BSN Annual Meeting in Exeter

Programme and Abstracts

#Exeter 2023

Contents

Programme

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Sunday 3 September 2023

Time	Activity	Room
1500	Arrival and optional registration	Forum Street Open Area
1700	**Public Lecture followed by drinks reception** Anna Murray - *Decoding the Biological Clock: Exploring the Genetics of Human Reproductive Ageing*	Forum Alumni Auditorium
1900	**Dinner (People staying in Holland Hall)**	Holland Hall

Monday 4 September 2023

Time	Activity	Room
0800	Arrival and registration	Registration and speaker preview area, Forum Building
0900	Opening Remarks – Neil Evans, BSN President **Symposium: *Stress - New frontiers*** • Eder Zavala - *Quantitative analysis of high-resolution daily profiles of HPA axis hormones* • Naresh Hanchate - *Mapping brain circuitry of stress using new single-cell genomic tools* • Michael Emmerson - *Acute thermal stress upregulates transcript expression of genes involved in cellular stress in the hypothalamus of nestling zebra finches depending on embryo acoustic experience* • Lora Martucci - *The endolysosomal cation channel TPC regulates social behaviour by controlling oxytocin secretion*	Forum Alumni Auditorium
1030	Coffee	Forum Street Open Area
1100	**Mortyn Jones Lecture:** Valerie Simonneaux *Neuroendocrine mechanisms of seasonal adaptation*	Forum Alumni Auditorium
1200	Lunch/networking/posters	Forum Street Open Area
1400	**Selected ECR oral communications** • Isabella Marinelli - *Uncovering physiological mechanisms modulating common circadian distributions of epileptiform discharges* • Sasha Howard - *Role of Variants in Methyl-CpG-binding protein 2 (MECP2) in GnRH regulation and Precocious Puberty* • Lauryn New - *Investigating the astrocyte neurone crosstalk involved in insulin sensing in the dorsal vagal complex* • Roongrit Klinjampa - *Salt loading reduces central osmoresponsiveness in magnocellular neurones in vivo* • Su Young Han - *In vivo GCaMP recordings of GnRH neuron activity in freely-behaving mice*	Forum Alumni Auditorium
1530	Coffee	Forum Street Open Area

1600	**Symposium: *Metabolism - Beyond the hypothalamus*** • Jenni Harvey on *Food for thought: Exploring the cognitive enhancing and therapeutic potential of leptin* • Dan Brierley on *Mapping GLP-1 signalling pathways in the gut-brain axis reveals novel strategies for obesity pharmacotherapy* • **Astrid Van Irsen** on *Activation of Drd1 MSNs in the medial shell of the nucleus accumbens projecting to the lateral hypothalamic area improves glucose homeostasis* • **Sophie Buller** on *Oligodendrocyte plasticity contributes towards the regulation of glucose homeostasis in adult mice*	Forum Alumni Auditorium
1900	**Dinner (People staying in Holland Hall)**	Holland Hall

Tuesday 5 September 2023

Time	Activity	Room
0700	Fun run or walk	
0800	Arrival and registration	Registration and speaker preview area, Forum Building
0900	**Symposium: *Rhythms of life*** • Lukasz Chrobok - *Circadian timekeeping in the brainstem satiety centre* • Beatriz Baño-Otálora - *Brighten up your circadian clock: Impact of daytime light intensity on behaviour and brain clock function in a diurnal mammal.* • Jarne Jermei - *The impact of time-restricted feeding on microglial function* • Callum Stewart on *The Molecular Architecture of a Circannual Timer in a Seasonal Rodent*	Forum Alumni Auditorium
1030	Coffee	Forum Street Open Area
1100	**Alison Douglas Lecture:** Manuel Tena-Sempere *Exploring the neuroendocrine basis of puberty and reproduction: Kisspeptins and beyond*	Forum Alumni Auditorium
1200	Lunch/networking/posters	Forum Street Open Area
1330	BSN AGM	Forum Alumni Auditorium
1400	**Symposium: *Neuroendocrine adaptations during pregnancy & development*** • Sharon Ladyman - *Neuroendocrine control of body temperature and physical activity during pregnancy* • Laura Dearden - *Early life programming of obesity via a hypothalamic miRNA involved in fatty acid sensing* • Helen Eachus - *Elevated glucocorticoid: from adaptive plasticity to allostatic overload*	Forum Alumni Auditorium
1530	Coffee	Forum Street Open Area
1600	**NC3R workshop** with Dr Jessica Eddy – NC3Rs Regional programme manager, Cardiff and the SouthWest of England • Embedding 3Rs principles into your experimental design and funding applications	Forum Alumni Auditorium
1800-2200	**Conference dinner (Pre-booking required)**	Reed Hall

Wednesday 6 September 2023

Time	Activity		Room
900	**Symposium: *Reproductive Neuroendocrinology*** • Allan Herbison - *Recording the GnRH pulse and surge generators in vivo* • Elodie Desroziers - *Unusual suspect: role of microglia in the neuroendocrine disorder polycystic ovary syndrome* • Deyana Ivanova - *The amygdala, a key upstream regulator of the hypothalamic GnRH pulse generator*		Amory Parker Moot Room
1030	Coffee		Xfi Student Study Area
1100	**Julia Buckingham Award and Michael Harbuz Prize Lectures** and prize giving • Julia Buckingham Award winner: Simon J. Guillot *Hypothalamus-driven sleep alterations in a neurodegenerative disease: Amyotrophic Lateral Sclerosis* • Michael Harbuz Prize awardee: Teodora Georgescu *Suppression of fever, but not sickness behaviours in late pregnancy in mice*		Amory Parker Moot Room
1200	Lunch/networking		Xfi Student Study Area
1330		ECR only event (hyperlink to web page with programme)	Seminar rooms, Building One
1400	Departure		
1500		Coffee (ECRs only)	

Speaker abstracts

Sunday 3 September 2023

Public Lecture

Anna Murray - Decoding the Biological Clock: Exploring the Genetics of Human Reproductive Ageing

As women age, their ability to conceive and carry a pregnancy to term declines, a process known as reproductive ageing. This phenomenon is governed by a complex interplay of genetic and environmental factors, and recent advances in genomics have shed new light on the underlying mechanisms.

Our research uses large scale genomics data to identify the genes and biological processes that govern reproductive ageing. Humans are born with all the eggs that they will produce in life and this pool reduces across the lifespan. Genetic variation influences the process of ovarian decline mostly by affecting how the ovary repairs damage to DNA, which could occur through environmental effects or be part of the normal way eggs are generated. Some genes when not expressed cause earlier entry into menopause, which could cause infertility. Reduced expression of other genes can extend reproductive lifespan and provide potential targets for extending fertility, through improved assisted conception technologies.

Understanding the genetics of reproductive ageing is not only important for fertility and family planning, but also has implications for broader health outcomes. For example, early menopause has been linked to an increased risk of certain diseases, including osteoporosis and cardiovascular disease. The genomics of human reproductive ageing represents a fascinating and rapidly evolving field of research with broad implications for human health.

Monday 4 September 2023

Symposium: Stress - New frontiers, 9am

Eder Zavala - Quantitative analysis of high-resolution daily profiles of HPA axis hormones

The Hypothalamic-Pituitary-Adrenal (HPA) axis is the key regulatory pathway responsible for maintaining homeostasis under conditions of real or perceived stress. Endocrine responses to stressors are mediated by adrenocorticotrophic hormone (ACTH) and corticosteroid (CORT) hormones. In healthy, non-stressed conditions, ACTH and CORT exhibit highly correlated ultradian pulsatility with an amplitude modulated by circadian processes. Disruption of these hormonal rhythms can occur as a result of stressors or in the very early stages of disease. Despite the fact that misaligned endocrine rhythms are associated with increased morbidity, a quantitative understanding of their mechanistic origin and pathogenicity is missing. Mathematically, the HPA axis can be understood as a dynamical system that is optimised to respond and adapt to perturbations. Normally, the body copes well with minor disruptions, but finds it difficult to withstand severe, repeated or long-lasting perturbations. Whilst a healthy HPA axis maintains a certain degree of robustness to stressors, its fragility in diseased states is largely unknown, and this understanding constitutes a critical step toward the development of digital tools to support clinical decision-making. This talk will explore how these challenges are being addressed by combining high-resolution biosampling techniques with mathematical and computational analysis methods. This interdisciplinary approach is helping us quantify the inter-individual variability of daily hormone profiles and develop novel "dynamic biomarkers" that serve as a normative reference and to signal endocrine dysfunction. By shifting from a qualitative to a quantitative description of the HPA axis, these insights bring us a step closer to personalised clinical interventions for which timing is key.

Naresh Hanchate – Mapping brain circuitry of stress using new single-cell genomic tools

The mammalian brain contains millions to billions of neurons highly interconnected in a vast array of neural circuits. The molecular identities and functions of individual neuronal components within specific circuits are yet undefined. Previously, we developed a new method, termed "Connect-seq", by combining retrograde viral tracing and single-cell transcriptomics to uncover the molecular identities of upstream neurons in a specific circuit. Application of Connect-seq to hypothalamic neurons controlling physiological responses to fear and stress revealed subsets of upstream neurons that express diverse constellations of signaling molecules and can be distinguished by their anatomical locations. However, it is still unable to reconstruct a molecular map of the entire circuit, largely due to the insufficiency of data. To overcome these limitations, we developed a significantly improved method, termed 'nuc-Connect-seq', by combining single-nucleus transcriptomics and retrograde viral tracing and employing droplet-based microfluidics. Due to its rapid tissue dissociation and barcoding strategy, nuc-Connect-seq enables rapid profiling of thousands of single-neuron transcriptomes in a specific circuit in a massively parallel fashion. Moreover, nuc-Connect-seq has added advantage over Connect-seq in reducing methodology-induced transcription of activity-regulated genes, enabling its application to investigate neuronal activation of individual neuronal components within specific circuits.

Michael Emmerson - Acute thermal stress upregulates transcript expression of genes involved in cellular stress in the hypothalamus of nestling zebra finches depending on embryo acoustic experience

Michael G. Emmerson1*, Mylene M. Mariette2, David F. Clayton3, Elisabetta Versace1, Kate L. Buchanan2, Julia M. George4

Embryos detect and respond to parent vocalisations, with different parent vocalisations shifting individuals onto alternative developmental trajectories and constructing different later-life phenotypes. For example, zebra finches exposed to parent heat calls (produced at temperatures >26°C) as embryos develop into adults with greater thermal tolerance than those exposed to control calls. What mechanisms underpin this is unclear, but differences in stress physiology are plausible. Vertebrates have similar neuroendocrine stress responses, like hypothalamic-pituitary-adrenal axis activation and secretion of stress coping hormones (e.g., catecholamines, glucocorticoids). Hypothalamic nuclei regulate stress and temperature responses, with heat calls possibly shaping hypothalamic gene expression to enhance later-life thermal tolerance. Zebra finch eggs (n = 68) were therefore exposed to parent heat or control calls during incubation, and twelve days after hatching birds were subject to an acute thermal challenge (40°C). Brains were collected before or after the acute thermal challenge. Hypothalamus samples were then collected and whole genome transcript abundance quantification was conducted via QuantSeq. An acute thermal challenge upregulated transcript expression of six genes, including a stress responsive transcription factor (ZBTB16), glucocorticoid receptor co-chaperone (FKBP5), and a marker of DNA damage (DDIT4). Embryo heat call exposure blunted the rise in FKBP5, but not DDIT4 or ZBTB16, and resulted in greater transcript expression of a gene inhibiting cell death (FAIM2) compared to tet call exposure. Heat calls therefore improve thermal tolerance by altering stress-responsive gene transcription in glucocorticoid and cell survival pathways, revealing that parent vocalisations shape the neurodevelopmental trajectories of their offspring to improve stress resilience.

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2. Deakin University, School of Life & Environmental Sciences, Centre for Integrative Ecology
3. Clemson University, Department of Genetics and Biochemistry
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Lora Martucci - The endolysosomal cation channel TPC regulates social behaviour by controlling oxytocin secretion

L. Martucci1, J.-M. Launay2, C. Grimm3, A. Galione1, J.-M. Cancela4;

Oxytocin (OT) is a prominent regulator of many aspects of mammalian social behaviour and stored in large dense-cored vesicles (LDCVs) in hypothalamic neurons. It is released in response to activity-dependent Ca2+influx, but is mainly dependent on Ca2+ release from intracellular stores, which primes LDCVs for exocytosis. Despite its importance, critical aspects of the Ca2+-dependent mechanisms of its secretion remain to be identified. In a recently published paper, using immunostaining, we showed that lysosomes are in close proximity with the OT LDCVs, and that the direct activation of endolysosomal two-pore channels (TPCs)provides the critical Ca2+ signals to prime OT release by increasing the releasable LDCV pool without directly stimulating exocytosis. Using radioimmunoassays, we observed a dramatic reduction in plasma OT levels in TPC knockout mice, and impaired secretion of OT from the hypothalamus demonstrating the importance of neuropeptide vesicles priming for activity-dependent release. Furthermore, we showed that activation of type 1metabotropic glutamate receptors sustains somatodendritic OT release by recruiting TPCs. The priming effect could be mimicked by a direct application of NAADP, the endogenous agonist of TPCs, or a selective TPC2agonist, TPC2-A1-N. Confocal calcium imaging revealed reduced aspects of Ca2+ responses evoked by glutamatergic stimulation in presence of pharmacological inhibitors of TPCs or TPC deletion. Finally, behavioural experiments revealed that mice lacking TPCs exhibit impaired maternal and social behaviour, which is restored by direct OT

administration. This study demonstrates an unexpected role for lysosomes and TPCs in controlling neuropeptide secretion, and in regulating social behaviour.

LL Martucci et al., PNAS, 2023
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Mortyn Jones lecture, 11am

Valerie Simonneaux - Neuroendocrine mechanisms of seasonal adaptation

The annual change in the nocturnal production of the pineal hormone melatonin has long been known to be pivotal for the seasonal adaptation of biological functions in mammals. The discovery that melatonin acts on the pars tuberalis cells to control the synthesis of TSH, which in turn acts on the hypothalamic tanycytes to modulate local thyroid hormone metabolism, has been a breakthrough in our understanding of the neuroendocrine mechanisms underlying seasonal adaptation. In this lecture, I will discuss how this melatonin/TSH/T3 signal impacts hypothalamic circuits to synchronize various biological functions with the seasons.

Oral Communications: Selected ECR abstracts, 2pm

- Isabella Marinelli - *Uncovering physiological mechanisms modulating common circadian distributions of epileptiform discharges*
- Sasha Howard - *Role of Variants in Methyl-CpG-binding protein 2 (MECP2) in GnRH regulation and Precocious Puberty*
- Lauryn New - *Investigating the astrocyte neurone crosstalk involved in insulin sensing in the dorsal vagal complex*
- Roongrit Klinjampa - *Salt loading reduces central osmoresponsiveness in magnocellular neurones in vivo*
- Su Young Han - *In vivo GCaMP recordings of GnRH neuron activity in freely-behaving mice*

Symposium: Metabolism - Beyond the hypothalamus, 4pm

Jenni Harvey - Food for thought: Exploring the cognitive enhancing and therapeutic potential of leptin

Evidence is accumulating that the endocrine hormone leptin is a potent regulator of hippocampal synaptic function. Indeed, recent studies have highlighted the cognitive enhancing actions of leptin as this hormone regulates many aspects of synaptic function including glutamate receptor trafficking, neuronal morphology and activity dependent synaptic plasticity. Furthermore, leptin-insensitivity is associated with impairments in hippocampal-dependent memory tasks and activity-dependent synaptic plasticity. However, there is significant decline in the ability of leptin to regulate hippocampal synaptic function with age and leptin dysfunction has been linked to neurodegenerative disorders like Alzheimer's disease. Here, we will review the impact of leptin-driven changes on hippocampal excitatory synaptic function, and how this is providing valuable insight into leptin's role in higher cognitive functions in health and disease.

Dan Brierley - Mapping GLP-1 signalling pathways in the gut-brain axis reveals novel strategies for obesity pharmacotherapy

Centre for Cardiovascular & Metabolic Neuroscience; Department of Neuroscience, Physiology & Pharmacology; University College London; UK

The anorexigenic peptide glucagon-like peptide-1 (GLP-1) is secreted from gut enteroendocrine cells and brain preproglucagon (PPG) neurons, which respectively define the peripheral and central GLP-1 systems. PPG neurons in the nucleus tractus solitarii (NTS) have been assumed to link the peripheral and central GLP-1 systems in a unified gut-brain satiation circuit, in which GLP-1 released from the gut in response to meals acts via vagal and/or hormonal gut-brain pathways to trigger central release of GLP-1 from PPGNTS neurons to suppress further eating. However, direct evidence for this hypothesis is lacking, and the gut-brain connectivity necessary for this circuit has not been demonstrated.

We tested this hypothesis using complementary circuit mapping and behavioural approaches in transgenic mouse models which allowed selective manipulation of neuronal populations within the peripheral and central GLP-1 systems. Contrary to the unified GLP-1 circuit hypothesis, we determined that PPGNTS neurons are not a major target of either vagal or hormonal signalling pathways from the peripheral GLP-1 system. Furthermore, PPGNTS neurons are not activated by or required for the anorectic effects of the GLP-1RA drugs semaglutide or liraglutide. Consistent with the alternative hypothesis that peripheral and central GLP-1 systems are anatomically and functionally distinct entities, we demonstrated that chemogenetic activation of PPGNTS neurons, concurrent with peripheral semaglutide administration, suppressed eating more potently than either manipulation alone. Furthermore, this apparently additive effect could be replicated pharmacologically, as the 5-HT2CR agonist anti-obesity drug lorcaserin required PPGNTS neurons for its anorectic effect, and individually anorexigenic doses of lorcaserin and liraglutide suppressed eating to a greater extent when administered concurrently than either monotherapy alone.

We therefore conclude that central and peripheral GLP-1 systems suppress eating via apparently independent gut-brain circuits, providing a rationale for investigation of strategies for additive pharmacological manipulation of these systems for the treatment of obesity.

Astrid Van Irsen - Activation of Drd1 MSNs in the medial shell of the nucleus accumbens projecting to the lateral hypothalamic area improves glucose homeostasis

Astrid A. S. van Irsen(*)1-4, Tess Kool1-4, Anouk M. Corstens1-4, Margo Slomp1-4, Andries Kalsbeek1-4, Susanne E. la Fleur1-4.

The nucleus accumbens (NAc) plays a critical role in reward and food-motivated behavior. It contains a core that is surrounded by a medial and lateral shell. The majority of accumbal neurons consists of medium spiny neurons (MSNs), which nearly all either express dopamine D1 receptors (Drd1) or dopamine D2 (Drd2) receptors. Previous findings showed that in rats deep brain stimulation of the NAc medial shell (mshNAc) increased blood glucose and plasma glucagon concentrations compared to sham or core stimulation. Moreover, infusion of vanoxerine, a dopamine reuptake inhibitor, into the mshNAc of rats decreased endogenous glucose production, blood glucose and plasma glucagon concentrations compared to vehicle. These effects may seem contradictory, however, this can likely be explained by the opposing effects of Drd1 and Drd2 activation under different conditions and their distinctive projections. Based on previous viral tracer experiments in rats (unpublished), we know mshNAc MSNsDrd1 mainly project to the lateral hypothalamic area (LHA).

To determine the role of this mshNAcDrd1>LHA connection in glucose metabolism, we employed chemogenetics in Drd1-Cre transgenic male rats to target MSNsDrd1 in the mshNAc. We compared overall activation of MSNsDrd1 in the mshNAc to specific activation of the mshNAcDrd1>LHA connection, on glucose tolerance. Activation of the mshNAcDrd1>LHA connection improved glucose tolerance (drug effect $p<0.05$ and interaction effect drug x time $p<0.01$, $n=9$), whereas overall mshNAc MSNDrd1 activation did not result in a significant effect. Overall, these results increase our understanding of neural circuitry underlying glucose metabolism. Currently we are replicating this experiment in female rats.

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Sophie Buller - Oligodendrocyte plasticity contributes towards the regulation of glucose homeostasis in adult mice

Sophie Buller1,*, Emily Staricoff1, Christine Riches1, Anthony Tsang1, Kentaro Ikemura2, Sara Kohnke1, Sam Virtue1, Antonio Vidal-Puig1, Satoshi Hirohata2, William Richardson3, Michael Schwartz4, Mark Evans1, Clemence Blouet1

Unlike what occurs in other brain regions, new oligodendrocytes (OL) and myelin are continuously produced and replaced in the adult median eminence (ME). While ME oligodendrocyte progenitor cell (OPC) proliferation, differentiation and myelin turnover are regulated by nutritional cues, detailed understanding of the physiological and functional significance of ME OL plasticity is lacking. Here, we demonstrate that manipulation of blood glucose levels rapidly alter ME OL lineage progression. To test the role of OL plasticity in the regulation of glucose homeostasis, we used Pdgfra-CreERT2;R26R-GFP;Myrffl/fl (Myrffl/fl) mice to blunt new OL production. Myrffl/fl mice rapidly become glucose intolerant and insulin resistant, show an exacerbated counter-regulatory response (CRR) to neuroglycopenia and exhibit significant hypercorticosteronaemia compared to controls. To disentangle the role of OPC differentiation vs. myelination in this phenotype, we used Pdgfra-CreERT2;R26R-GFP;Mbpfl/fl (Mbpfl/fl) mice to prevent new compact myelin generation. Glucose tolerance, insulin sensitivity and the CRR are unaffected by Mbp deletion despite profound hypocorticosteronaemia in this model, suggesting that compact myelin in the ME

is not essential for glucose homeostasis, and new OLs contribute towards the regulation of glucose homeostasis through myelin-independent mechanisms. We identified ADAMTS4, an aggrecanase exclusively expressed by myelinating OLs as a potential candidate mediating these effects. ADAMTS4 administration to the ME acutely decreased food intake, increased plasma corticosterone and reduced insulin sensitivity compared to vehicle in C57BL/6J mice. Collectively these data indicate a role for adult-born OLs, independent of new compact myelin generation, in the regulation of glucose homeostasis and the hypothalamic-pituitary-adrenal axis.

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Tuesday 5 September 2023

Symposium: Rhythms of life, 9am

Lukasz Chrobok - Circadian timekeeping in the brainstem satiety centre

Lukasz Chrobok, Charlotte Muir, Tanya Kaur, Jake Ahern, Hugh D. Piggins

Feeding is critical for survival and is orchestrated through the interplay of brain and gut signals. A subset of brain structures localised in the hypothalamus and brainstem coordinate both the amount and circadian times of day that food is consumed. Although the master circadian clock is localised in the suprachiasmatic nuclei of the hypothalamus (SCN), local extra-SCN timekeeping mechanisms are important for circadian physiology including the daily patterning of feeding.

