tracing from the RVLM to determine whether activated CRH-positive PVN neurones project to the RVLM to increase CSNA in DM.

Poster H47

17-α Estradiol ameliorates age-associated sarcopenia and improves late life physical function in male mice but not in females or castrated males

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Pharmacological treatments can extend mouse lifespan, but lifespan effects often differ between sexes. 17-α estradiol (17aE2), a less feminizing structural isomer of 17-β estradiol, produces lifespan extension only in male mice, suggesting a sexually-dimorphic mechanism of lifespan regulation. We tested whether these anti-aging effects extend to anatomical and functional aging – important in late-life health – and whether gonadally-derived hormones control aging responses to 17aE2 in either sex. While 17aE2 started at four months of age diminishes body weight in both sexes during adulthood, in late-life 17aE2-treated mice better maintain body weight. In 17aE2-treated male mice, the higher body weight is associated with heavier skeletal muscles and larger muscle fibers compared with untreated mice during aging. Maintenance of skeletal muscle in male mice is associated with improved grip strength and rotarod capacity at 25 months, in addition to higher levels of most amino acids in quadriceps muscle. We further show that sex-specific responses to 17aE2 are regulated by gonadal hormones in male mice. Castrated males have heavier quadriceps muscles than intact males at 25 months, but do not respond to 17aE2, suggesting 17aE2 promotes an anti-aging skeletal muscle phenotype similar to castration. Finally, 17aE2 treatment benefits can be recapitulated in mice when treatment is started at 16 months, suggesting that 17aE2 may be able to improve aspects of late life function even when started after middle-age.

Poster H48

Neuroimmune interaction in the ageing brain

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High fat diet (HFD) and obesity are triggers that cause widespread and lasting brain inflammation and this effect is mediated by activation of microglia, major immune cells of the brain. This neuroinflammation leads to cognitive dysfunction as we age. Since ageing itself also promotes brain inflammation we hypothesized here that HFD and ageing in combination are more detrimental to cognition than either alone and that microglia play a role in this. We therefore aimed to test if we could improve cognitive dysfunction in the aged by strategically suppressing microglial hyper-activity. To this end, we used CRISPR/Cas9 genome editing to develop a *Cx3cr1-Dtr* transgenic Wistar rat with a diphtheria toxin receptor (*Dtr*) gene expressed in the promoter for the fractalkine receptor (*Cx3cr1*), highly expressed on microglia and monocytes. Upon administration of diphtheria toxin (DT), microglia and monocytes are transiently depleted in *Cx3cr1-Dtr*, but not wildtype rats. This allows us to specifically investigate the role of microglia/monocytes in normal and disease states including ageing- and HFD-related cognitive decline. To directly assess the role of microglia in ageing-related vulnerability to HFD, we fed aged (and young) wildtype and *Cx3cr1-Dtr* rats HFD for 8 weeks and assessed cognitive function with and without microglial ablation. Our data suggest microglia are important contributors to ageing-related cognitive vulnerability that may be a useful potential target for treatment against cognitive decline.

Poster H49

Analysis of Ca^{2+} Imaging in Rat Adrenal Medullary Slices

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Adrenal medulla chromaffin cells secrete catecholamines and biological active peptides to assist in the adaptation to stress. The primary trigger for catecholamine secretion is the action of acetylcholine released from splanchnic nerve terminals onto nicotinic receptors. Chromaffin cells are also responsive to other stimuli including co-transmitters (PACAP), endocrine signals (angiotensin II) and immune derived cytokines (IL-6). The aim of the study is to investigate how these signals are integrated within the adrenal medulla using Ca^{2+} fluorescence as a readout. Studies have addressed this question using isolated cells in culture, but these cannot be related to anatomical organization of the medulla. Here we examined this issue in intact tissue.

Slices of the adrenal medulla (200 μm) were prepared from adult male rats using a vibratome. Slices (3-6 per adrenal) were transferred to oxygenated aCSF for 1 to 3 h. They were transferred to the recording chamber containing fresh aCSF where they were loaded for 20 min with 5 μM of Fluo-4 AM dye. They were then perfused with 1mL per min of aCSF. Individuals cells within slices were then recorded under fluorescence microscopy (475 nm) and analysed using Clampfit software and Prism. Slices were first perfused with aCSF to obtain the basal response (10 min) and then exposed to nicotine (100 μM, 10 min) followed by K^+ (60 mM) to test cell viability.

Under basal conditions, the cells showed a variety of Ca^{2+} profiles, with some showing small Ca^{2+} pulses of varying frequency while others showing no response. Nicotine exposure produced a rapid and much larger Ca^{2+} response which remained elevated for up to 10 min. Application of K^+ resulted in a similar increase in Ca^{2+} to nicotine but in a larger proportion. Ongoing experiments are now investigating the effects of other possible chromaffin cell stimulants.

Poster H50

A lipolytic model for the effects of oxytocin on 3T3-LI differentiated adipocytes

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A model of oxytocin and the regulation of metabolic status has described one of oxytocin synthesis and release from the neurohypophysis in response to leptin, to suppress further leptin release (reviewed by Eladb and Sabry, 2015). In addition, a lipogenic role for oxytocin has been suggested (Eckertova *et al.*, 2011) consistent with an insulinergic action. This model, however, may be incorrect. Oxytocin reduces fat mass in the absence of either leptin or leptin receptor signalling, thereby challenging the interdependence between leptin and oxytocin. An oxytocin induced production of the lipolytic/anti-lipogenic adipokine prostaglandin E_2 (PGE_2) might account for this. It was hypothesized that oxytocin treatment of 3T3-LI differentiated adipocytes would increase (PGE_2) secretion and induce lipolysis. Media from 3T3-L1 differentiated adipocytes treated with oxytocin (0, 10, 25 or 50 $nmol.L^{-1}$; n=4 independent treatments) for 24hrs were assayed for PGE_2 and leptin. Phase contrast images of treatment groups were taken and lipid droplet numbers compared. Harvested cells were analysed for triglyceride (Nile red detection) and free fatty acid (Nile blue detection) by flow cytometry (Boumelhem *et al*., 2017). Both [PGE_2] and [leptin] were significantly increased by oxytocin treatment. Lipid droplet numbers were also significantly increased following 10 $nmol.L^{-1}$ oxytocin treatment, suggesting a lipolytic effect. This was supported by an apparent decrease in triglyceride content but increase in free fatty acid content following 10 $nmol.L^{-1}$ oxytcin treatment. In conclusion, oxytocin stimulates lipolysis in adipocytes.

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