Daily timekeeping mechanisms in the hypothalamus are much researched, but circadian rhythms in the brainstem are under-explored. Thus, we are evaluating potential local clock control over the brainstem's dorsal vagal complex (DVC). Real-time bioluminescence recording of clock gene expression (PER2::LUC) ex vivo reveals that in developing and adult DVC, PER2 rhythms can be sustained for days to weeks, even when isolated from the SCN. Assessing neuronal activity in DVC brain slices on a multielectrode array recording platform, shows that the PER2 rhythms are accompanied by electrophysiological rhythms with peak neuronal firing at late day. Manipulating the rodent diet reveals that this temporal variation in DVC neurophysiological activity is notably dampened by consumption of a high-fat food. Importantly, assessment of gene expression in vivo by qPCR and RNAscope in situ hybridisation reveals that core clock genes are rhythmically expressed in the DVC with their expression present in neurochemically diverse cellular populations. This suggests that the potential for circadian timekeeping in the DVC is not due to a distinct subpopulation of cells, but rather arises from multiple different neuronal and non-neuronal groups.

Collectively, these studies point to how circadian mechanisms alter molecular and cellular activity in the DVC. Future research determining how diet and circadian signals interact to influence DVC function are necessary. Our research adds to a growing literature on the importance of understanding local extra-SCN clocks in the daily patterning of physiology and behaviour.

Beatriz Baño-Otálora - Brighten up your circadian clock: Impact of daytime light intensity on behaviour and brain clock function in a diurnal mammal

Centre for Biological Timing | Division of Diabetes, Endocrinology, & Gastroenterology | School of Medical Sciences | Faculty of Biology, Medicine & Health | The University of Manchester | Manchester

Circadian or ~24h rhythms constitute a fundamental feature of mammalian physiology and behaviour. These rhythms are orchestrated by a brain master clock, the suprachiasmatic nucleus of the hypothalamus (SCN), which is synchronized with the external world mainly by light. Circadian rhythms evolved under conditions in which there was a large difference in light intensity between day and night. However, the advent of artificial lighting has allowed humans unprecedented freedom to choose when to be active and exposed to light. So, we now live in a society in which we experience artificial light at night, but also, we deprive ourselves from exposure to bright light during the day by spending most of the waking hours in relatively dimly lit indoor environments. Over the past years, there has been a growing understanding of the potential negative effects that exposure to light-at-night can have on human health. However, our knowledge of the impact of exposure to low light intensity during the day is much more limited.

In this talk, I will present our work using a recently established diurnal rodent model, Rhabdomys pumilio to understand the impact of daytime light intensity and self-selected light exposure on daily rhythms in physiology and behaviour, and clock function. Our results show that bright daytime light enhances the

robustness of behavioural and physiological rhythms and increases the amplitude of circadian rhythms in electrical activity in the SCN and gene expression in central and peripheral tissues. These findings reveal an impact of light on circadian amplitude and highlight the potential importance of daytime light exposure for circadian health.

Jarne Jermei - The impact of time-restricted feeding on microglial function

Jarne Jermei*1, Han Jiao1, Chun-Xia Yi1

Microglia are the resident brain immune cells, initiating local immune responses. It is well established that an obesogenic high-fat diet (HFD) causes brain inflammation. HFD consumption stimulates microglial activity also in the hypothalamus, resulting in decreased numbers of the appetite-curbing pro-opiomelanocortin (POMC) neurons, which eventually leads to obesity. Previously, we observed that in rats fed a HFD, hypothalamic microglial cells are constantly activated, instead of showing daily rhythmicity, probably due to their constantly elevated 24-hour food consuming behaviour. The goal of this study was to investigate whether indeed eating at the wrong time of the day affects microglial activity. To answer this research question, a time-restricted feeding (TRF) experiment was performed. Specifically, Wistar rats fed with a chow diet or HFD were divided into three groups, i.e. ad libitum, only light-phase feeding or only dark-phase feeding. After 4 weeks of TRF, rats were sacrificed every 4 hours along the light/dark cycle to study the daily rhythm in microglial activity and immunometabolism. HFD dark-fed rats showed a reduction in adiposity compared to the light-fed and ad libitum-fed rats. Interestingly, first analyses of the RNA sequencing data showed that the amplitude of the daily rhythm of microglial clock genes was altered in light-fed rats, compared to the dark- and ad libitum-fed rats, especially in the HFD groups. In conclusion, our data suggest that HFD food intake restricted to the active period has beneficial metabolic outcomes, preventing obesity. Importantly, the microglial clock is clearly affected by the timing of food intake.

1.Laboratory of Endocrinology, Amsterdam University Medical Center, Amsterdam Gastroenterology & Metabolism, University of Amsterdam, Amsterdam, The Netherlands.

Callum Stewart – The Molecular Architecture of a Circannual Timer in a Seasonal Rodent

Calum Stewart* – University of Glasgow
Timothy A. Liddle – University of Glasgow
Gaurav Majumdar - University of Allahabad
Christopher J. Marshall – University of Bristol
Tyler J. Stevenson – University of Glasgow

Seasonal alterations in energy balance, reproduction and behaviour allow animals to survive harsh seasonal conditions. The neuroendocrine mechanisms allowing for the precise timing of the seasonal response are not fully elucidated. To date, there have been no in-depth studies into the molecular changes in hypothalamic nuclei over the entirety of the seasonal waveform. This study aimed to generate high frequency transcriptomic data from individual hypothalamic nuclei across the seasonal waveform. Siberian hasmters (N = 54) were held under constant short photoperiod (8L:16D) for up to 32 weeks. At 4-week intervals, groups of animals were culled (n = 6), and physiological measurements were taken. Body mass, adipose mass and testes mass were recorded. Physiological factors showed previously reported seasonal changes, initial reduction followed by spontaneous recovery ($P < 0.05$). Individual hypothalamic nuclei were then dissected, and the molecular programme was assessed by Oxford Nanopore sequencing. This revealed several hundred transcripts differentially expressed by exposure to short photoperiod (FDR < 0.1). The transcripts and patterns of expression were unique to individual nuclei. Many of these transcripts showed dynamic changes throughout the seasonal waveform, indicating a complex relationship between

central expression and the seasonal phenotype. This study is the first to generate a high dimensionality and high frequency seasonal transcriptome and uncovers the neuroendocrine mechanisms which may underlie the seasonal response.

Alison Douglas lecture, 11am

Manuel Tena-Sempere - Exploring the neuroendocrine basis of puberty and reproduction: Kisspeptins and beyond

Instituto Maimónides de Investigación Biomédica de Cordoba (IMIBIC); Department of Cell Biology, Physiology and Immunology, University of Cordoba; Hospital Universitario Reina Sofia; and CIBER Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, 14004 Cordoba, Spain

Pubertal maturation and reproductive function are intimately related phenomena, controlled by sophisticated developmental programs and regulatory circuits, centered around the hypothalamic-pituitary-gonadal axis. In this highly-hierarchical neuroendocrine system, hypothalamic neurons, producing gonadotropin-releasing hormone (GnRH), are masterpiece for timed pubertal onset and fertility, operating under the control of a wide variety of central transmitters and peripheral hormones, whose nature and mode of action have been partially exposed over the last decades. In this context, a major breakthrough was the identification, back in 2003, of the reproductive roles of kisspeptins, as major stimulators of GnRH neurons, responsible for their dynamic regulation along the lifespan. Of note, this seminal finding not only transformed our understanding of the mechanisms controlling puberty and reproduction, but boosted also a New Age in reproductive neuroendocrinology, which has led to the discovery of additional novel signals, neuroendocrine pathways and molecular regulatory mechanisms responsible for the precise control of puberty and fertility, and their interplay with other essential body functions, such as energy homeostasis and metabolism. In this lecture, I will discuss examples of major developments and recent findings in this area, aiming also to highlight new pathways for further progress of this very active domain of neuroendocrine research.

Symposium: Neuroendocrine adaptations during pregnancy & development, 2pm

Sharon Ladyman – Neuroendocrine control of body temperature and physical activity during pregnancy

Centre for Neuroendocrinology, University of Otago, Dunedin New Zealand

Numerous changes in physiology and behaviour are required during pregnancy as the maternal body adapts to met the demands of providing the fetus with the optimal nutrients and environment for healthy growth and development. A particularly striking behavioural change is a profound reduction in voluntary running wheel activity (RWA) that occurs as soon as mice become pregnant. The hormone prolactin increases due to mating and our recent work demonstrated that prolactin action in the preoptic area of the hypothalamus (POA) drives this pregnancy-induced suppression of voluntary physical activity. The POA is a brain region associated with multiple homeostatic and behavioural roles, including maternal behaviour, but another POA function of considerable interest is the control of thermoregulation. Pregnancy represents a significant challenge to maternal thermoregulation, as the thermogenic effects of the pregnancy hormone progesterone and the metabolic heat generated from fetal development must be dissipated to prevent teratogenic effects of hyperthermia. We have demonstrated a role of prolactin, acting in the POA, in influencing thermoregulatory responses in pregnancy. Firstly, AAV-Cre-mediated deletion of Prlr in the POA resulted in significant hyperthermia throughout pregnancy. Secondly, mice lacking Prlr in glutamatergic neurons (including heat-sensitive POA neurons) have poor pregnancy outcomes specifically when housed at elevated environmental temperature (30°C) while control pregnant mice are unaffected. We hypothesize that during pregnancy prolactin acts on thermoregulatory circuits in the POA to sensitise them to increases in temperature and more rapidly engage counter-regulatory actions to lower body temperature. Moreover, we predict that these thermoregulatory actions of prolactin may underlie the suppressive effect of prolactin on voluntary physical activity, such that pregnant mice terminate engagement in voluntary physical activity sooner to protect against activity-induced elevations in body temperature. These adaptive changes provide resilience to the thermal challenge of pregnancy, enabling mothers to cope with increases in environmental temperatures.

Laura Dearden - Early life programming of obesity via a hypothalamic miRNA involved in fatty acid sensing

In utero exposure to maternal obesity programs an increased risk of obesity. Animal models have shown that offspring obesity is often preceded by increased food intake, however, the mechanisms that mediate these changes are not understood. Using a mouse model of maternal diet-induced obesity we observed increased intake specifically of a high-fat pellet in adult offspring of obese mothers. Through small RNA sequencing, we identified programmed overexpression of miR-505-5p in the hypothalamus of offspring of obese mothers that is established in the fetus and remains to adulthood, and confirmed in vitro that fatty acid exposure increases expression of miR-505-5p in hypothalamic neurons. Pulsed SILAC analysis demonstrated protein targets of miR-505-5p are enriched in pathways involved in fatty acid metabolism. These include key components of neuronal fatty acid sensing pathways that we find to be associated with BMI in human genetic studies. Over-expression of miR-505-5p decreased neuronal fatty acid uptake and metabolism in neurons in vitro. Importantly, intra-cerebroventricular injection of a miR-505-5p mimic in mice resulted in increased intake specifically of a high-fat pellet. Collectively these data suggest that maternal obesity induces over-expression of miR-505-5p in offspring hypothalamus, resulting in altered fatty acid sensing and increased intake of high-fat diet. This represents a novel mechanism by which exposure to obesity in pregnancy programs obesity in offspring.

Helen Eachus - Elevated glucocorticoid: from adaptive plasticity to allostatic overload

Glucocorticoids (GC) are thought to be implicated in stress-related psychiatric disorders. Acute stress triggers adaptive responses allowing animals to respond appropriately to threat, however, exposure to chronic GC is associated with negative effects on the brain and behaviour in later life. This apparent dichotomy of effects exposes the question of whether the effects of GC might be temporally dynamic. It is known that environmental stress can increase organism growth rates but at a cost to fitness in later life. However the developmental trajectory of GC-induced effects on the brain is unclear. In an optogenetic zebrafish model, our recent work describes how elevated GC causes precocious hypothalamic development followed by failed maturation. In GC-exposed animals, hypothalamic proliferation is initially increased, the hypothalamus is larger, and one of its associated behavioural functions, feeding, develops early. However, precocious development is followed by a rapid decline. In GC-exposed animals, excess hypothalamic progenitor cells fail to differentiate and proliferative radial glia are lost from the hypothalamus. This correlates with altered numbers of differentiated neurons, which are known to regulate food intake, plus suppressed feeding and growth. Our data highlight the developmental dynamics of hypothalamic neurogenesis following GC exposure and indicate that the hypothalamus is a GC-sensitive brain region. Alteration of hypothalamic neurogenesis is likely a mechanism through which GC exerts its effects on behaviour and its associated pathologies. Further understanding of how stress and GC exposure can alter development trajectories at the molecular and cellular level is of critical importance to reduce the burden of mental and physical ill health across the life-course.

NC3Rs workshop: Embedding 3Rs principles into your experimental design & funding applications

Dr Jessica Eddy

A well-designed and correctly analysed experiment not only increases the scientific validity of your results but can also reduce the number of animals you use. This workshop will cover the principles of good experimental design for animal studies and will focus on the following.

- How to determine the experimental unit.
- Minimising bias in animal studies through randomisation and masking (blinding).
- The importance of using both sexes in animal studies.
- Sample size calculations and justifying animal numbers for grant applications.
    - What funders want to see.
- NC3R resources

Wednesday 6 September

Symposium: Reproductive Neuroendocrinology, 9am

Allan Herbison - Recording the GnRH pulse and surge generators in vivo

Allan E. Herbison
Department of Physiology Development and Neuroscience, University of Cambridge, UK

Fertility in mammals is critically dependent upon episodic gonadotropin hormone secretion. It is now clear that kisspeptin neurons located in the arcuate nucleus (ARN) represent the gonadotropin-releasing hormone (GnRH) pulse generator in both males and females. In rodents, these kisspeptin neurons target GnRH neuron processes termed dendrons in the ventrolateral ARN to drive pulsatile gonadotropin secretion. In females, a second episode generator operates once per cycle to drive the preovulatory luteinizing hormone surge. This appears to involve a population of kisspeptin neurons located in the rostral periventricular area that integrate endocrine and circadian signals and project to nearby GnRH neuron cell bodies. A clear understanding of these episode generators requires their activity patterns to be established in vivo. We have used GCaMP-based fibre photometry approaches combined with in vivo CRISPR gene editing and local neurotransmitter receptor modulation to determine the activity patterns of kisspeptin and GnRH neurons in freely behaving male and female mice. This has generated predictable as well as surprising observations and, together, enable the construction of a simple model explaining the cyclical control of fertility in females.

Elodie Desroziers - Unusual suspect: role of microglia in the neuroendocrine disorder polycystic ovary syndrome

Aisha Sati1,2, Mel Prescott1, Kay Potapov1, Rebecca Campbell1, Elodie Desroziers1,3.

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

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Deyana Ivanova - The amygdala, a key upstream regulator of the hypothalamic GnRH pulse generator

Stress profoundly impedes pubertal development, disrupts reproduction and suppresses pulsatile luteinizing hormone (LH) secretion in mammals. The amygdala is the integrative centre for processing emotions, regulating the stress response and controlling anxiety. The amygdala also sends inhibitory signals to reproductive centres where previous findings have shown an inhibitory influence of the amygdala on the timing of puberty as well as on pulsatile LH secretion in adults. Stress alters neuronal activity within the posterodorsal medial amygdala (MePD), increasing the expression of Urocortin3 (Ucn3) and its receptor while enhancing the inhibitory output from the MePD to key hypothalamic reproductive centres. In this work we investigate the function of the stress neuronal circuitry within the MePD, which is involved in modulating pubertal timing, pulsatile LH secretion and dynamic corticosterone secretion in the presence of stress in mice. We find a functional role for MePD Ucn3 neurons and efferents to the hypothalamic paraventricular nucleus in modulating the activity of the hypothalamus pituitary gonadal and the hypothalamus pituitary adrenal axes, thus the MePD may act as a central hub integrating anxiogenic cues with the stress and reproductive axis. We also employ mathematical modelling to dissect the involvement of neurokinin 3 receptor signalling within the MePD in mediating stress-induced suppression of pulsatile LH secretion in mice.

Julia Buckingham Award and Michael Harbuz Prize lectures, 11am

Julia Buckingham Award winner: Simon J. Guillot - Hypothalamus-driven sleep alterations in a neurodegenerative disease: Amyotrophic Lateral Sclerosis

ALS is a progressive motor neuron disease inexorably leading to a premature death. Sleep disturbances have been ascribed to respiratory insufficiency, muscle cramps, spasticity, or restless legs syndrome, all leading to increased wakefulness. However, a recent neuropathological study in ALS patients described a loss of orexin-producing neurons, a neuropeptide involved in sleep and metabolic regulation, undermining the idea that sleep alterations are linked to central and peripheral changes. Yet, sleep changes are poorly characterized in ALS, and their relationships to motor symptom onset, disease progression and orexin neurons remain unknown. Here, we used electroencephalography coupled with indirect calorimetry recordings to characterize sleep and energy metabolism in two mouse models of ALS -Superoxide Dismutase 1 G86R (Sod1G86R) and Fused in Sarcoma (FusΔNLS).

In both Sod1G86R and FusΔNLS mice, electroencephalograms showed an increase in wakefulness and a decrease in rapid eye movement (REM) as well as non-rapid eye movement (NREM) episodes before the onset of major motor troubles. We did not observe an altered number of Orexin-positive neurons in the lateral hypothalamus of these mice.

Moreover, Suvorexant®, a drug antagonizing both orexin receptors, induced an increase in REM sleep and a decrease in wake quantities compared to control in both mouse lines. Interestingly, Sod1G86R and FusΔNLS mice displayed an increase in body temperature, energy expenditure and locomotor activity, as well as a lower respiratory quotient that were successfully rescued in both mouse models by the drug.

Sleep analysis in presymptomatic gene carriers and ALS patients matched with healthy controls is ongoing. Hence, preliminary results tend to point at sleep impairments in ALS patients and presymptomatic gene carriers, following our previous results in mice.

Thus, our results show that two mouse models of ALS display sleep and metabolic impairments and provide pharmacological evidence for the involvement of the lateral hypothalamus in these defects.

Michael Harbuz Prize winner: Teodora Georgescu - Suppression of fever, but not sickness behaviours in late pregnancy in mice

Teodora Georgescu[1,3], Zin Khant Aung[1], David R. Grattan[1,2], Rosemary S. E. Brown[3]

Introduction
Postpartum aggression in females is conserved across many species and enables a mother to guard her young from danger and potential threats. This protective behaviour is typically exhibited by mothers and not virgin females, but it is unknown how hormones act to induce this behaviour after birth of offspring. We have previously identified a population of prolactin sensitive neurones in the hypothalamic ventromedial nucleus (VMN), a region known to direct aggressive behaviours. As prolactin is high during pregnancy and lactation, we hypothesised that prolactin acts in the VMN to regulate maternal aggression.

Results
By using c-fos immunoreactivity in a prolactin receptor (Prlr)-reporter mouse line, we found that VMN Prlr-expressing neurones increased their activity following exposure to a juvenile male intruder. These neurones were predominantly glutamatergic. Next, we used a conditional knockout model to delete Prlr from glutamatergic neurones (Prlrlox/lox/VGlut2-Cre). Surprisingly, in the absence of prolactin signalling in glutamatergic neurones, maternal mice display heightened aggression towards intruders. Lactating

Prlrlox/lox/VGlut2-Cre mice showed significantly reduced latencies to attack, increased episodes of attacking and a greater time spent attacking. To specifically knockout Prlr from the VMN, we bilaterally administered an AAV-Cre into the VMN of adult Prlrlox/lox mice. As seen with the vGlut2-knockout mice, these mice showed significantly heightened maternal aggression compared to control mice. To understand how prolactin regulates maternal aggression, we pharmacologically blocked prolactin action during lactation but saw no changes in maternal aggression. Utilising calcium imaging, we similarly found that the majority of the neurones that express the Prlr in the VMN (74.07%) did not respond to prolactin with changes in intracellular calcium. This suggests prolactin is not acutely acting in the VMN to regulate behaviour, but is likely to be acting through a transcriptional pathway to change responsiveness of these neurones to other inputs.

Conclusion

Together, these data demonstrate a novel role for prolactin in aggressive behaviour, with prolactin being important in moderating the level of aggression in a lactating mouse.

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Poster abstracts

Listed in alphabetical order by surname. Presenting author is marked with an asterisk *.

P1: Restorative properties of P. alba extract against pancreatic β -cell destruction and hyperlipidemia in streptozotocin-rat model of Type 2 diabetes mellitus

Tawakaltu Abdulrasheed-Adeleke[1], Bashir Lawal[2], Sani Saidu[3] and Yunusa O. Ibrahim[1]*

P. alba is a reputable medicinal plant employed for the treatment of diabetes and its associated complications. However, scientific literature on the potential of the extract from this plant is scanty. Herein, the protective effect of the methanolic extract of *P. alba* on an experimentally induced type 2 diabetes rat model was evaluated. Wistar rats with streptozotocin (STZ)-induced diabetes were randomly allocated into five groups containing five animals each as follows: a normal glycemic group (I), diabetic rats receiving distilled water group (II), diabetic rats treated with 150 mg/kg of *P. alba* group (III) diabetic rats treated with 300 mg/kg of *P. alba* group (IV), and diabetic rats treated with 100 mg/kg metformin group (V). All treatments were administered for 21 consecutive days through oral gavage. Results revealed that treatment with *P. alba* extract significantly restored alterations in levels of fasting blood glucose (FBG), body weight loss, serum and pancreatic insulin levels, and pancreatic histology. Result also showed that *P. alba* significantly attenuated the dyslipidemia (increased cholesterol, low-density lipoprotein-cholesterol (LDL-C), triglycerides, and high-density lipoprotein (HDL) in diabetic rats), serum biochemical alterations [alanine transaminase (ALT), aspartate transaminase (AST), alanine phosphatase (ALP), blood urea nitrogen (BUN), creatinine, uric acid, and urea] and full blood count distortion in rats with STZ-induced diabetes. The present study thus provided evidence of antidiabetic potential of the P. alba extract for the treatment of diabetes and its associated complications. Hence, P. alba maybe employed for the treatment of diabetes melitus.

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P2: Perinatal Dietary Protein Deficiency disrupts reproductive function across two subsequent generations (F_1 and F_2) of the rat model

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Early life exposure to a protein-deficient diet and general maternal/child crosstalk during the critical stage of development is known to increase susceptibility to dysfunction. Maintaining an efficient reproductive function is an important trait that depends on the proper alignment of the reproductive makeup. This study sought to unravel some pathophysiological associations between perinatal dietary protein deficiency and reproductive function across two generations. Rats in four (4) groups were fed different grades of protein diets (5%, 10%, 21% protein diets, and control (16-18%) perinatally. Reproductive function analysis (hormone profiling, onset of puberty, estrous cyclicity, and sexual response) and morphometric analysis of the ovarian structure were carried out to assess associated consequences. The expression profile of some selected ovarian folliculogenic genes was also assessed. There was significant reduction in the fertility index at F1, with increased severity at F2 ($P \leq 0.05$). A low protein diet posed suboptimal intrauterine condition, which was linked to increased prenatal morbidity and mortality, low birth weight at F1 and F2 respectively, delayed onset of puberty, followed by induced cycle irregularity, altered follicular maturation and endocrine dysfunction, evidence of ovarian degeneration and discernible cyst, more severe in 5%PD. There was downregulation of Inhibin-□, while Aromatase was upregulated across the generations. CEBPA, FGFR1, and ER□ remain modulated across the generations. Exposure to protein inadequacy at the perinatal age is consequently associated with ovarian gene modulations that may mediate the epigenetic effects underlining reproductive function, evidenced as life-long changes in endocrine events, ovarian maturation, late pubertal attainment, and irregular cycle.

P3: The Impact of Emotional State on Cognitive Processes: An Eye-Tracking Study on Facial Expression Recognition

*Keisuke Adachi**
Miyu Kashiwa
Asami Oguro-Ando
Hiroko Ichikawa

Research has shown that an individual's emotional state can significantly influence cognitive processes. For instance, previous studies reported that happy individuals perceive others' facial expressions more holistically than sad individuals. However, it remains unclear whether this finding holds in everyday situations, given that people do not always exhibit intense emotional expressions, and the use of facemasks during the COVID-19 pandemic has limited the visibility of facial expressions over the past two years. Thus, the current study aims to investigate how an observer's emotional state affects their eye-scanning pattern for subtle facial expressions and whether this pattern differs between expressions with and without a facemask.

In the experiment, participants viewed the emotion-arousing movie either of positive or neutral emotion. The former was the comedy video, and the latter was the weather forecasting news. After the movie watching, participants conducted the facial expression recognition test, and their eye scanning pattern were measured by the eye-tracker. During the experiment, we measured participant's emotional feeling by the questionnaire and facial EMG measurement to analyse the only data obtained from participants who successfully induced target emotion.

Our results showed that the scanning pattern did not differ depending on the observer's emotional state, while the recognition accuracy of facial expression did. Observers recognized the subtle happiness expressions as happiness when they are feeling happy more often than when they were not feeling happy. These results indicated that the observer's emotional state affect in recognition of subtle expression without modulating scanning pattern.

P4: Optogenetic stimulation of MePD kisspeptin neurones blocks the LH surge in female mice

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Ovulation, essential for reproduction, depends on production of a timely luteinizing hormone (LH) surge. Positive feedback actions of estradiol drive the generation of LH surge. The medial amygdala (MeA) is commonly known for its role in higher-order emotion processing and modulating responses to sexual olfactory cues. The MeA, particularly its posterodorsal subnucleus (MePD), contains kisspeptin (Kiss1) neurons, known to have an up-stream regulatory influence on gonadotrophin-releasing hormone (GnRH) pulse generator frequency. Early stimulation/lesion studies implicated the MeA in control of the LH surge, however, it's unclear whether this involves LH surge advancement or blockade.

In this study, we proposed that the MePD Kiss1 neurones play a role in LH surge generation. Female ovariectomized (OVX) Kiss-Cre mice with low-level estradiol capsule implants were injected with a viral construct containing stimulatory channelrhodopsin or control virus and implanted with fibreoptic canulae unilaterally in the MePD. Mice were injected with estradiol benzoate followed by progesterone 24-h later to induce LH surges. Blood samples were collected at 30-min intervals from 13:00-21:00h with or without optogenetic stimulation (473nm, 5Hz, 10mW, 5-ms pulse width) from 18:00-19:30h.

Selective optogenetic stimulation of MePD Kiss1 neurones completely blocked the steroid-induced LH surge (area under curve [ng/(ml.min)]: no stimulation control group, 1208±34.45 (n=6) vs MePD Kiss1 stimulation group, 190.93±12.04 (n=6); control virus with optical stimulation, 877.23±19.24 (n=5); mean±SEM; $P<0.05$ vs MePD Kiss1 stimulation group).
These findings offer novel insight on how amygdala kisspeptin may regulate the preovulatory LH surge in mice with implications for olfactory or stress related infertility.

P5: Conductance mechanisms and the role of electrotonic coupling in generating the TIDA oscillation in the male rat

Jake Ahern [1,2], David Lyons [1,3], Alan R. Champneys [2], Hugh Piggins [1]*

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2 – Engineering Mathematics, University of Bristol, UK
3 - School of Medical Sciences, University of Manchester, UK

The anterior pituitary hormone prolactin (Prl) is critical for pregnancy and parental behaviors. In non-pregnant female and male rats, low Prl levels result from inhibitory dopamine (DA) released by tuberoinfundibular dopaminergic (TIDA) neurons. These hypothalamic neuroendocrine cells exhibit synchronized bursting oscillations that are lost following the pharmacological blockade of electrical coupling. The conductance mechanism and role of network coupling in TIDA oscillations remain unclear.

Here, we present a Hodgkin-Huxley model exploring conductance mechanisms underlying oscillations in single cell and 2-cell networks. We show that slowly activated persistent sodium and calcium-dependent potassium currents can drive the oscillation and electrical coupling synchronizes intrinsically oscillating cells. The model compares favourably to single-cell and network wide electrophysiological recordings, strengthening our proposed mechanism. In two-cell networks, coupling supports sustained network oscillations between an oscillating and a silent cell or two silent cells, highlighting the possibility that coupling may be critical for generating network oscillations, as well as synchronizing them. Physiologically relevant neuromodulators induce a phasic to tonic switch in TIDA activity, and our model confirms that this could be driven by a TRPC current.

Understanding TIDA oscillations and network coupling is critical, given their impact on Prl regulation. Our findings elucidate the underlying mechanism and emphasize the role of electrotonic coupling in the generation and maintenance of TIDA oscillations. By shedding light on these processes, this study advances our understanding of the cellular and circuit mechanisms governing neuroendocrine dopamine release - important new information regarding the central regulation of reproductive control.

P6: Variants in the Neurodevelopmental Gene Bone Morphogenic Protein/Retinoic Acid Inducible Neural-Specific 2 (BRINP2) are Associated with Severe Delayed Puberty

Dr Yasmin Al-Sayed, Dr Sasha Howard, Dr Roberto Oleari and Professor Leonardo Guasti.*

Gonadotropin-releasing hormone (GnRH) is the master hormone regulating the reproductive axis and its pulsatile secretion is crucial for puberty onset and fertility. Disruption in GnRH neuron development or hypothalamic function can lead to absent or delayed puberty (DP), with a phenotypic spectrum from severe DP to partial or complete Hypogonadotropic Hypogonadism (HH). We aimed to identify novel genetic etiology of severe DP by screening and identifying variants in associated genes in our cohort of patients; and ascertain the functional effects of identified variants of interest. Whole exome sequencing (WES) was performed on DNA samples from 180 probands with DP from our patient cohorts to identify potentially pathogenic novel, or rare coding variants in relevant gene pathways. Integrative analysis was performed on genomic data from human patients combined with transcriptomics analysis of rodent immortalized and primary GnRH neurons to determine novel regulators of GnRH neuronal development and function. BRINP2 was identified as a candidate gene of interest as it was found to be significantly upregulated during GnRH neuronal development in these single cell transcriptomics analyses. BRINP2 is localised to the olfactory bulb, a key site during GnRH neuron migration, and has been associated with neurodevelopmental disorders (NDD). WES analysis identified three rare predicted pathogenic variants in BRINP2 in four unrelated probands with severe DP or partial HH, in combination with NDDs. We have investigated the role of BRINP2 in GnRH biology via wildtype and mutant protein expression and sub-cellular localization, as well as tissue expression in mouse hypothalamic tissue across development.

P7: The role of dopamine signalling in age-related cognitive decline

Junior Bowen[1], Katie Hanna[2], Andrew MJ Young[3], Gisela Helfer[2] and Samantha L McLean[1]*

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Age-related cognitive decline is a major concern for the ageing population, with deficits in learning and memory particularly impacting the quality of life. The neurotransmitter dopamine is strongly involved in the regulation of cognitive functions including learning and memory. These neuroendocrine processes are coordinated by the pre-frontal cortex and the hippocampus in the brain. Previous studies have shown a link between aging and cognitive decline, however, the relationship between dopamine, aging and cognitive function is poorly understood and remains a clinical unmet need.

Our study aimed to understand the impact of ageing on memory function and dopamine signalling in the pre-frontal cortex and hippocampus by elucidating the mechanisms underlying cognitive decline.
Using in vivo microdialysis, we found changes in dopamine and metabolites in the pre-frontal cortex of young (6 months) and aged (20 months) adult female Lister Hooded rats. These changes were accompanied with an impairment in cognition in the aged group measured by the novel object recognition task, a test of short-term episodic memory. Young rats explored the novel object significantly more than the familiar object ($P<0.05$) whereas aged rats spent equal time exploring both objects. Furthermore, qPCR and Western blot analyses showed changes in dopamine signalling pathway (receptors, transporters, catabolism) and markers of synaptic integrity and plasticity (PSD-95, synaptophysin, BDNF) in aged rats in the pre-frontal cortex. Interestingly, in the hippocampus some of these markers showed opposite effects.

Our findings suggest that dopamine signalling plays a role in learning and memory and may underpin the age-related cognitive decline.

P8: Leptin Signalling Promotes Axonal Regeneration

Jessica S. Chadwick[1,2,] Elisabeth Serger[1], Guiping Kong[1], Luming Zhou[1], Franziska Müller[1], Ilaria Palmisano[1], Phoebe Liddell[1], Linshan Chu[3], Yuyang Yan[1], Simone Di Giovanni[1]*

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The mammalian central nervous system (CNS) lacks regenerative capacity whereas the peripheral nervous system is critically limited by slow axonal regenerative rate and delayed target re-innervation, leading to irreversible loss of function of target organs. However, understanding the biological drivers within the limited peripheral system may highlight pivotal translational targets to manipulate in the CNS. Neuronal regenerative ability is influenced by environmental factors such as exercise and diet. Several of these pathways, including modifications in gene transcription and protein synthesis, mitochondrial metabolism and release of neurotrophins, can be activated by intermittent fasting (IF). IF has in turn been shown to increase synaptic plasticity, neurogenesis, and peripheral axonal regeneration. However, the molecular mechanisms underlying IF- dependent pro-regenerative signalling remains to be investigated. Here we show that IF promotes leptin signalling within dorsal root ganglia (DRG) sensory neurons, initiating a transcriptional programme to enhance axonal regeneration following peripheral and central injury in the mouse. Leptin signalling via the leptin receptor is required for efficient axonal regeneration, and treatment with leptin after sciatic nerve injury significantly enhances axonal regeneration, accelerating recovery of sensory function. Our results indicate for the first time, a unique role of leptin within the nervous system distinct from its activity as a homeostatic regulator. RNA sequencing of sciatic DRG neurons following leptin overexpression and spinal cord injury reveal a large number of differentially expressed genes and an exciting role of neuronal leptin treatment in driving pro-regenerative transcriptional changes, offering a promising avenue in the treatment of human axonal injuries.

P9: Bilateral Oophorectomy Activated Hortega and Inflammatory Cells Response Exacerbating Long Term Continuous Unpredictable Stress Mediated Anxiety, Depression, And Memory associated ageing in Rat Cerebral Cortex

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Menopausal women have an increased risk of depression, anxiety, and memory dysfunction, which may be due to a loss of the oestrogen hormone, which has antidepression, antianxiety, and memory enhancement functions in the body. Unfortunately, the specific process by which oestrogen enhances neurological functions is still unknown. Present research involves in the bilateral oophorectomy, linked with a long term continuous unpredictable stress (LTCUS) model in SD female rats. Different behavioural experiments demonstrated that bilaterally oophorectomized rats receiving LTCUS had significantly higher anxiety, memory loss, and depression levels than control group rats, and sucrose concentrations were clearly lowered. The elevated plus maze test experiment reduced the time and duration of open arm entries. In the open field test, the frequency of boundary crossings, rearings, centre square entries, and centre square duration were significantly reduced; grooming duration was enhanced. The forced swimming test raised the rate of rat immobility while decreasing the number of rats swimming and crawling. Further, bilateral oophorectomy reduced the blood concentrations of oestrogen and corticosterone in rats. In the process, immunofluorescence results indicated that bilateral oophorectomy significantly increased the number of activated hortega cells in the cerebral cortex as well as the concentrations of hortega cell markers, and iNOS. Finally, polymerase chain reaction results showed that after bilateral oophorectomy stimulation, inflammasome and pro-oxidative markers such as iNOS, IL-1, IL-6, TNF-alpha, and CX3CR1 were upregulated in the cerebral cortex of bilaterally oophorectomy rats, whereas the anti-inflammatory markers Arg1 and hortega cell negative regulatory factor CD200 were downregulated. To conclude this research, bilateral oophorectomy increases LTCUS-mediated anxiety, memory loss, and depression in rats. The activation and polarisation of hortega cells and inflammasome further activate the inflammatory cytokine, which causes neural network dysfunction associated with ageing.

P10: Predicting druggable target for obesity from human hypothalamic cellular models

Hsiao-Jou Cortina Chen[1], Andrian Yang[1, 2], Simone Mazzaferro[1], Iman Mali[1], Peter Kirwan[1], Sanya Aggarwal[1], Venkat Pisupati[1], Matthew Livesey[3], Katherine Kentistou[4], Eleanor Wheeler[4], Claudia Langenberg[4], John Perry[1,4], John Marioni[2], and Florian Merkle[1]*

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Melanocortin neurons located in the hypothalamus, including those expressing proopiomelanocortin (POMC), have been identified as one of the primary regulators of energy homeostasis. Although mutations of the POMC gene in both mice and humans lead to hyperphagia and obesity, the limited availability of a human hypothalamic POMC neuronal model has hampered investigation of human obesity-related disease mechanisms. In the present study, we aim to identify obesity-associated genes that are differentially expressed in POMC-expressing neurons. To achieve this, we have utilised an improved protocol to differentiate POMC neurons from human induced pluripotent stem cells (iPSC) by including an enhanced media that synchronises the neurogenesis and accelerates synaptogenesis in addition to co-culturing with astrocytes. By combining electrophysiological, morphological, and transcriptome analysis, we have demonstrated higher differentiation efficiency and greater functional maturation of iPSC-derived POMC-expressing neurons differentiated using this enhanced protocol. Using single cell RNA sequencing, we have then identified genes enriched within the specific POMC neuronal population and integrated these data with genetic association results from large-scale population studies of childhood and adult obesity. We then prioritised candidate genes that are enriched and associated with obesity based on the availability of FDA-approved drugs and selected these for in vivo follow-up experiments. Candidates were advanced to mouse models of diet-induced obesity and we have identified a drug that significantly reduced body weight on its own and potentiated the effects of glucagon-like peptide 1 agonist. Together, this unique approach has the potential to identify candidate genes for disease modelling and therapeutic targeting.

P11: Molecular characterization in human neurons of rare loss of function variants in BSN gene, associated with severe obesity.

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Obesity is a major risk factor for many common diseases and has a significant heritable component. While previous studies have identified many rare variants with large effects on obesity risk, there are likely many unknown genes with highly penetrant effects. Therefore, we performed whole exome-sequence analyses for adult BMI in up to 587,027 individuals. Through that rare loss of function variants in the BSN gene were identified. One in ~6500 individuals carry a heterozygous protein truncating variant (PTV) in BSN, which confers a 6.6, 3.7 and 3-fold higher risk of severe obesity (BMI >40kg/m2), non-alcoholic fatty liver disease and type 2 diabetes, respectively, but no apparent effect on childhood adiposity. Furthermore, we found the common polygenic score to exhibit an effect on BMI twice as large in BSN PTV carriers than non-carriers. BSN is a scaffold protein of the presynaptic cytoskeletal matrix, where synaptic vesicles dock, fuse, release their neurotransmitter content and are recycled. Together with its interaction partner Piccolo, BSN participates in the formation of Golgi-derived Piccolo-Bassoon transport vesicles that are transported along axons to the synapse. It is also relevant for the maintenance of presynaptic structures, and regulation of communication between the nucleus and presynaptic boutons. Therefore, we explored the functional consequences of BSN deletion in CRISPR-Cas9 edited human iPSC hypothalamic neurons. That highlighted a network of differentially expressed genes that were collectively enriched for genomic regions associated with BMI, and suggest a role for degenerative neuronal synaptic function and neurotransmitter release in the etiology of obesity.

P12: Investigating the Role of CaSR in Mediating Effect on Glucose Tolerance via α-cell Signalling

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Protein ingestion stimulates both insulin and glucagon release. Recent studies have shown chronic glucagon receptor agonism may have beneficial effects on glucose homeostasis, possibly through stimulating insulin secretion via intra-islet signalling. The Calcium sensing receptor (CaSR) is reported to modulate gastroenteropancreatic hormone secretion. CaSR also acts as an amino acid sensor, thus, CaSR may function as an amino acid sensor mediating the effects of protein ingestion on glucose tolerance.

We investigated the role of CaSR on PPG-expressing cells in protein-induced improvements in glucose tolerance.

The effects of amino acids on α-cells were investigated using islets isolated from PPG-Cre; GCaMP6 mice, which specifically expresses the cytosolic calcium indicator GCaMP6f in α-cells, following tamoxifen induction. The role of CaSR signalling in mediating the effects of oral administration of whey on glucose tolerance was assessed in vivo using PPG-Cre; CaSR flox mice in which CaSR is knocked out of PPG-expressing α-cells and gastrointestinal L-cells.

Glutamic acid had the most potent stimulatory effect on intracellular calcium concentration followed by ornithine and alanine. L-Phenylalanine, the most potent amino acid activator of the CaSR, also increased intracellular calcium levels, though to a lesser extent. The effect of whey on glucose tolerance was blunted in PPG-Cre CaSR flox mice, suggesting that CaSR signalling in α-cells and/or L-cells is involved in the effect of protein on glucose tolerance. These data suggest that CaSR in PPG-expressing cells partly mediates the beneficial effects of protein on glucose tolerance. Further work is required to identify the involvement of other complementary mechanisms.

P13: Oligodendrocyte lineage cells, growth hormone deficiency, and regulation of the neuroendocrine hypothalamus.

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The neuroendocrine hypothalamus regulates the secretion of pituitary hormones, which in turn control many important physiological processes. Deficiencies in pituitary hormones are therefore sources of considerable morbidity. Specifically, regarding radiation-induced hypopituitarism, oligodendrocyte precursor cells (OPCs) are a prime target for research, given their proliferative nature and sensitivity to radiation. In this work, we show that clinically relevant doses of X-irradiation targeted to the juvenile mouse brain result in ablation of OLIG2+ cells, including OPCs, and a reduction in pituitary growth hormone (GH) consistent with a lack of post-natal pituitary differentiation and a lack of normal weight gain. In adult mice, while irradiation ablates OLIG2+ cells, the effect on pituitary GH is less severe. At the hypothalamic level, the structure of the growth hormone-releasing hormone (GHRH)+ neuronal network is unchanged, but electrophysiological recordings reveal that irradiated GHRH+ neurons are hyperpolarised. In a separate mouse model, we observe that loss of the transcription factor SOX8 also results in a reduction of pituitary GH. Using in situ hybridisation, we find that SOX8 is not expressed in the pituitary, but is primarily expressed throughout oligodendrocyte lineage cells in the brain. Interestingly we also saw that loss of SOX8 causes an unexpected increase in the number of OPCs and oligodendrocytes, in contrast to the effects of X-irradiation, suggesting that loss of a discrete function or sub-type of oligodendrocyte lineage cell is sufficient to cause GHD. Ongoing work seeks to characterise mechanistic interactions between oligodendrocyte lineage cells and GHRH+ neurons in our models of GHD.

P14: Selective activation of GFRAL neurons is sufficient to promote sickness behaviour and metabolic adaptation.

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The growth/differentiation factor 15 (GDF15) is a stress responsive cytokine circulating at high levels in a number of pathological conditions. Previous work has shown that high GDF15 levels lead to a reduction in body weight and food intake. In rodents, GDF15 mediates sickness behaviour including nausea, conditioned taste avoidance (CTA), anorexia and delayed gastric emptying[1,2]. GDF15 acts through its receptor GFRAL localised in the area postrema and nucleus of the tractus solitarius in the brainstem1. In addition to its anorexic effect, it is also proposed that GDF15 has an adaptive role during inflammatory conditions by supporting critical organ function[3].

We used a Cre-dependent hM3Dq designer receptor model to selectively activate GFRALAP/NTS neurons. We confirmed that the designer drug, clozapine N-oxide, induced c-Fos in GFRALAP/NTS cells, as well as downstream targets in the lateral PBN, CeA, oval BNST, PVH and SON.

Selective activation of GFRAL neurons was sufficient to elicit a reduction in body weight, suppress food intake, delay gastric emptying and produce a strong aversive response measured as a CTA. Thus, we completely recapitulated the response to exogenous GDF15. Furthermore, chemogenetic activation of GFRALAP/NTS neurons led to a significant reduction in body temperature, respiratory exchange ratio and energy expenditure, measured by indirect calorimetry. These data suggest that GFRAL neurons are involved in all aspects of sickness behaviour, including metabolic adaptation.

[1] *Worth et al., 2020, eLife 9:e55164*
[2] *Borner et al., 2020, Cell Reports 31:107543*
[3] *Luan et al., 2019, Cell 178:1231*

P15: Fasting and the 5:2 diet, a role in adult hippocampal neurogenesis and spatial memory?

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New neurones are generated throughout life in the mammalian brain in a process known as adult hippocampal neurogenesis (AHN). Since this phenomenon grants a high degree of neuroplasticity influencing learning and memory, identifying factors that regulate AHN may be important for ameliorating age-related cognitive decline and neurodegeneration. We show that calorie restriction (CR) enhances AHN and improves hippocampal-dependent memory, mediated by the hormone, ghrelin. Intermittent fasting (IF), which offers more flexibility than conventional CR, also promotes aspects of AHN. The 5:2 diet is a popular form of IF linked to health benefits, however its effects on AHN and spatial memory are not well characterised. We hypothesised that the 5:2 diet would enhance AHN in a ghrelin-dependent manner.

To assess this, we quantified new adult-born neurones and new neural stem cells (NSCs) in the hippocampus of adolescent and adult wild-type and ghrelin-null mice following six weeks on a 5:2 diet. We report an age-related decline in neurogenic processes and identify ghrelin-receptor mediated regulation of new adult born NSC formation. However, the 5:2 diet did not affect new neurone or NSC formation. Consistent with this finding, 5:2 diet did not alter performance on a spatial learning and memory task. These data demonstrate that the 5:2 diet used in this study does not increase AHN or improve associated memory function.

Our findings suggest that distinct dietary restriction regimens differentially regulate neurogenesis in the adult hippocampus and that further studies are required to identify optimal protocols to support cognition during ageing.

P16: Spatial mapping of GLP-1R cell populations in the human hypothalamus

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The hormone and neurotransmitter GLP-1 acts on receptors in multiple organs in the body, including the brain. Previous studies in animal models show that GLP-1 acts in several brain regions, including the hypothalamus, to modulate food intake. With GLP-1R agonists such as liraglutide and semaglutide used for treating obesity, it is important to understand the mechanisms of GLP-1 action in the human brain. We previously used single-cell RNA-sequencing and RNAscope to characterise hypothalamic GLP1R populations in mice. More recently, developments in spatial transcriptomics now allow for whole transcriptome detection across a section of tissue at a resolution of 1-10 cells. Here, using single nucleus RNA-sequencing (NucSeq) in combination with spatial transcriptomics (ST) we provide a census of cells from the human hypothalamus. Using NucSeq, we profile over 400,000 single nuclei, capturing canonical hypothalamic neuronal populations, as well as rarer cell types. In addition to NucSeq, we utilise Visium spatial transcriptomics on 7 human hypothalamic sections. Using bioinformatic tools, we integrate these two datasets to create a spatial multi-omics map of the human hypothalamus. Through this we characterise distinct GLP1R populations based on their transcriptome, and spatially map these populations to several different areas throughout the hypothalamus. These separate GLP1R populations were confirmed in near adjacent sections using RNAscope. Using this, we can begin to delineate the cellular targets of GLP1R agonists which mediate their weight loss effects.

P17: Hypothalamic ZFHX3 regulation of growth and energy balance in mice

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Zinc-finger homeobox-3 (ZFHX3) is a transcriptional regulator implicated in energy balance in a human genome-wide association study, where a protein altering variant is associated with low BMI. In this study we investigated the metabolic effects of ZFHX3 in mice harbouring a similar missense mutation in Zfhx3 (Zfhx3Sci/+). Zfhx3Sci/+ mice were shorter and weighed less than wildtype littermates at 1 year old and had lower lean and fat mass. Fasted insulin, leptin and insulin-like growth factor-1 concentration were lower in Zfhx3Sci/+ mice. Secondly, male and female Zfhx3Sci/+ mice had reduced food intake from 10 weeks old. Finally, female Zfhx3Sci/+ and Zfhx3+/+ mice already had lower lean mass (EchoMRI) from 6 weeks old, and these mice had also had lower energy expenditure. We mapped ZFHX3 expression in the hypothalamus, and in situ hybridisation analysis expression revealed altered hypothalamic growth axis component genes only in the arcuate nucleus (ARC); with increased somatostatin (Sst) and decreased growth hormone-releasing hormone (Ghrh) and growth hormone-receptor (Ghr) expression in Zfhx3Sci/+ mice. This was accompanied by decreased neuropeptide-y (Npy) expression in the ARC and increased G-protein coupled receptor-50 (Gpr50) expression in the ventricular ependymal layer. These data demonstrate a metabolic and growth effect of ZFHX3 for the first time and supports its role in human bodyweight regulation. Many of the identified genes altered in Zfhx3Sci/+ mice contain an AT-motif in their promotor which ZFXH3 has been demonstrated to bind to, and therefore is a likely mechanism for this effect.

P18: Super-resolution ultrasound imaging approaches to visualise the neuroendocrine regulation of gut structure

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The gut plays an important role in glucose and energy homeostasis and gut function is under intensive neuroendocrine control. The non-invasive imaging techniques contrast enhanced ultrasound (CEUS) imaging and super resolution ultrasound (SRUS) have the potential visualise the gut and provide novel insight into its structure and function.

We aimed to demonstrate that changes in duodenal villi structure can be monitored using CEUS and SRUS, providing an insight into neuroendocrine regulation of the gut.

Rats (n = 8) received twice daily vehicle control or the GLP-2 analogue teduglutide (0.3mg/kg; sc), known to drive small intestinal growth, for six days. In a separate experiment, rats (n=5) received once daily vehicle control or the gut-damage inducing drug methotrexate (2.5mg/kg; sc) for three days.

Animals were imaged before and after using a high-frequency L22-14Vx probe (Verasonics, Kirkland WA).

Following image analysis, villi length increased by 22% by day 6 of the teduglutide treatment compared to a 9% decrease in the control group, in accord with gold standard histological measurements. Villi length was found to have decreased by 12.0% (SEM=4%) following MTX treatment, while the controls showed no significant change (2.8%; SEM=2.6%) from baseline. These results were in line with histology.

This work demonstrates that SRUS can track structural changes in the duodenum and has potential, as a tool, to longitudinally study the gut and its role in homeostasis.

P19: Refining mouse glucose tolerance testing using micropipette-guided oral glucose dosing

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An oral glucose tolerance test (oGTT) is routinely used clinically for diagnosis of pre-diabetes, gestational diabetes, and insulin-resistance. The assay is also adapted for rodent metabolic phenotyping. A GTT measures the ability of the subject to clear excess blood glucose in a specified time frame, after a bolus glucose treatment. In the rodent oGTT this is typically administered via oral gavage, an invasive method that carries risk of injury when performed incorrectly. Intraperitoneal injection (ipGTT) is often used instead to administer the glucose in a GTT; however, this bypasses the gastrointestinal tract and therefore is less reflective of the human clinical assay. Both oral gavage and i.p. injection also bypass the cephalic glucose-sensing via the mouth and can cause stress, a confounding variable potentially necessitating the use of more animals. To refine the rodent GTT for both animal experience and physiological relevance, we developed a mouse oGTT protocol, where the glucose bolus is administered via a simple, non-invasive micropipette-guided dosing (MDA) method. Mice are habituated to voluntarily drinking a flavoured glucose solution from a pipette without prior water-restriction or restraint, limiting stress. A statistically significant blood glucose excursion is seen in mice after MDA-glucose dosing compared to animals that just received handling; this is a change comparable to that seen after oral gavage of the same glucose dose. Blood levels of glucoregulatory and stress hormones, and pattern of cFOS-immunoreactivity in the brain are currently being compared between dosing routes and will be validated in lean and obese mice.

P20: Gpr10 mutant mice are prone to obesity and have disrupted autonomic drive

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Prolactin-releasing peptide (PrRP) is expressed in three neuronal populations, including one in the dorsomedial hypothalamus. The receptor for PrRP, GPR10, is expressed at sites involved in appetite and autonomic regulation. We have shown that mice with a null mutation in the PrRP gene have late-onset obesity.[1] Furthermore, in rats, central administration of PrRP increases mean arterial blood pressure (BP),[2] while we have found polymorphisms in human GPR10 linked to both obesity and BP.[3,4]

Here we report that null $Gpr10^{-/-}$ mice exhibit an obese phenotype. Importantly, pre-obese $Gpr10^{-/-}$ mice have reduced energy expenditure, measured as a difference in oxygen consumption (VO2) by indirect calorimetry, with no difference in food intake. This reduction persists with age compared with wild-type (WT) littermates. Using tail-cuff plethysmography, baseline BP recordings were made in conscious, pre-obese $Gpr10^{-/-}$ mice, which are hypotensive compared with WT littermates. To test the physiological consequence of human variants on body weight, we generated a knock-in mouse model harbouring the most common functional GPR10 variant found in individuals with severe obesity (Gpr10P193S). Gpr10P193S mice exhibit greater weight gain than WT littermates. As with $Gpr10^{-/-}$ mice, pre-obese Gpr10P193S mice show no difference in food intake, but reduced energy expenditure. Together, the data suggests that PrRP/GPR10 signalling plays an important role in energy balance and cardiovascular regulation.

1 Dodd et al., 2014, Cell Metab 20: 639.
2 Samsom et al., 2000, Brain Research, 858(1):19-25.
3 Bhattacharyya et al., 2003, Diabetes 52(5):1296–1299
4 Talbot et al., 2023, Nat Commun 14: 1450.

P21: Manipulating mitochondrial dynamics in the NTS Astrocytes alters insulin sensitivity and metabolism

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The Dorsal Vagal Complex (DVC) of the brain is an essential regulator of glucose metabolism, food intake and energy expenditure. In rodents, the Nucleus of the Solitary Tract (NTS) in the DVC senses insulin and triggers a neuronal relay to decrease rodents' hepatic glucose production (HPG) and food intake. While this pathway is well studied in rodents, clear evidence is emerging that intranasal insulin delivery in humans to target the brain can modulate feeding behaviour and blood glucose levels. Interestingly, in high-fat diet (HFD)- fed rodents, the DVC becomes insulin resistant and loses the ability to regulate glucose levels and food intake. We discovered that HFD-feeding causes fragmentation of the mitochondria (Mitochondrial fission) in the NTS, thus triggering insulin resistance and is sufficient to block mitochondrial fragmentation in the NTS astrocytes to prevent HFD-dependent insulin resistance and body weight gain. Most recently we discovered that also the brown adipose tissue (BAT) ability to uptake glucose is modulated by altered mitochondrial dynamics in the NTS astrocytes. More specifically, the BAT uptakes and metabolises both glucose and triglycerides to produce heat and is activated by the central nervous system (CNS) through direct noradrenergic sympathetic innervation. Dysregulation of signalling modules in selective CNS areas such as the nucleus of tractus solitarius (NTS) are linked with altered BAT activity, obesity and diabetes. We showed that short-term HFD-feeding decreases the ability of BAT to uptake glucose and that inhibiting mitochondrial fragmentation in NTS-astrocytes of HFD-fed rats increase BAT glucose uptake while lowering blood glucose and insulin levels. Tyrosine Hydroxylase (TH) labelling showed that compared with HFD-fed rats, HFD fed animals, where mitochondrial fragmentation was inhibited in the NTS-astrocytes, had higher levels of catecholaminergic innervation of BAT, and did not present HFD-dependent infiltration of enlarged white fat droplets in the BAT. In regular chow-fed rats, increasing mitochondrial fragmentation in the NTS-astrocytes reduced BAT glucose uptake, TH and β3-adrenergic receptor levels.

Our data suggest that targeting mitochondrial dynamics in the NTS-astrocytes could be a beneficial strategy to decrease body weight and food intake and to increase glucose utilization in BAT in order to prevent the development of obesity and diabetes.

P22: Translocator protein 18kDa (TSPO) regulation of astrocyte function

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Background: Translocator protein 18kDa (TSPO) is an outer mitochondrial membrane protein implicated in (neuro)steroidogenesis and regulating cellular metabolism. Astrocytes are involved with many processes including neurosteroid production, regulation of brain tissue homeostasis and cellular energy use. We aimed to determine the role of TSPO in regulating astrocyte function, focussing on bioenergetics.

Methods: Mouse primary astrocytes (MPAs) cultured from TSPO deficient mice ($TSPO^{-/-}$; $Tspo^{tm1b(EUCOMM)Wtsi}$), or wild-type (WT) littermate controls, and a human astrocytoma cell line were used. Cellular bioenergetics (oxygen consumption [OCR] and extracellular acidification rates [ECAR]) were examined using the Seahorse XFe96 bioanalyzer. Co-immunoprecipitation using lysate from cells transfected with a Myc-tagged TSPO plasmid, followed by immunoblotting, was used to identify protein complexes involving TSPO.

Results: Compared to controls, $TSPO^{-/-}$ astrocytes showed reduced baseline OCR and ECAR. Unlike WT MPAs, when metabolically challenged with glucose-free media, $TSPO^{-/-}$ MPAs did not increase their OCR and ECAR in response to reduced glucose availability. Co-immunoprecipitation studies revealed TSPO forms protein complexes with key metabolic regulators including voltage dependent anion channel, carnitine palmitoyl-transferase 1a, and hexokinase 2.

Conclusions: TSPO-deficiency in cultured astrocytes reduced basal cellular metabolism and attenuated the metabolic response to reduced glucose availability. This may be mediated via interactions between TSPO and key metabolic regulatory proteins. These data suggest that TSPO may play a role in astrocyte metabolism by regulating enzyme activity or import of substrates into the mitochondria for downstream utilisation. Our future work will focus on defining TSPO-containing protein complexes to characterise interactions of TSPO with steroidogenic machinery in astrocytes.

P23: Heterogeneity as a determinant of pulsatile insulin secretion in pancreatic beta-cell networks: a mathematical modelling study

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The pancreatic islets of Langerhans are key for maintaining glucose homeostasis throughout the body. Over recent years, high-speed multi-cellular calcium imaging studies from pancreatic islets have facilitated the study of coordinated insulin secretion from a network perspective. These studies have uncovered a wide range of phenotypical variability, both in cell-intrinsic and network-level features, and motivated the question of what is the role of this heterogeneity in the glucose-stimulated insulin responsiveness (GSIS) of beta-cell networks. To address this question, we have developed mathematical models for predicting the effects of cell-intrinsic heterogeneity, cytoarchitecture, and gap-junction coupling in coordinating beta-cell network dynamics. As a case study, we focus on the impact of heterogeneous excitability due to its critical role in characterising subpopulations of beta-cells, including first-responder cells in the transient phase and wave-initiator cells in the pulsatile phase of GSIS. We find that the spatial aggregation of excitable cells can tune the sensitivity of the network to glucose but that it also introduces trade-offs with the precision and strength of the collective response. This work highlights the importance of considering cytoarchitecture when studying heterogeneity in beta-cell networks and provides a general framework for quantifying its impact. We envisage that this mathematical framework will facilitate understanding of the interplay between beta-cell-intrinsic and network-level features in modulating secretory function.

P24: A novel role for JAKMIP1 in neuronal cytokine signalling via transcriptional modulation of STAT3 expression

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Dysregulated expression of Janus Kinase and Microtubule-Interacting Protein 1 (JAKMIP1) has previously been associated with autism spectrum disorder (ASD) and related neurodevelopmental disorders. Previous studies have reported important roles for JAKMIP1 in neurodevelopment and Jakmip1-deficient mice display striking behaviour phenotypes reminiscent of mouse models of ASD. Yet, the molecular functions of JAKMIP1 and how its dysregulation leads to ASD-associated behaviours remain unclear.

JAKMIP1 possesses multiple functional domains. Its C-terminus interacts with the Janus Kinases (JAKs) that associate with cytokine receptors to trigger Signal Transducer and Activator of Transcription (STAT) signalling downstream of receptor activation. However, it is uncertain whether JAKMIP1 regulates neuronal cytokine/JAK/STAT signalling as the functional consequence of JAKMIP1-JAK interactions has not been explored.

Using JAKMIP1-knockout human neuroblastoma SH-SY5Y cells generated by CRISPR-Cas9 technology, we demonstrate a novel role for JAKMIP1 in modulating IL-6/JAK1/STAT3 signalling through transcriptional regulation of STAT3 expression. JAKMIP1-deficient SH-SY5Y cells show impaired responses to IL-6, and transcriptional profiling reveals that JAKMIP1 may regulate the expression of multiple cytokine signalling-related genes. Furthermore, we show that the JAKMIP1 C-terminus can alter STAT3 expression, likely via a nuclear mechanism, and use proteomic techniques to characterise the JAKMIP1 interactome. We show that JAKMIP1 interacts with RNA-binding proteins that control various aspects of mRNA synthesis and processing, many of which are already implicated in neurodevelopmental disorders.

Our work suggests further areas for convergence between JAKMIP1, its regulated genes and protein interactions among ASD-relevant pathways. As such, further investigation into JAKMIP1-modulated IL-6/STAT3 signalling and its consequences in neurodevelopment is clearly warranted.

P25: Preconceptional/in-utero exposure to biosolids- real-life source of environmental chemical mixture- disrupts key metabolic pathways in the medial preoptic area and paraventricular nucleus of the hypothalamus of adult rams

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Daily exposure to environmental chemicals (ECs) can adversely affect human metabolic health. Yet, the exact mechanisms of action of the ECs are not understood. We have already found that gestational exposure to biosolids (B)- a source of real-life mixture of ECs- decreases body weight, increases fat mass and compromises energy metabolism in adult rams. As a follow up, in this study we evaluated the effects of in-utero exposure to ECs on the transcriptome profile of two key metabolic regulatory regions of the hypothalamus- medial preoptic area (mPOA) and paraventricular nucleus (PVN).

Hypothalamic samples were collected from male offspring of either control (C) or B-treated pasture grazing (from one month prior to pregnancy until around parturition) ewes at 11months of age. RNA sequencing was performed on the mPOA and PVN, using Oxford Nanopore GridION sequencer and an R9.4.1 flow cell. KEGG pathway analysis was performed for the differentially expressed genes (DEGs) list in DAVID 2021.

With $p<0.01$ threshold, 201 and 231 DEGs were detected in the mPOA and PVN, respectively. KEGG pathway analysis revealed that some vital metabolic pathways in the mPOA e.g., “Fatty acid biosynthesis” and “metabolic pathways” and the “Drug metabolism” pathway in the PVN were enriched as a result of B exposure.

Changes in these pathways by reason of in utero exposure to a real-life mixture of ECs could have contributed to phenotypic effects observed in the B rams across the first year of life which included decreased body weight, increased subcutaneous fat mass and compromised glucose metabolism.

P26: Divergent modulation of neuropeptide-Y (NPY) neurones by insulin

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Neurones in the brain that express neuropeptide Y (NPY) have been linked to regulation of appetite and body metabolism. As part of that function, it is possible that these neurones possess the ability to directly sense metabolic signals such as insulin. This ability has already been demonstrated for NPY cells of the arcuate nucleus of the hypothalamus, but it is not known if NPY cells in other brain regions are also capable of sensing insulin. Here, we investigated insulin sensitivity in NPY cells of the lateral hypothalamic area (LHA), the amygdala (AMY), and the intergeniculate leaflet (IGL) of the thalamus, because these cells have been implicated in modulating several aspects of metabolism. Using whole-cell patch-clamp electrophysiology in acute brain slices from mice, we found that insulin inhibited NPY cells in the AMY but was excitatory on IGL(NPY) cells, as measured by changes in membrane potential and firing frequency. Insulin had no overall effect on LHA(NPY) neurones. The shape of the action potential was also affected differently: insulin increased the after-hyperpolarisation in AMY(NPY) but not in IGL(NPY) or LHA(NPY) neurones, whereas the width of the action potential increased with insulin in IGL(NPY) cells but did not change in AMY(NPY) or LHA(NPY) cells. Our observations suggest that insulin acts on distinct effector mechanisms in different populations of NPY neurones. These effector mechanisms perhaps involve diverse K currents. It will be interesting to investigate in vivo if these differences are functionally relevant for maintaining homoeostasis.

P27: Abstract withdrawn.

P28: Novel phenotypic and exonic variants for Neuroendocrine Neoplams: a UK Biobank study

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Neuroendocrine neoplasms (NENs) are a heterogeneous tumour classification including indolent neuroendocrine tumours (NETs), aggressive neuroendocrine carcinomas (NECs). NECs and small cell lung cancer (SCLCs) are poorly differentiated tumours and life expectancy following metastatic diagnosis is less than 1 year. Currently, there are known variants in germline DNA that associate with bronchial and pancreatic NENs, but not intestinal. We conducted an exome-wide association study in the UK Biobank (N=500,000) to test for phenotypic and rare coding variations in germline DNA that associate with NETs, NECs and SCLC.

We used histology and ICD10 data from the UK Biobank's cancer registry linkage to define phenotypes for NET (N=591), NEC (N=328), and SCLC (N=477) and a cohort of cancer-free controls (N=395,914). We used regenie to perform single-variant and rare (<0.1%) variant gene-based tests for cancer-causing germline variants for each of the three NEN phenotypes.

We found significant phenotypic associations between baseline BMI and HbA1c with all three NEN phenotypes. SCLC further associated with environmental pollution (OR=1.31 (1.21-1.41) p=2.9e-12) and Townsend deprivation index (OR=1.61 (1.49-1.74) p=4.2e-33). In the single-gene tests, a single variant in the *DST* gene (6:56482593) associated with SCLC (Beta=6.0 (4.4-7.6), p=4.9e-8). In the gene-based tests, loss-of-function variants in *MEN1* (Beta=6.9 (4.9-8.9), p=2.2e-7) associated with NECs.

Germline mutations in *MEN1* are known to associate with NETs, but this is the first study to show an association with NECs. We also identified a novel variant for SCLC in the *DST* gene. Further investigation could help understand how NECs and SCLCs develop and progress.

P29: In vivo GCaMP recordings of GnRH neuron activity in freely-behaving mice

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Gonadotropin-releasing hormone (GnRH) neurons drive gonadotropin secretions essential for fertility. The GnRH neuron cell bodies are scattered throughout the hypothalamus however their distal dendrons converge within the ventrolateral arcuate nucleus (vlARN) before forming short axons that innervate the median eminence. The distal dendron is thought to represent an autonomous regulatory site for the initiation of pulsatile GnRH secretion while also carrying action potentials from the cell bodies that evoke the GnRH surge. We used GCaMP6 fiber photometry to examine GnRH neuron population activity at the distal dendron in freely-behaving mice for up to 24 hours. Intact male and female mice exhibited abrupt episodes of activity, termed dendron synchronization events (dSEs), lasting 8-12 minutes, tightly coupled to a pulse of luteinizing hormone (LH). In males, the dSEs occurred with an interval of 157 min, while the inter-dSE frequency varied throughout the estrous cycle in females between 38 and 95 minutes. In addition to dSEs, female mice exhibited a large and sustained increase in activity for 10-12 hours, starting in the afternoon of proestrus. The rise began with multiple oscillatory activities each lasting around 30-60 min before reaching its plateau after 3-6 hours. The subsequent decline also showed similar oscillatory patterns of activity. Serial blood sampling revealed a sustained increase in LH secretion during this period. These observations indicate that both pulse and surge patterns of GnRH neuron activity can be recorded at the level of the distal dendron and represent the first direct recordings of GnRH neuron activity in freely-behaving animals.

P30: Life in the dark: Circadian rhythms of naked mole rats

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Naked mole rats (NMR) are subterranean rodents that live in large eusocial colonies. They have an extraordinary lifespan of more than 35 years and seemingly display no age-related diseases such as metabolic disorder and cognitive decline. Interestingly, NMRs are often cited as having no circadian rhythms and/or being active around the clock. However, only few studies have investigated circadian rhythms in NMRs and these have reported contradicting results.

To demonstrate that NMRs retain a functional circadian clock, we investigated clock gene rhythms in the hypothalamus, where the master circadian clock (SCN) is located, and peripheral tissues every 4 hours in a 24-hour period. qPCR analysis showed striking circadian oscillation of clock genes (*BMAL1*, *Cry1* and *Per2*) and melatonin synthesis genes. To determine whether these molecular rhythms are reflected in a behavioural output of the circadian clock, we measured locomotor activity of NMR colonies kept in 24-hour darkness. The results clearly show daily locomotor rhythms with high activity during the subjective 'night'. This is remarkable because NMRs live in total darkness and are therefore not influenced by the light/dark cycle, which is the most common Zeitgeber for circadian rhythms.

Our data provide novel insights into the NMRs ability to live underground, with no or little exposure to day light. Understanding the extent of the circadian system in NMRs, which, in theory, do not need a functional circadian clock, and how the system is entrained will give fundamental biological insights into the relevance of the circadian system using a mammalian model.

P31: Preliminary investigation of the role of glutamatergic projections from the MePD to the ARC in regulating LH pulsatility

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The mechanisms by which psychological stress can induce infertility are not well characterised. The presence of kisspeptin neurones in the posterodorsal medial amygdala (MePD) has led to interesting discoveries as to how this region, implicated in stress as well as social and sexual behaviour, can regulate the hypothalamic GnRH pulse generator. Neuropharmacological manipulation whilst optogenetically stimulating these MePD kisspeptin neurones led to the hypothesis that there are both GABAergic and glutamatergic outputs to the KNDy network in arcuate nucleus (ARC) of the hypothalamus directly responsible for regulating the GnRH pulse generator frequency. We have previously shown that optical stimulation of the MePD GABAergic projections to the ARC dramatically suppressed luteinising hormone (LH) pulse frequency (McIntyre et al., Endocrinology, 2022, 164, 1-11). In the current study channelrhodopsin2 was selectively expressed in glutamatergic neurones originating in the MePD of adult female Vglut2 $Flp^{+/-}$ mice. Optogenetic stimulation (5 - 20 Hz, 10 ms, 10 mW, 5 sec on/5 sec off for 1 hour) was applied in the ARC to selectively activate glutamatergic projections from the MePD, but no effect on LH pulse frequency was observed, 3.89 ± 0.22 pulses/ hour prior to stimulation and 3.29 ± 0.35 during stimulation (mean ± SEM). These data suggest GABAergic projections may be the only regulator originating from the MePD, or that glutamate may play an indirect role to regulate the GnRH pulse generator.

P32: The 5:2 diet in mice: a sexist and ageist weight loss intervention

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The 5:2 diet (2 non-consecutive fast days and 5 normal feeding days) is a fashionable weight-loss strategy. Despite reported health benefits, its effectiveness in mice and the impact of sex, age and the ghrelin system remain unknown.

In this study, adolescent (7-week old) and adult (7-month old) male and female loxTB-GHSR (GHSR-null) mice and wild-type (WT) littermates received standard rodent chow in *ad-libitum* or 5:2 patterns for 6 weeks.

The 5:2 diet reduced body weight gain by 20% in adolescent WT males, and induced minor weight loss in adult males, without affecting body weight gain in females. Tibial epiphyseal plate width (EPW), an accurate marker skeletal growth rate, was elevated by 13% and 9% in growing adolescent males and females, but unaffected in adult mice. Tibial marrow adiposity was elevated by 39% in adult males but reduced by 42% in adolescent females, with comparable effects in adult females. In adolescent GHSR-null males, the 5:2 diet-induced effects on weight gain, EPW and marrow adiposity were abolished. In adult GHSR-null males, the weight loss impact of the 5:2 diet was unaffected, but the 5:2-induced elevation in marrow adiposity was reversed, being reduced by 40% in adult 5:2-fed GHSR males. While weight gain in adolescent 5:2-fed GHSR-null females increased by 26%, the effects of the 5:2 diet on EPW and adiposity were largely retained. The lipogenic effect of the 5:2 diet in adult females was abolished in the absence of GHSR.

Thus, the 5:2 diet has surprising sex-, age- and GHSR-dependent pleiotropic effects.

P33: Role of Variants in Methyl-CpG-binding protein 2 (MECP2) in GnRH regulation and Central Precocious Puberty

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Whilst several key genetic contributors to the phenotype of central precocious puberty (CPP) have been recognized, many familial cases remain without clear genetic aetiology. Our recent high-impact large cohort study (Lancet Diabetes & Endocrinology, 2023 *accepted*) identified CPP-associated variants in Methyl-CpG-binding protein 2 (MECP2), a chromatin-associated transcriptional regulator, with known roles in neuronal maturation. This X-linked gene is highly expressed in hypothalamic nuclei (arcuate, suprachiasmatic, and paraventricular) and co-localises with GnRH within GnRH neurons, suggesting a role in regulation of the GnRH axis.

Loss-of-function mutations in MECP2 are usually associated with Rett syndrome, a severe neurodevelopment disorder characterized by developmental regression and intellectual disability. Interestingly, multiple studies report Rett syndrome presenting with precocious puberty.

We investigated the *in vitro* impact of 5 CPP associated and 2 Rett syndrome associated *MECP2* variants in a GT1-7 mouse neuronal GnRH producing cell line. Immunocytochemistry of MECP2 variant overexpressing GT1-7 identified differential expression of CPP-associated and Rett-associated MECP2 variants. Western blotting confirmed differential expression of overexpressed MECP2 variants of interest compared to wildtype MECP2. Preliminary studies in a GnRH reporter system demonstrated differential ability of MECP2 variants to supress GnRH promoter activity suggesting a possible regulatory role in the GNRH neuronal network.

Here we present the first functional data building on our human patient discovery, suggesting that CPP-associated variants in MECP2 alter protein biology. There may be key differences in expression and activity of CPP-associated MECP2 variants, compared to those associated with Rett syndrome, which may aid in differential diagnosis and treatment of patients.

P34: Discovery and functional characterisation of bombesin-type neuropeptide signalling in an echinoderm

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Bombesin (BN) is a peptide that was first isolated from the skin of the toad *Bombina bombina*. Subsequently, structurally and evolutionarily related neuropeptides known as gastrin-releasing peptide (GRP) and neuromedin B (NMB) were discovered in mammals, where they act as regulators of feeding, gut physiology and other processes. GRP and NMB have also been identified and functionally characterised in non-mammalian vertebrates, but BN-type peptides have not been identified in non-chordates. Here we report the discovery and functional characterisation of a BN-type neuropeptide (ArBN) in an echinoderm - the starfish *Asterias rubens*. A transcript encoding a putative precursor of BN-type peptide was identified in *A. rubens* (ArBNP) and analysis of its sequence and the exon-intron structure of the gene encoding this protein revealed similarity with vertebrate BN-type precursors. Furthermore, using cell-based assays, an A. rubens G-protein coupled receptor that is closely related to vertebrate GRP/NMB-type receptors was identified as the receptor for ArBN. Using mRNA *in situ* hybridization and immunohistochemistry, ArBN expression was revealed in the central nervous system, locomotory system (tube feet) and digestive system of *A. rubens. In vitro* pharmacological experiments revealed that ArBN causes does-dependent contraction of cardiac stomach and tube foot preparations from *A. rubens*, whilst *in vivo* injection of ArBN causes cardiac stomach retraction. This study has revealed that the evolutionary origin of BN-type neuropeptide signalling can be traced back to the common ancestor of deuterostomes and it has an ancient role in regulation of feeding processes.

P35: Functional characterization of kisspeptin-type signalling in an echinoderm

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The neuropeptide kisspeptin regulates reproductive maturation and function in mammals by stimulating hypothalamic release of gonadotropin-releasing hormone (GnRH), mediated by a G-protein coupled receptor (GPR54/KiSS1R). Phylogenetic analysis of genome sequence data indicates that kisspeptin signalling originated in a common ancestor of the Bilateria, but little is known about kisspeptins in invertebrates. Recently, four kisspeptin-type neuropeptides (ArKP1.1, ArKP1.2, ArKP2.1, ArKP2.2) derived from two precursor proteins (ArKPP1, ArKPP2) and their cognate receptors were discovered in the starfish *Asterias rubens* (Phylum Echinodermata; Escudero Castelán et al., 2022). To obtain insights into the evolution of kisspeptin signalling as a regulator of physiology and behaviour, we are investigating the expression and pharmacological actions of kisspeptin-type neuropeptides in *A. rubens*. Use of mRNA *in situ* hybridisation and immunohistochemistry has revealed expression of ArKPP1- and ArKPP2-derived neuropeptides in the central nervous system, sensory organs, digestive system (e.g., stomach) and locomotory system (tube feet). Furthermore, on-going studies are comparing expression ArKPP1- and ArKPP2-derived neuropeptides in *A. rubens* by immunohistochemical analysis of adjacent sections of the arms and central disk region. *In vitro* pharmacological experiments revealed that ArKPP1- and ArKPP2-derived neuropeptides have opposing effects on stomach preparations from *A. rubens*, with ArKP1.1 and ArKP1.2 causing relaxation and ArKP2.2 causing contraction. Accordingly, on-going experiments are investigating the *in vivo* effects of kisspeptin-type neuropeptides on feeding-associated processes in *A. rubens*. Our discovery that kisspeptin-type neuropeptides derived from different precursor proteins exert opposing effects on the starfish stomach provides a new perspective on the evolution of kisspeptin function in the Bilateria.

P36: Exploring the effect of increased vasopressin expression on the hypothalamic pituitary adrenal axis

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The hypothalamic-pituitary-adrenal axis is the primary neuroendocrine system responsible for providing a rapid response to stress. Corticotropin-releasing hormone (CRH) is the primary secretagogue of the HPA and is released from the hypothalamic PVN parvocellular neurons to stimulate the corticotrope cells to release ACTH and the subsequent synthesis of glucocorticoids at the adrenal gland. Vasopressin (AVP) is the system's secondary secretagogue; AVP expression is very low in basal conditions compared to CRH and plays a less crucial role in diurnal glucocorticoid and ACTH rhythms, but it appears to be particularly important in stressful conditions where it has been shown to potentiate ACTH/CORT release. These two secretagogues have distinct expression and activation mechanisms from one another and thus generate different electrical and calcium signals upon binding to the corticotrope. Interestingly, individual corticotrope calcium responses to secretagogue are reproducible but there is a high degree of heterogeneity at the population level. While the degree of heterogeneity has only been studied with a fixed dose of 2nM AVP and 200pM CRH; we have investigated how varying AVP and CRH concentrations effects the responsivity and heterogeneous characteristics of the corticotrope population.

To do so, primary pituitary cells were collected and corticotropes identified using a POMC-mCherry adenovirus. The corticotropes were treated with Fura for recordings of calcium activity in response to treatment with varying concentrations of AVP, ranging from 2pM to 3nM, either in isolation or in combination with CRH. Our results showed a dose dependent AVP-response in pituitary corticotropes. Co-administering CRH and AVP caused a significant increase in response amplitude compared to AVP-only stimulations. Certain cells displayed synergy in the combination stimulations where the amplitude of the combined stimulation exceeded the total sum of the individual AVP and individual CRH stimulation responses.

Our results have demonstrated a greater range of corticotrope sensitivity to AVP beyond the commonly used 2nM AVP dose which is cited in much literature. We aim to repeat this protocol in aged animals, who are known to have significant AVP upregulation in the hypothalamus, to understand how this dose-dependent sensitivity changes and/or shifts when the HPA environment becomes AVP-enriched.

P37: Neural circuit basis underlying prepubertal alloparental care

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In mice, parental behaviour is controlled by brain-wide circuits, the functional organisation of which is increasingly well understood. However, it remains unknown when these circuits become functional in early life, and whether they also control alloparental behaviour, i.e. parental behaviour displayed by non-parents.

We have observed that prepubertal mice show an onset of spontaneous alloparental behaviour at postnatal day 15, and that these animals exhibit levels of parental care similar to those of adult virgin females. Using hypothalamus-wide immediate early gene mapping, we found that prepubertal alloparenting is associated with activation of galanin-expressing neurons in the medial preoptic area ($MPOA^{Gal}$ neurons), suggesting that these neurons control parenting in both adults and juveniles.

We next carried out both anterograde, and retrograde trans-synaptic viral tracing to outline $MPOA^{Gal}$ projections and inputs in juveniles. Surprisingly, while the projections are mostly the same, $MPOA^{Gal}$ neurons received more extensive inputs in juveniles than in adults. This highlights an extensive remodelling of the $MPOA^{Gal}$ circuit during adolescence.

We are currently using fiber photometry and slice electrophysiology to identify the neural mechanisms underlying the prepubertal parental switch. This work, thus, highlights an adult-like behaviour that emerges before puberty, and which seems to be controlled by adult-like circuitry.

P38: Developing a mathematical framework to parametrise electrical activity in pituitary lactotrophs.

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Model optimization has focused on parameter estimation for extracting relevant information from complex systems by time series observations of voltage and current data. In neuroendocrinology, these parameters represent the fingerprints of ion channel subtypes and may identify ion channel mutations from changes in the patterns of electrical activity. The pituitary lactotrophs exhibit significant heterogeneity in calcium influx and electrophysiological properties. Thus, a critical challenge in building computational models is to obtain ion channel parameters with sufficient accuracy and consistency to provide new information about the patterns of electrical activity regulating diverse physiological functions such as hormone secretion, neurotransmitter release, and contractility. Given the complex behaviour exhibited by the endocrine axes and the limited feasibility of the experimental studies to measure ion channel parameters, the present study implements interior point optimization method, for the first time, to estimate ion channel parameters in a pituitary lactotroph model derived from Hodgkin-Huxley formulation. To validate the framework, the estimated parameters were then used to successfully predict the dynamics of the membrane potential. The reliability of the system was confirmed by estimating the behaviour of individual currents present in the lactotroph model. Furthermore, to check the robustness of the presented method, voltage time series was generated by adding noise to the process and validated by successfully predicting the subsequent response using the extracted parameters. The tools explored here are computationally efficient and have a potential to allow exploration of biophysical properties of individual cell and complicated networks.

P39: Mapping peptidergic motoneurons in an echinoderm

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Echinoderms (e.g. starfish) are unique amongst bilaterian animals in exhibiting radial symmetry as adult animals and consequently they do not have a "brain", but they do have a central nervous system (CNS). For example, in the starfish *Asterias rubens* the CNS comprises a circumoral nerve ring linking five radial nerve cords (RNCs). From a comparative and evolutionary perspective it is of interest, therefore, to determine how a radially symmetrical CNS is organised at the molecular/cellular level in echinoderms. However, little is known about the molecular and functional neuroanatomy of neurons in the CNS of echinoderms. Here, we developed whole-mount immunohistochemical methods to map peptidergic motoneurons in the segmental hyponeural ganglia of the RNCs in *A. rubens*. Antibodies to five *A. rubens* neuropeptides have been tested: calcitonin-type (ArCT), corticotropin-releasing hormone-type (ArCRH), pedal peptide-type (ArPPLN1 and ArPPLN2), and somatostatin-type (ArSS2). These neuropeptides were found to have distinctive segmentally repeated patterns of expression in subpopulations of hyponeural neurons in the RNC of *A. rubens*. For example, ~10 ArCRH-immunoreactive cell bodies are sparsely distributed in the centre of each segmental ganglion, with axons projecting to the lateral margin of the RNC. These findings indicate that subpopulations of hyponeural motoneurons in *A. rubens* express different neuropeptides, although it is possible that there is co-expression of multiple neuropeptides in some/all motoneurons. Future studies will compare the peripheral distribution of motor axons containing different neuropeptides so that the functional neuroanatomy of subpopulations of hyponeural motoneurons in starfish can be determined.

P40: Spatiotemporal expression of ZFHX3 in the mouse dorsal vagal complex

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Physiological processes align to the solar day through a light-responsive internal timing mechanism in the suprachiasmatic nuclei (SCN), the principal circadian clock. Notably, circadian clock genes are rhythmically expressed in multiple brain regions and peripheral organs to maintain 24h oscillations in physiology and behaviour including sleep, hunger, and food consumption. Disrupted synchrony among these extra-SCN clocks contributes to metabolic disorders, such as obesity and Type 2 diabetes. The brainstem's dorsal vagal complex (DVC) is a key integrative centre for the control of food intake and we recently identified that its subcomponents (the area postrema, nucleus of the solitary tract, dorsal motor nucleus of the vagus and ependymal cells surrounding the 4th ventricle), behaved as autonomous circadian oscillators but their inter-temporal regulation remains poorly understood. We adopted a molecular and neuroanatomical approach to assess whether ZFHX3, a transcription factor with important roles in regulating the neurochemistry of the SCN circadian clock, is expressed in potential clock cells of the DVC and if its expression alters over the day-night cycle. Using both RNAscope and conventional RT-qPCR techniques, we reveal Zfhx3 expression in neuronal and ependymal cells of the DVC, identify its co-localization with certain neurotransmitters, and find that its expression does not appear to vary over 24 hours despite co-localisation with Per2. We surmise that, while ZFHX3 potentially influences transcription in Per2 expressing DVC cells, further studies are necessary to assess whether it plays a prominent role in regulating circadian patterns of DVC gene expression and DVC function.

P41: Metformin and AMP kinase activators elevate circulating levels of GDF15 through distinct tissue-specific mechanisms

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Objective: GDF15 acts on its hindbrain-localized receptor, GFRAL-RET, to reduce food intake and body weight, and is a key mediator of the weight loss effects of metformin. Using mice with tissue-specific deletions of *Gdf15*, on a range of dietary backgrounds, we sought to determine the relative contributions of two classical metformin target tissues—the gut and liver—to this effect. We additionally performed comparative studies with another pharmacological agent, the AMPK pan-activator MK8722.

Methods: Cohorts of *Gdf15*-gut-KO, *Gdf15*-liver-KO, and C57Bl/6N mice were fed a number of antecedent diets before receiving metformin or MK8722.

Results: In response to acute metformin administration, circulating levels of GDF15 were reduced by ~53% in *Gdf15*-gut-KO mice compared to wild-type littermates. In contrast, *Gdf15* ablation in the intestine appeared to have no significant effect on the rise in GDF15 after MK8722 treatment. Liver-specific *Gdf15* deletion had no impact on metformin-stimulated GDF15 levels. In contrast, the GDF15 response to MK8722 was reduced by ~68% in *Gdf15*-liver-KO mice versus wild-type counterparts. In both models, metformin treatment resulted in a robust increase in kidney-specific *Gdf15* expression.

Conclusions: The gut, but not the liver, is a major source of metformin-stimulated circulatory GDF15. The kidney is likely the principal source of the remainder. In contrast, the liver is the major source of GDF5 that is stimulated by the ingestion of a pan-activator of AMPK. These findings add to the growing body of evidence implicating the intestinal epithelium in key aspects of the pharmacology of metformin action.

P42: Salt loading reduces central osmoresponsiveness in magnocellular neurones *in vivo*

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It is well known that magnocellular vasopressin and oxytocin neurones in the supraoptic (SON) nucleus are directly osmosensitive and are modulated by osmotic pressure. Previous *in vitro* studies have indicated that, in magnocellular neurones, the direction of GABA actions reversed from inhibition to excitation in chronic salt-loading conditions. This *in vivo* study aimed to investigate the neural plasticity of magnocellular vasopressin and oxytocin neurones in the SON of adult male Sprague-Dawley rats in response to acute osmotic challenges after salt-loading. We found that prolonged salt-loading led to significant changes in the firing properties of SON neurones. Specifically, the mean basal firing rate of the SON neurones was significantly higher in the salt-loaded group (6.08 spikes/s) than in the euhydrated group (5.06 spikes/s), and the proportion of phasic vasopressin neurones in the salt-loaded (41.91%) group was also higher when compared with euhydrated (19.26%) and rehydrated (19.53%) groups. Basal pituitary vasopressin stores and plasma vasopressin levels were significantly reduced, but plasma sodium levels were significantly higher in salt-loaded than in euhydrated rats. In response to an acute hyperosmotic challenge, the mean change in firing rate of SON neurons was significantly lower in salt-loaded rats (0.81 spikes/s) compared to euhydrated rats (2.68 spikes/s). We confirmed that the neuronal electrical activity of SON is strongly modulated by hyperosmotic stimulation, and this modulation persists across euhydrated and salt-loaded and rehydrated conditions. These results suggest that salt-loading induces significant changes in the neural properties of SON neurones and impairs their ability to respond to acute osmotic challenges.

P43: Age-dependent mechanisms in seasonal and developmental programs underpinning neuroendocrine control of reproductive physiology in Japanese quail (*Coturnix Japonica*)

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The seasonal changes in photoperiod are a vital environmental cue, used by temperate seasonal breeders to time investment into physiology across life history transitions and maximise fitness. Neuroendocrine changes in the hypothalamus and pituitary gland are required for the connection of the perception of environmental cues (e.g., daylength) with adaptive change in developmental and reproductive physiology. This study aimed to identify similarities and differences in molecular substrates involved in both development and seasonal timing of reproduction. Adult and juvenile Japanese quail (*Coturnix japonica*) were collected from either long photoperiod breeding (16L:8D) or short (8L:16D) photoperiod non-breeding conditions. Key molecular targets in the mediobasal hypothalamus (MBH) and the pituitary gland were examined using Oxford Nanopore RNA-sequencing and targeted qPCR analyses. qPCR assays showed that patterns of adult deiodinase type-2 and type-3 (*DIO2* and *DIO3*) expression were consistent with historical research in the MBH across long and short photoperiods, correlating with growth in reproductive physiology. Despite showing similar physiological growth, juveniles showed contrasting patterns of *DIO2* and *DIO3* expression, inconsistent with previous literature. Pituitary gland RNA-sequencing analyses revealed a total of 340 differentially expressed transcripts across differing photoperiods, and 1189 transcripts showing age-dependent differential expression. Prolactin (*PRL*) and follicle-stimulating hormone subunit beta (*FSHß*) are significantly upregulated during long photoperiods, whereas growth hormone (*GH*) expression is significantly upregulated in juvenile quail, regardless of photoperiod. The results presented here identify a suite of photoperiod- and age-dependent transcripts and indicate a specificity of pituitary gland cells for photoperiod and developmental programs in Japanese quail.

P44: The methyl-CpG-binding protein 2 (Mecp2) regulates body energy balance by modulating hypothalamic-white adipose tissue axis

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The hypothalamus is the major brain area controlling body energy balance and to that requires to maintain high levels of plasticity to integrate and respond to peripheral signals that account for energy levels. The maintenance of this high levels of plasticity requires a large amount of energy, provided primarily as ATP by cellular energy metabolism. Mecp2 is a molecular bridge that binds to methylated CpG dinucleotides to orchestrate gene expression in response to environmental factors, and loss-of-function mutations cause overweight and mitochondrial dysfunction. However, the role of Mecp2 in the control of cellular energy metabolism and its impact on hypothalamic function are not completely understood. In this study, we used a Mecp2-null mouse model to evaluate cellular parameters associated with energy sensing and body energy metabolism.

We found that the lack of Mecp2 alters the expression of proteins involved in mitochondrial function and cellular energy metabolism in the hypothalamus. Besides, in the adipose tissue, we found increased adiposity and alterations in the lipid composition, results associated with changes in the expression of genes related to lipid metabolism. In addition, Mecp2-null mice show a body energy homeostasis disruption reflected by reduced locomotor activity and increased respiratory exchange ratio.

In conclusion, our results show that the absence of Mecp2 alters cellular energy metabolism in the hypothalamus which impacts on white adipose tissue metabolic control and body energy homeostasis.

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P45: Hippocampal neuroprotection is enhanced by cherry consumption in a photoperiod dependent manner in F344 rats

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Seasonal animals have evolved diverse variations in physiology, reproduction, and behaviour to accommodate yearly changes in environmental and climatic changes. These changes are initiated by changes in photoperiod (daylength). Here we investigated the effect of photoperiod and fruit consumption on the expression of key genes involved in photoperiod regulation in the hypothalamus and neuroprotection in the hippocampus in photoperiod-sensitive F344 rats. Fruit consumption is associated with beneficial health effects due to the antioxidative and anti-inflammatory effects of polyphenols. However, while there is evidence suggesting that polyphenols can influence seasonal rhythms, the link between polyphenols, photoperiod and neuroprotection is unknown.

To test this, F344 rats were exposed to short (L6, 6h of light/6h of dark), intermediate (L12, 12h of light/12h of dark) and long (L18, 18h of light/12h of dark) photoperiods and fed a standard chow diet supplemented with either control, or lyophilized cherry 1, or cherry 2 with distinct phenolic hallmarks. Physiological parameters (body weight, eating pattern index, testosterone and T4/T3) and hypothalamic key genes (*Dio2, Dio3, Raldh1, Ghrh*) were strongly regulated by photoperiod and/or fruit consumption. Importantly, we show for the first time that neurotrophs (*Bdnf, Sod1* and *Gpx1*) in the hippocampus were also regulated by photoperiod. Furthermore, the consumption of Cherry 2, richer in total flavanols, but not Cherry1, richer in total anthocyanins and flavanols, enhanced neuroprotection in the hippocampus.

Our results show that the seasonal consumption of cherry with specific phenolic composition plays an important role in hippocampal activation of neuroprotection in a photoperiod dependent manner.

P46: Uncovering physiological mechanisms modulating common circadian distributions of epileptiform discharges

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People with epilepsy often report stress and sleep deprivation as key precipitants of their seizures. Moreover, recent research has shown that epileptiform discharges (including both seizure and interictal epileptiform activity) follow temporal organizations over periods ranging from hours through months. Despite these observational studies, at present relatively little is known about the physiological mechanisms underlying these patterns.

We analysed epileptiform discharge distributions derived from 24-hour EEG recordings from 107 people with idiopathic generalized epilepsy, finding two dominant subgroups with distinct distributions of epileptiform discharges. To explore possible physiological contributors to these distinct variations in seizure likelihood, we developed a mathematical model that describes the transitions between background activity and seizure-like states in large-scale brain networks. This model includes a time-dependent forcing term to simulate the impact of external physiological factors on node excitability. The parameters of this forcing term were calibrated by using independently collected human cortisol recordings and sleep-staged EEG from healthy human participants.

We found that sleep accounted for the majority of observed variability in one group, whereas the dynamic variation in cycling hormone levels accounted for the majority of observed variability in the second group. We further found that combining both measures improved the explained variability in the first group, whereas it did not in the second group.

Our findings provide conceptual evidence for the presence of underlying physiological drivers of rhythms of epileptiform discharges. We propose that future research should explore these mechanisms in carefully designed experiments using animal or human models.

P47: Examining the influence of sex and cycle in mouse torpor

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Sex differences in hibernation have been observed across a number of species where females typically express deeper and longer bouts, and have a greater propensity to hibernate. Recent work has investigated the role of Esr1 expressing neurons in the hypothalamic preoptic area in generating torpor in mice, suggesting a possible link between hormonal status and torpor. Surprisingly little has been reported on sex differences in mouse torpor, though many studies report findings from female mice only. The aim of the present study was to investigate features of torpor in male and female mice, as well as examining torpor in different phases of the mouse oestrous cycle.

To examine sex differences, surface temperature FLIR recordings were taken from male (n=8) and female (n=8) wild-type mice during fasting-induced torpor. Female mice expressed a greater torpor score over 24 h ($p < 0.05$) that was driven by a lower nadir temperature ($p < 0.05$), but not longer bouts. The effect of oestrus cycle was examined in female TRAP2 mice in diestrus (n=5) and estrus (n=4) during fasting-induced torpor. Mice in diestrus displayed significantly longer ($p < 0.05$) and deeper ($p < 0.01$) bouts of torpor than mice in estrus.

These findings support a body of evidence that implicate females as more ‘energetically savvy’ with regards to hibernation and torpor, which may be driven in part by circulating gonadal steroid hormones. Future work will test this by administration of exogenous hormones or blockade of hormone signalling to support these findings.

P48: A novel role for *CYFIP1*, an Autism spectrum disorder candidate gene, in regulating IL-6/STAT3 signalling

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Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder with large genetic and phenotypic heterogeneity. One of the most common forms of syndromic ASD, Chromosome 15q-Duplication Syndrome (Dup(15q)), is associated with increased expression of *Cytoplasmic FMRP-Interacting Protein 1* (*CYFIP1*). Notably, CYFIP1 associates with and modulates the expression of another ASD candidate gene, *Janus Kinase and Microtubule-Interacting Protein 1* (*JAKMIP1*). However, the exact roles that CYFIP1 and JAKMIP1 play in brain development are yet to be fully understood.

We have recently discovered a novel role for CYFIP1 in regulating cytokine signalling, specifically, the Interleukin-6 (IL-6)/Signal transducer and activator of transcription 3 (STAT3) pathway. Using HEK293 cells, we demonstrate that *CYFIP1*-overexpression (OE) leads to reduced STAT3 expression and enhanced STAT3 transcriptional activity following stimulation with IL-6. We have also confirmed reduced *Stat3* expression in cortical tissue from *Cyfip1*-OE mice. Importantly, in both *CYFIP1*-OE cells and tissue, this was associated with a reduction in *JAKMIP1* expression.

To better understand the roles of CYFIP1 and JAKMIP1 in modulating STAT3 activity, we have generated CRISPR constructs to endogenously tag CYFIP1, JAKMIP1 and STAT3 with fluorescent proteins, and we have confirmed these by immunocytochemical staining in HEK293 cells. We aim to use these constructs to investigate the localisation and interactions of these proteins following IL-6 stimulation. We also plan to confirm our findings using induced pluripotent stem cell lines obtained from individuals affected by Dup(15q) with ASD. This will allow us to determine whether alterations in neural cytokine signalling pathways are characteristic of Dup(15q) with ASD.

P49: A canine obesity GWAS reveals a new role for *DENND1B* in the regulation of human MC4R signaling

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The top association in a canine genome wide association study (GWAS) for obesity is with DENN domain containing 1B gene (DENND1B). DENN domains are highly conserved and play a key role in membrane trafficking, specifically receptor-mediated endocytosis for DENND1B. There is a well documented role of DENND1B in T cell receptor down-modulation and its loss leads to increased signaling and allergic disease. Ubiquitous expression of DENND1B meant we hypothesised genetic variants could dysregulate receptors with known roles in energy homeostasis, such as melanocortin 4 receptor (MC4R).

We identified DENND1B as significant in a GWAS for human BMI (UKBB) with variants reducing expression associated with reduced human BMI. In murine single nuclei RNA-sequencing data (HypoMap) we showed robust co-expression of DENND1B with hypothalamic receptors MC4R and Growth Hormone Secretagogue receptor (GHSR). RNAScope in human hypothalamus samples is ongoing. We tested whether DENND1B affects activation and internalisation of MC4R and GHSR in response to ligand activation in vitro. A luciferase-based GloSensor cAMP assay revealed DENND1B overexpression significantly reduced MC4R signaling and knock down increased signaling. Internalisation of labelled receptors was quantified from HILO images. Overexpression of DENND1B significantly increased MC4R internalisation to the endosomes and knock down reduced internalisation. In contrast, DENND1B expression did not alter GHSR signaling or internalisation suggesting receptor specificity.

These data show the power of canine genomics to prioritise human GWAS loci for further study. We identify a novel mechanism linking canine and human GWAS associations to obesity via DENND1B altering MCR4 trafficking and signalling.

P50: Neurotensin regulates glucose homeostasis via a specific population of enteropancreatic neurons

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The neuropeptide Neurotensin is predominately expressed in the brain and the gastrointestinal tract, and has well established central effects on appetite, thermoregulation and analgesia. Peripherally, Neurotensin increases lipid absorption from the gastrointestinal lumen, but its role in glucose homeostasis is unclear. Neurotensin acts via three receptors, with most reported effects being mediated via the NtsR1, and is endogenously released following the ingestion of olive oil.

Previously, we have found that Neurotensin acutely improves glucose tolerance in both lean and diet-induced obese mice using a peripherally mediated effect to stimulate the secretion of insulin. Studies from the 1990s have reported a population of neurons which span the gut-pancreas connection in both mice and humans and we investigated whether Neurotensin may exert its glucoregulatory effect via these neurons.

Using a NtsR1Cre::Ai9 reporter mouse model and RTF tissue clearing, we found that a subpopulation of these enteropancreatic neurons at the proximal duodenum are NtsR1-positive.
To assess their role, we ablated these neurons using a Diptheria Toxin Receptor (DTR) ablation approach. Cre-dependant DTR-expressing AAV was surgically injected into the pancreas adjacent to the proximal duodenum of NtsR1-Cre mice. Glucose tolerance tests revealed that ablation of these neurons prevents the glucoregulatory effects of both Neurotensin, at pharmacological doses, and olive oil, revealing a functional role for NtsR1-positive enteropancreatic neurons in the neuroendocrine control of glucose homeostasis. Future work will aim to further characterise these neurons to investigate the potential of pharmacological exploitation of this system to treat metabolic disease.

P51: Human, Rodent and Pharmaceutical Studies to Investigate Intranasal Administration of Kisspeptin as a Novel Delivery Route for the Management of Reproductive Disorders

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Background: Kisspeptin is a potent regulator of hypothalamic GnRH neurons with potential to treat reproductive, psychosexual and bone disorders. However, administration is limited to the subcutaneous or intravenous routes. Herein, we comprehensively examine the translational potential of intranasal kisspeptin administration for the first-time using human, rodent and pharmaceutical studies.

Methods: *Human studies:* Healthy men (n=12) and a patient group of women with Hypothalamic Amenorrhoea (HA) (n=5) completed a randomised, double-blinded, crossover, placebo-controlled study investigating the effects of intranasal kisspeptin-54 administration (doses: 3.2-25.6nmol/kg [healthy men] and 12.8nmol/kg [women with HA] vs placebo) on reproductive hormone secretion over 4hrs. Thereafter, we conducted *rodent studies* in adult C57BL/6J male mice to elucidate possible mechanisms for kisspeptin's effects. Finally, we undertook *pharmaceutical studies* to evaluate the chemical stability of kisspeptin-54 in solution for nasal delivery.

Results: *Human studies:* in healthy men, intranasal kisspeptin dose-dependently increased serum LH at doses 6.4-25.6nmol/kg (p<0.01 for all doses vs placebo) with maximal rises at 30mins. Likewise, in women with HA, intranasal kisspeptin acutely increased serum LH (p=0.004 vs placebo). *Rodent studies:* We demonstrate that GnRH neurons located in the olfactory bulb express kisspeptin receptors and that intranasal administration of fluorescently-tagged kisspeptin-54 binds to the olfactory epithelium. *Pharmaceutical studies:* Kisspeptin-54 in 0.9% saline remained within pharmaceutically accepted limits for stability for 60days at 4°C, demonstrating realistic pharmaceutical potential.

Conclusion: We identify robust clinical effects and provide mechanistic and pharmaceutical data for intranasal delivery as a novel, non-invasive and effective kisspeptin administration route for the management of common reproductive disorders.

P52: Profiling duodenal enteric nervous system typology changes in prediabetes and type 2 diabetes in human and mouse

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Type 2 diabetes (T2D) is associated with Western diet high in saturated fats and low in plant-based foods. Long-term exposure of the gut to Western diet can induce neuroplastic changes in the enteric nervous system (ENS). Rodents fed high-fat diet experience loss of ileal and colonic myenteric neurons. The extent of neuroplastic changes in the ENS in response to Western diet and the relationship to T2D remains unexplored. At least 20 classes of enteric neurons are currently distinguishable, by combinatorial chemical coding. A major challenge is to systematically analyse the different classes of enteric neurons in pre-diabetic and T2D context, in humans and pre-clinical models of T2D. Hypothesising that the ENS could be implicated in T2D aetiology and pathology, the aim of this work was to explore ENS changes in pre-diabetic and T2D duodenum. Human formalin fixed duodenum tissue from T2D, pre-diabetic and non-diabetic patients was obtained from gut resection surgeries, sectioned and immuno-stained. Mouse duodenum tissue was obtained from C57BL/6J mice fed for 12 weeks on high-fat or control diet and oral glucose tolerance tested to ascertain the diabetic phenotype. Mouse duodenum tissue was fixed by perfusion with 4% paraformaldehyde solution, cryo-sectioned and immuno-stained. We present results of profiling human and mouse duodenal ENS typology changes in pre-diabetes and T2D using acquisition of fluorescence signals with ZEISS Axioscan 7 Microscope Slide Scanner and QuPath software analyses of combinations of antibodies against 18 different kinds of neural markers.

P53: A fresh view of the somatostatin/cortistatin system's therapeutic potential in neuroendocrine neoplasms

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Neuroendocrine neoplasms (NENs) are a heterogeneous group of malignancies with growing incidence, due in part to improvements in diagnosis and increased awareness. Surgery is usually effective for local disease, whereas disseminated or metastatic disease requires medical treatment, which is not always successful. Gastroenteropancreatic (GEP)-NENs), the most prevalent NENs, commonly express somatostatin receptors (SSTs), which provide a suitable target for pharmacological treatment based on the use of SSAs, the synthetic, long-lasting analogues of somatostatin (and its natural analogue cortistatin). In this context, the main aim of this work was to test and dissect the effects of the compounds of the somatostatin/cortistatin system in different GEP-NENs models. Firstly, SSTs profile was assessed in the new and 'classic' NEN cell models (primary and metastatic pancreatic neuroendocrine tumours [Pan-NETs] and gastrointestinal neuroendocrine carcinoids [Gi-NECs]), revealing relevant differences that could explain their distinct response to each compound. Secondly, effects of somatostatin, cortistatin and SSAs (1nM-1μM) on key functional parameters related to tumour aggressiveness were tested. A promising efficacy of specific compounds in specific models was observed, associated to the distinct features of each model, providing a precise guide in the search for personalised treatments. Finally, we explored what mechanisms could underle the impact of the drugs in tumour behaviour in each cell model. Overall, this study provides a fresh vision of the somatostatin/cortistatin system as a therapeutic approach in new NEN cell models that could help bringing personalised medicine closer to neuroendocrine neoplasm patients.

P54: Investigating regulators of melatonin synthesis by combination of viral and RNA interference tools in rat models

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Melatonin is a neuroendocrine hormone which regulates circadian and seasonal rhythms. It is synthesised and secreted by the pineal gland during night-time following noradrenergic stimulation. Rats are the preferred animal model to study melatonin synthesis, due to the location and the size of the rat pineal gland, but also because most laboratory mice do not produce melatonin. However, there are fewer genetic tools developed for use in rats compared to laboratory mice.
Here, we have combined short-hairpin RNA (shRNA), a type of RNA interference that knocks down the expression of a gene by interfering with either the transcription or translation, with adeno-associated viral (AAV) infection of living cells to manipulate gene expression. We have validated shRNA that targets the LIM homeobox 4 (*Lhx4*) gene, which was previously demonstrated to regulate expression of genes encoding melatonin-producing enzymes in primary cultures of rat pinealocytes. We then optimised the production of AAVs expressing this shRNA, as well as a scrambled control, sequence using AAV-producing HEK293 cells. This provides us with the tools to optimise and develop AAV-shRNA mediated knockdown of *Lhx4 in vivo* through intra-cranial injection of the virus product to the rat pineal.

Infection of the rat pineal gland by AAVs expressing shRNA, and production of AAVs in-house, is a novel application that allows us to delineate the roles of a variety of homeobox genes in addition to *Lhx4* and their roles in melatonin synthesis *in vivo.*

P55: Brainstem Clocks: growing apart in postnatal development

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Introduction
The dorsal vagal complex (DVC) of the brainstem plays key roles in energy homeostasis and ingestive behaviour. Recently, we showed that the adult rodent DVC contains at least three distinctly phased circadian oscillators that are sensitive to metabolic and dietary cues. Similar to humans, rodents undergo marked postnatal changes in the pattern of intake and composition of their diets, but when in ontogeny the DVC oscillators initiate rhythms in clock genes is unknown.

Methods
To assess ontogeny in molecular clock rhythms, expression of the circadian clock gene protein, PERIOD2, was monitored through bioluminescent signals resulting from the production of a luciferase reporter. Recordings were taken from DVC explants prepared from PERIOD2::LUCIFERASE (PER2::LUC) mice at postnatal days (P)3, P7, P10, P14, P21, and >P60 (adult). Bioluminescence intensity was analysed with the ImageJ Stacks T-Functions plugin. Circadian period, rhythm amplitude dampening, and phase of peak expression were analysed and compared across early postnatal development to adulthood.

Results and conclusions
Rhythmic PER2 production in the DVC was detected at P3, with circadian period as well as the phasing among DVC oscillators altering across postnatal development. Further research is required to determine whether changes in responsiveness to metabolic and dietary cues also accompany this ontogenetic progression. In conclusion, properties of brainstem circadian oscillators alter as the mouse matures.

P56: Computational modelling of the interaction between kisspeptin and GABA-glutamate in the posterodorsal medial amygdala

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Kisspeptin is considered a main regulator of reproductive function in mammals. While the role of kisspeptin has been extensively studied in the hypothalamic region of the brain, little is known about its importance in other parts. In particular, large population of kisspeptin and its receptors have been found in the posterodorsal medial amygdala (MePD), where it acts as an upstream input for the sub-populations of neurotrasmitters of gamma-aminobutyric acid (GABA) and glutamate. These neurotransmitters then provide upstream input to the kisspeptin in the arcuate nucleus, affecting the function of gonadotropin-releasing hormone (GnRH) pulse generator. We propose a coarse-grained mathematical model that captures the cooperative and competitive dynamics between the sub-populations of GABA and glutamate neurotransmitters in the MePD using a phenomenological framework. By performing the continuation analysis, we investigated how the changes in the afferent input from kisspeptin and connectivity strength between the sub-populations influence the dynamics of the system. This insight is key to understand the functional role of GABA-glutamate neuronal circuitry, which can be used to design interventions to normalise the abnormal function of reproductive system under the influence of stress.

P57: Investigating the astrocyte neurone crosstalk involved in insulin sensing in the dorsal vagal complex

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The central nervous system (CNS) plays an important role in controlling metabolic functions, where obesity and diabetes impact CNS function and thus cause metabolic imbalance. The nucleus of the solitary tract (NTS) located in the dorsal vagal complex (DVC) of the brain is an important regulator of glucose metabolism and feeding behaviour. Little is known about which NTS cell types respond to changes in insulin levels to trigger a neuronal relay to the liver to affect hepatic glucose production (HGP), although work from our lab shows that the majority of $INSR^+$ cells are either $VGAT^+$ neurones or $GFAP^+$ astrocytes. Our study therefore aimed to investigate this further. Eight-week-old male Sprague Dawley rats received DVC stereotactic surgery and vascular cannulation to perform pancreatic-euglycemic clamp experiments. Firstly, our data show that GABAaR antagonists mimic the effect of insulin and decrease HGP, even in the presence of HFD-induced insulin resistance. In addition, insulin's ability to decrease HGP was prevented by direct NTS-infusion of GABAaR agonists. Knockdown of the insulin receptor specifically in NTS astrocytes also prevented the ability of insulin to decrease HGP, illustrating that glial-GABAergic neuronal crosstalk may be essential for NTS insulin sensing. This is the first characterization of the neuronal population responsible for insulin sensing in the NTS and the first elucidation of the NTS neuronal-glial crosstalk associated with insulin action. Novel treatments are needed for diabetes and obesity and our work further illustrates how targeting the CNS may be the answer.

P58: Investigating Kisspeptin Neurons in the Amygdala

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The medial amygdala (MeA) is a brain region involved in the integration of socio-sexual stimuli into hypothalamic and cortical circuits governing reproduction and social decision making respectively. Kisspeptin, a neuropeptide with a critical role in the regulation of fertility, is produced by neurons in two regions of the hypothalamus, the arcuate (ARC) and the anteroventral periventricular (AVPV) nuclei. However, a smaller population of Kisspeptin neurons is also present within the MeA, where they have been shown to have a role in the modulation of sexual behaviour in males. Much less is known about their function in females as well as their basic physiological and molecular properties in both sexes. Given their location within the amygdala and the reproductive circuits, as well as being modulated by sex steroids, this population represents an intersection of behaviour and reproductive function. An assessment of the distribution and number of Kiss1MeA neurons was conducted in a transgenic Kiss-Cre:Tdt mouse model which revealed a significant difference in the number and distribution of Kiss1MeA between males and females, as well as temporal changes at different ages of adult life. Preliminary whole-cell patch-clamp recordings indicate differences in the excitability of Kiss1MeA neurons between male and female mice.

P59: Desynchronization of the hypothalamic tuberoinfundibular dopaminergic neuronal network in lactating rats

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The tuberoinfundibular dopaminergic (TIDA) neurons play a critical role in the regulation of prolactin secretion. Their synchronous network activity and slow but highly rhythmic oscillatory pattern of firing activity is known to greatly influence their dopamine release in rats1. While those network properties of TIDA neurons are crucial in establishing the prolactin negative feedback mechanism during non-lactating conditions, the behaviour of the network is unknown during lactating conditions, where prolactin is on high demand. We hypothesised the TIDA neuronal network of lactating rats to desynchronise leading to cessation of the negative feedback loop. Here we aimed to use *ex-vivo* Ca2+ imaging to achieve population-wide simultaneous real-time monitoring of TIDA neuron activity from non-lactating (NL) and lactating (L) tyrosine hydroxylase-cre recombinase rats (n=10) injected with an adeno-associated virus (AAV) containing Ca2+-indicator, GCaMP6s into their arcuate nucleus.

Correlation coefficient analysis revealed significantly lesser synchronized TIDA neuronal populations in lactating compared to non-lactating rats (NL:0.87±0.02 vs L:0.22±0.03, $p<0.001$). Additionally, the oscillatory patterns of activity of these desynchronised TIDA neurons displayed significantly lower cell rhythmicity index values compared to non-lactating animals (NL: 0.70±0.02 vs L: 0.17±0.01, $p<0.001$). Intriguingly, among these low rhythmicity neurons, some display higher while others lower firing frequencies compared to that of non-lactating TIDA neurons. In summary, our findings demonstrated TIDA neurons with altered cellular and network activity properties during lactation, characterised by cells exhibiting low rhythmicity oscillations and individual variable firing patterns, as well as diminished intercellular synchrony, which may abolish dopaminergic output, thus allow prolactin release to support lactation.

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P60: Recurrent low glucose exposure (RLG) induces direct metabolic adaptations in pancreatic alphaTC1.9

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Background and aims:
Recurrent hypoglycaemia is a severe complication associated with insulin-treated diabetes. For poorly defined reasons, the pancreatic α-cells that release glucose-raising hormone glucagon can fail to sufficiently respond to recurrent hypoglycaemic bouts. Previous studies have focused on how decreased brain-pancreas communication can diminish hypoglycaemia counter-regulation. However, there is little known about if and how direct α-cell metabolism/function is altered following recurrent hypoglycaemia. We therefore investigated if recurrent bouts of low glucose (RLG) can alter intrinsic α-cell bioenergetics in a murine α-cell line, alphaTC1.9.

Methods
To mimic recurrent hypoglycaemia seen in diabetes, alphaTC1.9 cells were exposed to one (acute low glucose; ALG) or four bouts of RLG (0.5 mmol/l) and recovered overnight in euglycaemic-like glucose levels (5.5 mmol/l). Glycolytic rate was measured using a Seahorse XFe96 flux analyser by calculating proton efflux rate derived from glycolysis (glycoPER) and extracellular acidification rate (ECAR). Mitochondrial respiration was calculated by measuring cellular oxygen consumption rate (OCR). Total ATP was measured by luminescence.

Results:
RLG augmented α-cell basal glycolysis and oxidative respiration compared to ALG. After four bouts of low glucose, RLG increased acute glucose utilisation in a concentration-dependent manner and had retained total ATP levels.

Conclusion:
We demonstrate, for the first time, direct metabolic adaptations in α-cells are induced by prior bouts of RLG. These data could suggest defects in intrinsic α-cell glycolytic and mitochondrial respiration, following RLG, to retain cellular energy status. Therefore, over time, such alterations may play a role in driving α-cell dysfunction associated with defective hypoglycaemia counterregulation.

P61: Insulin-like growth factor 2, a potential regulator of prolactin receptors in the choroid plexus

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Insulin-like growth factor 2 (IGF2) is expressed in the choroid plexus (ChP), within the subventricular zone (SVZ) neurogenic niche. Global loss of *Igf2* in adulthood reduces SVZ mitogenesis and increases the number of new neurons in the olfactory bulb causing increased anxiety [1]. We have observed increased levels of IGF2 in mouse cerebrospinal fluid in lactation, a particularly vulnerable time for post-partum mood disorders. In the mouse ChP, *Igf2* increases 6-fold during lactation, but returns back to baseline on suppression of prolactin, suggesting that it is induced by the high levels of prolactin present in lactation. To gain insight into the role of prolactin-induced *Igf2* expression in the ChP during this time, we developed mice in which *Igf2* is conditionally removed from the ChP and measured mood resilience post-partum. Behavioural testing, including a buried food test (BFT), elevated plus maze (EPM) and light/dark transition test (LDTT) were carried out to assess anxiety-like responses and qPCR analysis investigated *Igf2* and *Prlr* levels in the ChP of knockout mice. In the BFT, mice with conditional loss of *Igf2* in the ChP took longer to find a buried fruit loop as compared to controls, however, overall anxiety levels were comparable between knockout and controls in the EPM and LDTT. Interestingly, qPCR analysis of ChP tissue revealed a 50% reduction in *Prlr* expression following deletion of *Igf2*, suggesting *Igf2* is a potential regulator of *Prlr* expression in the ChP. The functional consequences of this reciprocal regulation between *Prlr* and *Igf2* remain to be elucidated.

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P62: Impairment of neuronal morphology and function in *Snord116*^{del} Prader-Willi Syndrome mice

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The mechanisms of cognitive impairment in Prader-Willi syndrome (PWS), remain poorly understood. Since cortical and hippocampal complexity is a determinant of cognitive performance, we have charted the development of neuronal morphology in Snord116p-/m+ (Snord116del) mice, a model for PWS displaying impaired cognitive function.

Cortical and hippocampal neurones were visualised in P10 and P15 brains from Snord116del mice and their wild-type littermates using the Golgi-Cox method, with neurones reconstructed in 3D using Imaris Microscopy Software. Basal dendritic arborisation was impaired in layer V cortical pyramidal neurones of P10 Snord116del mice, the number of branching points and mean dendritic length reduced by 20% and 15% respectively. These impairments, which are similar in males and females, are more exaggerated in P15 mice, the number of branching points being halved. In contrast, hippocampal CA1 pyramidal neurones in P10 and P15 Snord116del mice show a 15-25% elevation in apical dendritic branching.

Since cortical dendrites receive information from neighbouring neurones, our findings imply that the integrational capacity of the layer V cortical pyramidal cells in Snord116del mice may be compromised by restricted connectivity with layer VI and sub-cortical inputs. Conversely, elevated apical dendritic arborization of CA1 neurones may augment signals received from Schaffer collaterals. Thus, loss of Sno-RNA expression from the PWS locus at this critical moment for neuronal development, may have a significant impact on cognitive flexibility in PWS. Establishing the contribution of endocrine and growth factor disturbance to these phenomena will predicate therapeutic strategies for alleviating this distressing aspect of human PWS.

P63: Adipose triglyceride lipase as a regulator of neuroinflammation

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Neuroinflammation associated with obesity is implicated in the development of comorbidities such as diabetes and mood disorders. Microglia have been shown to accumulate lipid droplets (LD) during inflammatory insults. LD act as stores of excess lipids as triglycerides. Adipose triglyceride lipase (ATGL) catalyzes the first step of triglycerides hydrolysis. Loss of ATGL reduces inflammation in macrophages, suggesting an active role for LD dynamics in inflammatory signaling. Taking these data together, we hypothesized that loss of microglial ATGL reduces neuroinflammation.
Mouse primary microglial cultures treated with ATGL inhibitor Atglistatin and/or lipopolysaccharide (LPS) were used to assess the role of ATGL in LD accumulation, cell lipidome and inflammatory signalling. A novel mouse model with an inducible loss of microglial ATGL (CX3CR1-CreER/ATGL$^{lox/lox}$) was generated to study the role of ATGL on inflammation *in vivo.* To model neuroinflammation, animals were administered with LPS or fed HFD for 12 weeks, prior to measurements of parameters of energy balance, glucose tolerance and anxiodepressive behaviors.
LPS-induced inflammation led to LD accumulation *in vitro*. Inhibition of ATGL activity *in vitro* decreased the expression and secretion of pro-inflammatory cytokines induced by LPS. Loss of microglial ATGL *in vivo* also attenuated expression of pro-inflammatory cytokines and anxiety-like behavior in response to LPS. Loss of microglial ATGL increased body weight during high-fat feeding compared to control animals, without affecting food intake or glucose tolerance. Together these data suggest that LD lipolysis by ATGL regulates neuroinflammatory responses in microglia and may play a causal role in diet-induced obesity.

P64: Activation of NTSR1-expressing enteropancreatic neurons improve glucose tolerance

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Chronic adherence to a Mediterranean diet, a diet rich in olive oil, improves remission rates in T2DM patients. Olive oil and its major monounsaturated fatty acid component, oleic acid, stimulate release of neurotensin, a 13-amino acid expressed in the brain and gastrointestinal tract. Neurotensin acts on three neurotensin receptors, but the majority of its biological effects have been shown to be mediated via the NTSR1. Previous studies have reported inconsistent effects of neurotensin on glucose homeostasis.

We found olive oil and neurotensin to acutely improve glucose tolerance in mice. This was driven by an increase in circulating insulin, and the effect was lost in the presence of a NTSR1-specific antagonist. Using in vitro and in vivo models, we confirmed neurotensin did not improve glucose tolerance via activation of pancreatic-, vagal- or central-NTSR1.

Previous work identified NTSR1 expression in the myenteric plexus of the human duodenum. Using NTSR1-cre:tdTomato mice, we identified NTSR1 in the murine enteric nervous system and confirmed NTSR1-mediated neurotensin-activation of these neurons using in vitro calcium imaging. We hypothesised that enteric-NTSR1 may be expressed on a population of enteropancreatic neurons that extend from the gut wall to pancreas and found injection of cre-dependent retrograde Gq-DREADDs into the pancreas of NTSR1-cre mice labelled NTSR1-enteric neurons in the proximal duodenum. Chemogenetic activation of this neuronal population significantly improved glucose tolerance.

These data suggest enteropancreatic-NTSR1 neurons drive the glucoregulatory effects of olive oil and identifies the first functional role for neurons that extend from the proximal duodenum to the pancreas.

P65: Elevated peripheral oxytocin levels in lambs compared to adult domestic sheep, a consequence of bonding or something more?

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The neuropeptide hormone oxytocin (OXT) has been well documented to impact on a range of social behaviours, with crucial roles in the formation and continuation of mother-offspring bonds. While maternal OXT dynamics have been extensively explored to date, there is a current lack of data on infant OT levels. There is evidence that positive OXT feedback loops exist in dependant offspring, motivating them to remain close to their mothers and preventing separation or other negative fitness costs, and that peripheral OXT levels are linked to mass change dynamics during early life. Domestic sheep (*Ovis aries*) have been a model species for studying the central oxytocin changes that accompany birth and bonding processes. In this study we validated an ELISA protocol for detecting oxytocin in a peripheral substrate, plasma, and generated a dataset of basal OXT levels in cohorts of different ages and sexes. The ELISA was successfully validated with extracted plasma, however low sample volume coupled with low OXT concentration levels in some cohorts generated detection issues with some samples. OXT concentrations were higher in young lambs that were still dependant on their mothers than in samples taken from adults. This pattern of dependant infant plasma containing approximately double the OXT levels detected in other age classes is consistent with the few existing studies documenting infant OXT levels in the periphery. Elevated peripheral infant OXT levels have been linked to physical developmental advantages, and the potential role of high OXT in the periphery during infancy needs to be explored.

P66: Dynamic transcriptional heterogeneity in pituitary corticotrophs

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Cellular heterogeneity is an inherent property of biological systems resulting from stochastic intracellular events. Heterogeneous behaviour at the cellular level has been proposed as a means to increase the dynamic range of biological systems allowing for emergent coordinated behaviour at the population level.

The hypothalamic-pituitary-adrenal (HPA) axis is the central controller of the neuroendocrine stress response; at the pituitary level, corticotrophs integrate internal and external signals allowing the generation of pulses of stress hormones. Yet, multiple lines of evidence show that corticotrophs are a heterogeneous cell population, for example, in their cellular morphology, basal and stimulated electrical activity, calcium responses, intracellular pathway activation and ACTH secretion.
Here we conduct a bioinformatics meta-analysis of published single-cell RNAseq data of the pituitary gland to identify different transcriptional sub-populations of corticotrophs. Our analysis shows marked heterogeneity in the transcriptome of corticotrophs, showing the existence of multiple transcriptional states; using a range of bioinformatics analyses, we show the presence of dynamic transcriptional states through which corticotrophs might be transitioning in response to specific environmental cues. However, scRNAseq experiments only capture snapshots of these dynamic changes, meaning a different subset of these states is present in different studies.

Overall, this suggests an intricate regulation of the transcriptional cell state of corticotrophs, allowing them to work as a population to respond to a large and diverse range of stimuli. Finally, it demonstrates the importance of performing and analysing multiple studies when using single-cell RNAseq data to study the transcriptional complexity of neuroendocrine systems.

P67: An *in silico* study of altered expressions in neuroendocrine and cell death-related genes in human pituitary gonadotroph tumours

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AIM: This study aimed to investigate the pathophysiological factors of pituitary gonadotroph tumors (PGTs) using gene expression analysis. PGTs are characterized by symptoms of hypogonadism, hypopituitarism, and visual impairments due to tumor mass effects. Currently, surgical intervention and radiation therapy are primary treatment options, as no effective medical treatments exist. Additionally, *in silico* analysis holds promise for understanding the underlying mechanisms of various diseases.

METHODS: GSE26966[1] dataset obtained from GEO (Gene Expression Omnibus) database was re-examined in the R program for this research. (In the dataset, 10 PGTs with 9 normal pituitary samples obtained at autopsy are recruited.) Based on Benjamini-Hochberg correction, adjusted p-values <0.05 were accepted as significant.

RESULTS: The analysis revealed significant up-regulation of genes involved in neuroendocrine functions, such as neuromedin B (NMB), relaxin-2 (RLN2), chromogranin A (CHGA), and clock circadian regulator (CLOCK). Additionally, genes associated with autophagy, including beclin-1 (BECN1) and various autophagy-related genes (ATG2B,3,4B,5,7,9A,10,13,14), were up-regulated. Furthermore, genes responsible for mitophagy, such as mitofusin (MFN1,2), ubiquitin-specific peptidase-30 (USP30), voltage-dependent anion channel-1 (VDAC1), and ras homolog family member T1 (RHOT1), exhibited up-regulation. Conversely, genes associated with neuroendocrine functions (proopiomelanocortin, cholecystokinin, prolactin, etc.), apoptosis (caspases, Fas receptor, somatic cytochrome c), and necrosis (tumor necrosis factor-related genes) indicated down-regulation in the PGT group compared to the healthy pituitary group.

CONCLUSION: Results from this *in silico* analysis indicate imbalances in expression levels (up- and down-regulation) of genes, implicating involvement of impaired neuroendocrine signalling, besides frequently increased autophagy and mitophagy processes and decreased apoptosis and necrosis processes in human PGTs.

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P68: Vitamin K: Its Antioxidant Effect on Testicular Oxidative Stress and Male Reproductive Hormone Level in Sprague-Dawley Rats

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Oxidative stress is a common cause of testicular dysfunction that leads to disturbance in the endocrine system, thereby affecting the production of male reproductive hormones. Evidence suggests that vitamin K exhibits antioxidant potentials that mitigate the effect of oxidative stress in tissues like liver. The roles of vitamin K in testicular oxidative stress have not been well elucidated. In this study, diets were modified to investigate the effects of a warfarin-induced vitamin K-deficient diet and different dietary forms and concentrations of vitamin K1 and K2 (menaquinone-4, MK-4) on testicular oxidative stress and serum levels of reproductive hormones in Sprague-Dawley rats. Different vitamin K1 and MK-4 dietary levels were achieved by the addition of the pure vitamers to a vitamin K-deficient diet. All animals were randomly assigned to a group (five groups in all) and were fed on their respective group diet for either four or eight weeks. Both forms of vitamin K reduced the level of testicular malondialdehyde and increased the activities of testicular antioxidant enzymes. The findings of this study showed that high dietary concentrations of K2 exhibit more antioxidant potential than K1 in the testes. The serum level of testosterone was higher in the group receiving MK-4 compared to K1. Consistently, the warfarin-induced vitamin K-deficiency group showed an elevated level of testicular oxidative stress and a decreased serum testosterone level.

P69: Neuronal Replacement Therapy for Polycystic Ovarian Syndrome

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Polycystic Ovarian Syndrome (PCOS) is the leading cause of infertility affecting 10% of the childbearing age population. Symptoms range from acne and weight gain to insulin resistance, irregular periods, and ovarian cysts. Birth control pills, diabetes medications, and lifestyle changes can treat the symptoms of PCOS, but none of these are shown to affect the central dysfunction in the neuroendocrine system. This survival-of-the-species system relies on gonadotropin releasing hormone (GnRH) neurons in the hypothalamus synchronizing to generate a pulse. GnRH cells integrate information from various upstream hypothalamic neurons and pass a pulse or surge signal to the pituitary gland. A normal GnRH pulse occurs hourly in humans, while surges induce ovulation. If any part of the brain-body feedback system is damaged, the GnRH neurons will amplify this dysfunction into a system-wide hormone disorder like PCOS. This makes GnRH neurons an attractive target for neuronal replacement therapy. We have generated a mouse line with an inducible GnRH cell knockout. This mouse will be used to produce our PCOS model. We will replace these neurons in the symptomatic adult with alternatives derived from human stem cells. These cells are functionalized to act as pacemakers. Either filtering out dysfunctional inputs from the hypothalamus or ignoring them and only delivering regular GnRH pulses to the body. Using this approach, we hope to reverse PCOS dysfunction in mice.

P70: Changes in somatotroph organisation but not ultrastructure after exposure to prenatal glucocorticoid

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Stress and glucocorticoid exposure in prenatal and neonatal life is recognised to cause long-term programming alterations to the HPA axis. However, influences on other endocrine axes are less well. Glucocorticoids negatively impact growth and GH production but also stimulate somatotroph development and growth hormone expression. We have investigated the effect of prenatal in-vivo dexamethasone in drinking water (1 μg/ml) on gestational days 16-19 in pregnant rats (John et al 2006, J Neuroendocrinol 18:949-959) on immunolabelled GH-secreting somatotroph cells in male and female adult offspring. By use of transmission electron microscopy and Image J-analysis software; the number, distribution, cellular/nuclear size, in addition to organelle appearance were compared between dexamethasone-treated and control rats to determine whether cellular and ultrastructural differences reflect changes in GH-processing. We also monitored the distribution of secretory granules in somatotrophs, in particular in relation to a vascular border if applicable. Neighbouring contacts with corticotrophs and somatotrophs were counted. Results showed no significant differences in any somatotroph ultrastructure parameters. In somatotrophs located next to a blood vessel, secretory granules were found to be distributed to the vascular pole of the cell but there was no significant difference in this distribution between male and female, control or prenatal dexamethasone groups. A larger number of corticotroph-somatotroph and somatotroph-somatotroph contacts were observed in prenatally exposed dexamethasone rats compared to controls. Although glucocorticoids are required for somatotroph development and differentiation, perinatal dexamethasone exposure does not significantly alter adult somatotroph ultrastructure but does influence neighbouring cell relationships.

P71: $Ca_v3.1$: a novel leucine sensor in hypothalamic neurons that mediates protein appetite and energy balance control

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In this study we explore the molecular underpinnings of leucine sensing in the mediobasal hypothalamus (MBH). Using the unbiased PhosphoTRAP technique, we found that *Cacna1g,* which encodes for the T-type voltage-gated calcium channel $Ca_v3.1$, is enriched in leucine-activated neurons. $Ca_v3.1$ is expressed in discrete subsets of hypothalamic populations, including POMC neurons which are known to sense leucine and mediate its anorectic actions.

Systemic $Ca_v3.1$ inhibition protects against diet-induced obesity and *Cacna1g* expression is significantly downregulated in the hypothalami of Prader-Willi patients, but the role hypothalamic $Ca_v3.1$ in the regulation of energy balance is unknown. We hypothesised that $Ca_v3.1$ in MBH POMC neurons contributes to leucine sensing and mediates the effects of dietary protein on energy balance.

Pharmacological inhibition of $Ca_v3.1$ activity blunted leucine-induced activation of POMC neurons in murine primary MBH neurons, brain slices and human induced pluripotent stem cell (hiPSC) derived MBH neurons. In vivo, pharmacological and genetic inhibition of MBH $Ca_v3.1$ significantly blunted the appetite-suppressing effects of local leucine injections. Importantly, genetic ablation of MBH *Cacna1g* abolished the feeding and metabolic responses to high protein feeding. Selective ablation of *Cacna1g* in MBH POMC neurons broadly recapitulated the behavioural and metabolic phenotypes observed following whole-MBH *Cacna1g* KO. Mechanistically, evidence obtained in HEK293 cell models suggests that leucine binds to $Ca_v3.1$ and enhances the voltage-dependent activation of the channel. Finally, pharmacological activation of $Ca_v3.1$ in the MBH promotes substantial weight loss alone and potentiates liraglutide-induced weight loss in diet-induced obese mice.

Together, these results indicate that $Ca_v3.1$ is necessary for MBH leucine sensing and the effects of dietary proteins on appetite and energy balance, and identify a novel nutrient-sensing mechanism in POMC neurons. $Ca_v3.1$ represents a viable target for anti-obesity treatments.

P72: Brain structure and behavioral changes in a mouse model of human chromosomal deletion disease with Autism Spectrum Disorders

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The prevalence of Autism Spectrum Disorders (ASD) has been on the rise in recent years. Its etiology remains unknown due to the involvement of various genes and the diversity of phenotypes. Copy number polymorphisms are known to be present in ASD individuals, among other changes that occur in individuals with ASD. We established a mouse model of human chromosomal deletion disease (3p del) in which three risk genes (CHL1, CNTN6 and CNTN4) for ASD are simultaneously deleted in the 3p26 deletion region of chromosome 3.

Previous studies have used models with single-gene deletion, which resulted in mild phenotypes. However, the actual effect of 3p26 deletion on these three defective region genes remains unclear. Therefore, we used 3p del mice to assess whether it shows human ASD-like behavior, which is known to be altered in the one-gene deletion model, via behavioral tests. Our results showed that 3p del mice exhibited reduced anxiety and repetitive behavior, but reduced exploration time towards other mice, indicating decreased socialization. In addition, Histological analysis of brain sections by Nissl staining revealed morphological abnormalities in the hippocampus, which is involved in memory and emotion, and enlargement of the lateral ventricle in some individuals. By elucidating the changes in 3p del mice and their mechanisms, including the relationship between ASD-like behavior, genes, and anatomical changes, we hope to further understand ASD and contribute to the establishment of a new treatment for ASD associated with 3p26 deficiency.

P73: The role of oxytocin in the neuroendocrine control of reproduction

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The hypothalamic-pituitary-gonadal (H-P-G) axis controls reproductive function, as hypothalamic gonadotrophin-releasing hormone (GnRH) regulates hypophysial release of luteinizing hormone (LH), which is essential for ovulation and steroidogenesis. A limited number of *in vitro* studies suggest the nonapeptide oxytocin (OT) may influence LH release, however, the precise mechanisms involved are yet to be delineated. This study aimed to elucidate the role of sex steroids in modulating the OT-stimulated LH response by measuring ELISA-determined whole blood LH concentrations in C56BL/6 WT mice, 15, 30 and 60 minutes following intracerebroventricular OT administration in ovariectomized (OVX; n=5) and ovariectomized oestrogen-treated (OVX + E_2; n=4) females, as well as, gonadectomized (GND; n=7) and gonadectomized testosterone-treated (GND + T; n=5) males. In females the presence of oestrogen elicited a significant rise in LH concentrations 15 min following OT administration (from 0.34 +/- 0.1 to 0.83 +/- 0.25, p=0.049) compared with a tendency to decrease LH concentrations in the absence of oestrogen (from 1.71 +/- 0.54 to 1.25 +/- 0.45, p=0.054). This contrasted with males, whereby GND mice displayed a significant increase in LH concentration 15 min following OT administration (from 1.64 +/- 0.29 to 3.19 +/- 0.17, p=0.003) but with no change in LH concentration observed in the presence of testosterone (from 0.57 +/- 0.05 to 0.64 +/- 0.09, p=0.6). These findings suggest that OT acts on the H-P-G axis to influence LH release in a sex- and sex steroid-dependent manner, but further research is required to delineate the underlying mechanisms.

P74: Chemogenetic Manipulation of Brainstem Noradrenergic Neurons Modulates the Activity of the Kisspeptin GnRH Pulse Generator in Mice

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Kisspeptin neurons in the arcuate nucleus (ARN^{KISS}) represent the GnRH pulse generator driving pulsatile luteinizing hormone secretion. These neurons serve as a hub to integrate multiple cues, such as metabolic and stress-related inputs. Brainstem noradrenergic (NA) neurons have long been implicated in the control of GnRH pulse generator, but the precise mechanism remains unclear.
Here we used intersectional *Cre-lox* and FLP-FRT recombination approaches to couple ARN^{KISS} neuron GCaMP6 fiber photometry with the brainstem NA DREADD modulation to investigate the role of NA in controlling the ARN^{KISS} neurons.

Adult female Kiss1-Cre,GCaMP6s mice were crossed with a mouse line expressing FLP recombinase selectively in NA neurons. Mice were injected bilaterally with an FLP-dependent retrograde viral vector (AAVrg-hSyn-fDIO-hM3D(Gq)-mCherry-WPREpA) into the mid-caudal ARN to express excitatory (hM3Dq) DREADD receptors selectively in brainstem NA neurons that project to the ARN. An optical fiber was implanted to detect the calcium-dependent population activity of $KISS^{ARN}$ neurons. Histology revealed m-Cherry/DREADD expression in small sub-populations of tyrosine-hydroxylase neurons located in the brainstem A1, A2 and A6, confirming direct innervation from these regions to the ARN. Selective activation of these NA neurons with 1.5 and 3 mg/kg CNO (s.c.) in diestrous-stage mice, dose-dependently inhibited the frequency of the pulse generator synchronization episodes by 48-76% over 4 hours.

These observations demonstrate that, when activated, brainstem NA neurons exert an inhibitory influence on the kisspeptin GnRH pulse generator in intact female mice. The new data may help to dissect how key components of the stress-responsive circuitry impact the central regulation of fertility.

P75: Regulation of neurosteroids in the mouse social behaviour network

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JD and DD: equal contribution to the work.

Steroid hormones such as testosterone and oestradiol are typically thought to be produced by the gonads and the adrenals but may also be produced in the brain i.e. neurosteroids. One of the best studied neurosteroids is oestrogen, produced from testosterone by the enzyme aromatase. Aromatase is widely distributed in the male and female rodent brain, including in the nodes of the social behaviour network (SBN), an interconnected and evolutionary conserved set of brain nuclei that drive social behaviours such as sex, aggression, partner preference. Pharmacological inhibition of aromatase in rodent hippocampal slices leads to a decrease in long term potentiation, concomitant with decrease in spine density. Recently, a forebrain-specific knockout of aromatase showed reduced cognition and social behaviour, underscoring the relevance of the local production of oestrogens in the brain.

We show, consistent with other reports, that oestradiol in both male and female mouse brains is higher than oestradiol in the plasma. Our novel slice culture system also allows us to further demonstrate ex vivo, the increase in neurooestrogen and neurotestosterone production within 24 hours of incubation in all nodes of the SBN nuclei in both males and female mice. However, there is considerable sexual dimorphism in the expression of steroidogenic enzymes in each of the nodes. Interestingly, we show that regulation of neurosteroid production in the female is dependent on androgen synthesis. The significance of aromatase regulation in mice will be further discussed.

P76: Progesterone Regulation of the GnRH Pulse Generator

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The arcuate nucleus (ARN) kisspeptin neurons, which represent the 'GnRH pulse generator", display synchronized episodes (SEs) of activity approximately every hour for most of the cycle but these dramatically reduce in frequency following the LH surge. As these ARN kisspeptin neurons express the progesterone receptor it is hypothesized that they may be the site at which P4 negative feedback occurs.

Given the lack of detailed information in the mouse, we first assessed the profile of circulating P4 levels in mice at 8-hr intervals throughout the estrous cycle using LCMS. The concentration of P4 was found to peak at 6 pm on proestrus (21.94 ± 3.78 ng/ml) and was at its lowest at diestrus 10 am (0.42 ± 0.11 ng/ml).

We next assessed the impact of circulating P4 on the activity of the GnRH pulse generator using ARN kisspeptin neuron GCaMP fibre photometry in freely behaving mice. A prior study found that IP administration of 8 mg/kg P4 resulted in circulating P4 concentrations reaching 34 ng/ml (Wong et al., J Pharm Pharmacol 64, 2012). Hence, in this study, diestrus mice equipped for monitoring the activity of the kisspeptin pulse generator were injected with either 8 or 4 mg/kg P4 at 10 am and SEs monitored for 24 hrs. Both doses resulted in a significantly decreased frequency of SEs compared to the vehicle ($p<0.05$), which persisted for 6 hrs.

These results indicate that P4 in physiologically relevant levels exerts powerful inhibitory control over the activity of the kisspeptin pulse generator.

P77: GWAS in Labrador retrievers identifies novel obesity genes in dogs and humans.

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Obesity is a complex disorder with far-reaching effects on human health. Despite heritability being estimated at 40-70%, the majority of genetic variants responsible are yet to be uncovered. In dogs, selective breeding and population bottlenecks simplify gene mapping for disease. Similarities between canine and human obesity means genetic associations in dogs can prioritise candidates from human genomic studies for further investigation. A genome wide association study for obesity in Labrador retrievers identified multiple novel obesity genes. Genetic scoring for obesity predicts phenotypes in the closely related breed, golden retriever, but not the unrelated pug breed. This polygenic background influences phenotypic penetrance of well characterised mutations in the leptin-melanocortin pathway. In large human cohorts, we found that syntenic canine obesity genes are associated with both common and monogenic forms of obesity. Finally, selective sweep mapping highlighted regions containing known obesity genes including a missense variant in MC4R which affects receptor function. We have therefore identified novel obesity-related neuroendocrine genes in humans by studying a canine obesity model. This demonstrates the benefits of studying complex disease in non-traditional animal models such as the dog.

P78: Metabolic effects of acute circadian desynchronization

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Aims: Shift workers have an increased risk to develop type 2 diabetes. Recently, a human study showed that an acute 12h phase shift has acute negative effects on muscle insulin sensitivity at the onset of the active period. To disentangle the underlying neuroendocrinological and metabolic mechanisms we subjected rats to acute circadian desynchronization.

Methods: First, male Wistar rats were placed in metabolic cages for 3 days in a 12h light: 12h dark (L:D) cycle with standard chow available ad libitum. Then a complete LD reversal (12h: 12h D:L cycle) was done from day4 in three different feeding time groups. Multiple metabolic parameters were measured continuously. The second experiment with food available ad libitum, jugular vein cannulation surgery was performed in rats, 7-10 days later an intravenous glucose tolerance test (ivGTT) was performed at Zeitgeber Time 2 (ZT2) and ZT14. After 7-10 days rats underwent a complete 12h phase L:D reversal, after 3 days the ivGTTs were repeated.

Results: The inverted L:D cycle leads to an expected gradual adaptation of daily rhythms in rats. Rats adapt their rhythm of locomotor activity faster to the inverted L:D cycle compared to their rhythms of food and water intake and glucose tolerance. And the daily rhythm of glucose tolerance has adapted within three days to the inversed light exposure.

Conclusion: Our results indicate that behavioural and metabolic rhythms show differential responses to simulated shift work. This circadian disruption may be related to the high incidence of metabolic disease in shift workers.

P79: The growth-promoting effects of meal-feeding in male mice are mediated by GHSR, the receptor for ghrelin

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The physiological impact of temporal feeding patterns remains a major unanswered question in nutritional science. Using a CLAMS-based automated feeding system, we have demonstrated that meal-feeding, but not grazing, protects skeletal growth rate in male rats and mice, despite reduced caloric intake, and that these effects are reversed in *ghrelin*-null mice. However, deletion of *ghrelin* removes acylated/unacylated ghrelin and obestatin.

To clarify the role of ghrelin signalling, 6-week old male loxTB-GHSR (GHSR-null) mice and wild-type (WT) littermates were singly housed in CLAMS cages and received standard rodent chow either *ad libitum* (AL), nocturnal grazing (GR; 1x 24th of daily AL consumption every 30mins) or nocturnal meal-feeding (MF; 3x 1hr periods of AL feeding) patterns for 3 weeks. MF alone reduced cumulative caloric intake in WT mice by 17% (P=0.018), with body weight gain and body- femoral- and tibial-length remaining unaffected. However, tibial epiphyseal plate width (EPW), an accurate marker skeletal growth rate, was elevated by 19% in MF WT mice (P=0.0007 *vs* AL and P=0.004 *vs* GR), due to 22% and 26% increases in proliferative and hypertrophic zone widths. Multiple indices of abdominal adiposity were elevated in GR and MF WT mice. These effects on growth and adiposity were abolished in male GHSR-null mice, tibial EPW in MF GHSR-null males being 104% of that in AL GHSR-null mice (P>0.999).

Our data indicate that the growth-promoting influence of meal-feeding is mediated by activation of GHSR and that the contemporary switch away from regular meals may be detrimental to growth outcomes.

P80: Impaired microglial activation in the *Snord116*^{del} mouse model for Prader-Willi Syndrome

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Aberrant neuronal morphology is emerging as a defining characteristic of the *Snord116*^{del} mouse model for Prader-Willi Syndrome (PWS), however the mechanisms giving rise to this phenotype remain poorly understood. Microglia, the resident immune cells of the CNS, regulate neuronal development, refinement and maturation. Whilst adult glial cells do not express *Snord116*, altered microglial activity in response to *Snord116*-deficient neurones could impair neuronal morphology in *Snord116*^{del} mice.

Immunohistochemistry and 3DMorph was used to quantify microglial morphology in P60 male and female *Snord116*$^{p-/m+}$ (*Snord116*^{del}) mice and wild-type littermates. Microglia from the *Snord116*^{del} neocortex and medial prefrontal cortex (mPFC) display increased cell volume (neocortex: 17%, $P<0.0001$; mPFC: 11%, $P<0.0001$), branch point number (neocortex: 19%, $P<0.0001$; mPFC: 10%, $P<0.0001$) and average branch length (neocortex: 46%, $P<0.0001$; mPFC: 26%, $P<0.0001$).
Since homeostatic microglia exhibit long, highly arborised processes and reactive microglia display shortened, less arborised processes with larger somas, our results suggest that the microglia in *Snord116*^{del} mice display a more homeostatic than reactive state. These less active microglia could contribute to the aberrant morphology of both embryonic- and adult-born neurones. The integration of adult-born neurones is essential for cognitive flexibility, pattern separation and behavioural inhibition all of which are altered in the cognitive phenotype of PWS. Our observations, which are consistent with the development of hyperghrelinaemia in PWS, suggest a potential mechanism by which aberrant neuronal morphology might arise and be maintained in this condition. Further characterisation of these mechanisms will ultimately predicate potential therapeutic strategies for improving the cognitive aspects of PWS.

P81: Brainstem PrRP and PPG projections to the dorsomedial hypothalamus reduce food intake without reducing the activity of "hunger" neurons

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Anorectic signals from the gut reach the brain through the brainstem nucleus of the tractus solitarius (NTS). Two phenotypically-defined anorectic neuron populations in the brainstem contain either prolactin-releasing peptide (PrRP) or preproglucagon (PPG). While $PrRP^{NTS}$ neurons are responsive primarily to meal-related satiety signals, PPG^{NTS} neurons respond to more aversive stimuli. Both neurons induce anorexia when stimulated and, thus, may converge on common downstream targets. Hunger-promoting agouti-related peptide neurons in the hypothalamic arcuate nucleus ($AgRP^{ARC}$) are inhibited by a variety of anorectic stimuli. The central pathways leading to $AgRP^{ARC}$ inhibition are yet to be fully defined, but potential relays in the NTS and dorsomedial hypothalamus (DMH) have been implied. Using a combination of chemo- and optogenetics, we have identified central pathways utilised by $PrRP^{NTS}$ and PPG^{NTS} neurons, including direct projections from the NTS to the DMH. Selective activation of either population, using Cre-dependent stimulatory designer receptors, reduces both normal night-time feeding and fast-induced refeeding, as well as inducing cFos in the DMH and other potential downstream targets. To demonstrate these projections are sufficient to reduce feeding, we used Cre-dependent optogenetics to activate terminals in the DMH. Selective optogenetic stimulation of $PrRP^{NTS \rightarrow DMH}$ or $PPG^{NTS \rightarrow DMH}$ projections reduced feeding in hungry mice. Finally, by combining chemogenetics and fibre photometry, we demonstrated that stimulation of $PrRP^{NTS}$ or PPG^{NTS} neurons reduced food intake without inhibiting the activity of $AgRP^{ARC}$ cells. In summary, these two NTS→DMH projections represent parallel pathways that can cause anorexia without necessarily switching off hunger.

P82: Exploring the role of Janus kinase and microtubule-interacting protein 1 (*JAKMIP1*) in the activity of cortical glial cells and development of Autism Spectrum Disorder (ASD).

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder with unclear underlying mechanisms. In two types of syndromic ASD, abnormal expression of the gene encoding *JAKMIP1* has been found. *JAKMIP1* knockout (KO) mice exhibit impaired social and excessive restricted behaviour, resembling ASD-like behaviour, suggesting a crucial role of *JAKMIP1* in ASD development. However, the downstream function of this protein is elusive.

We examined the contribution of *JAKMIP1* to brain morphogenesis and identified an increased density of astrocytes and microglia in the somatosensory cortex of adult *JAKMIP1* KO mice. This is supported by qPCR data which showed increased gene expression of astrocyte and microglial markers. Inflammation is a major environmental risk factor for ASD, and clinical reports document aberrant glial cell activity. To gain a more complete understanding of how *JAKMIP1* affects glia, we aim to assess the morphology of astrocytes and microglia in *JAKMIP1* KO mice.

JAKMIP1 interacts with Janus Kinases 1 (JAK1), a component of the IL6-JAK1-*STAT3* pathway, which is known to be involved in inflammation. To clarify the effects of *JAKMIP1* at the single cell-type level, we performed magnetic-activated cell sorting to isolate populations enriched in astrocytes, microglia, neurons, and oligodendrocytes. *JAKMIP1* was highly expressed in neurons and all other cell types. Additionally, *STAT3* was found to be most highly expressed in microglia. We plan to investigate how *JAKMIP1*-deficiency affects *STAT3* expression in all cell types and how it regulates inflammatory responses. As JAK1-STAT3 is also implicated in hormone signalling, these studies may also be relevant to neuroendocrine systems.

P83: Delineating the surge GnRH neuron ensembles in adult female mice

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Neuronal ensembles related to a stimulus can be identified using the activation of immediate early genes (IEGs), such as c-*fos*. Here, we use the robust activity marking (RAM) technique to mark the specific clusters of the gonadotropin-releasing hormone (GnRH) neurons activated exclusively during the luteinizing hormone (LH) surge. During the proestrus LH surge, approximately 46% of GnRH neurons (N=3) in the preoptic area were activated. We hypothesised that the same group of GnRH neurons is involved in all LH surges. To test this, we used the adeno-associated virus (AAV) carrying the RAM construct with a doxycycline-dependent Tet-Off system, AAV-RAM-tdTomato, that can only be activated in the absence of doxycycline. The Tet-Off system enabled the precise control of the activation time frame for "tagging" GnRH neurons over three consecutive LH surges. Immunostaining was performed using the IEG marker c-Fos and another component of the AP-1 transcription factor, c-Jun, to identify the RAM+ GnRH neurons that were activated in the final LH surge when the animals were culled. Careful analysis of seven animals culled at 0-3.5 hour time points post-LH surge showed that 41.6 ± 0.2% of GnRH neurons located around the AAV injection site were RAM+ over 3 oestrous cycles. However, only 41.3 ± 0.4% of these RAM+ GnRH neurons (N=3 animals) were co-labelled with c-Fos and 38.7 ± 0.3% co-labelled with c-Jun. This indicates that only approximately 40% of GnRH neurons active in prior LH surges were activated at the time of the final LH surge just prior to death. This data suggests that different, partly overlapping, subpopulations of GnRH neurons are responsible for activating the LH surge on subsequent oestrous cycles.

P84: Chemogenetic activation induces dopamine secretion from the TIDA neurons of lactating rats

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Prolactin activates the tuberoinfundibular dopaminergic (TIDA) neurones to secrete dopamine which inhibits further prolactin secretion from the pituitary thereby forming a negative feedback loop. During lactation, the TIDA neurones remain prolactin responsive, but the high circulating prolactin promotes them to synthesise the opioid peptide enkephalin instead of dopamine. Unlike dopamine, enkephalin stimulates prolactin secretion from lactotrophs, essentially switching into a prolactin positive feedback system. We aim to address the hypothesis that during lactation, the TIDA neurones can be activated to release enkephalin thus promote prolactin secretion.

Using tyrosine hydroxylase-Cre rats injected with Cre-inducible AAV-DREADDs(hm3dq)-mCherry into the arcuate nucleus, we measured the prolactin levels of day 6 postpartum dam, pre- and post-stimulation of TIDA neurones by CNO. Blood samples (10min intervals) were collected from the tail vein of dams deprived of pups for 1h, 40min before and after CNO (3mg/kg, i.p) or vehicle. Sampling was repeated two days later with the alternative treatment on the same cohort of animals.

Our result showed that there's a further reduction from basal levels after pups removal. This suggests that the TIDA neurones release dopamine when activated "chemogenetically". To confirm this, dams with pups in place were treated with CNO followed by the dopamine antagonist, domperidone (0.2 mg/kg) 40min later. We showed that CNO again suppressed the high prolactin levels in these dams (n=5) but domperidone restored prolactin to pre-CNO treatment levels compared to vehicle group (n=3). Our findings show, for the first time, that TIDA neurones are still able to release dopamine during lactation.

P85: Chemerin signalling pathways, receptor trafficking and regulation in the hypothalamus

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Chemerin peptide has multiple roles, functioning as a chemoattractant adipokine regulating energy balance in the hypothalamus and provides an important link between neuroinflammation and obesity. Chemerin's actions are mediated by its receptors CMKLR1, GPR1 and CCRL2, activating MAP kinase pathways in peripheral tissues. Here, we tested cell-specific expression of chemerin and its receptors in mice hypothalamic tanycytes, astrocytes, microglia, and neurons and found that chemerin colocalises with CMKLR1 in tanycytes. *Chemerin*, *Cmklr1* and *Gpr1* mRNA are constitutively expressed over 24 hours, but *Ccrl2* showed diurnal rhythms with the highest expression at ZT8. To assess CMKLR1 activation, we treated hypothalamic E44 cells with chemerin (10nM) and/or α-NETA (10nM), a selective antagonist against CMKLR1. We show that chemerin treatment induces ERK1/2 and AKT phosphorylation, and chemerin/α-NETA administration significantly reduces the ratio of ERK1/2 and AKT phosphorylation. Interestingly, chemerin administration in the E44 cells modulates the mRNA expression of anorexigenic neuropeptides (*Agrp* and *Npy*) and inflammatory markers (*Il-6* and *IκBα*), and *Gpr1* mRNA expression was increased after chemerin and/or α-NETA treatment in the E44 cells. To test if chemerin affects CMKLR1 trafficking, we performed surface biotinylation assay on E44 cells by comparing the surface and total receptor fraction. Chemerin treatment induced CMKLR1 endocytosis after 5 mins suggesting a dynamic regulation of receptor. Our results suggest that chemerin contributes to hypothalamic inflammation and the neuroendocrine control of appetite, including cell proliferation/differentiation and survival. Our data demonstrate that chemerin is a promising target for urgently needed pharmacological treatment strategies for obesity.

P86: Intrinsic neuronal plasticity in kisspeptin neurons of the arcuate nucleus of female mice at puberty

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Puberty is a transition period from infertility to fertility and is governed by specific neuronal circuits in the hypothalamus, including kisspeptin neurons of the arcuate (ARC) nucleus that exert pulsatile control of the hypothalamic-pituitary-gonadal axis. The aim of this study was to test the hypothesis that arcuate kisspeptin (*Kiss1*^{ARC}) neurons from female mice alter their physiological properties from an immature to a mature phenotype during puberty, with puberty defined here as vaginal opening. Pre-puberty mice were divided into two age groups: 3-4 week old and 4-5 week old. Post-puberty mice were 6-9 weeks old and had a stable estrous cycle. Whole cell current clamp recordings were made from *Kiss1*^{ARC} neurons expressing TdTomato in brain slices and action potentials were evoked by depolarising current injections. *Kiss1*^{ARC} neurons from 3-4 week old pre-puberty mice showed a significantly lower maximum number of action potentials and a significantly higher firing frequency than those from 4-5 week old pre-puberty mice or post-puberty mice. *Kiss1*^{ARC} neuron action potential waveform was significantly different between 3-4 week old pre-puberty mice and post-puberty mice in their amplitude, width and afterhyperpolarisation amplitude, suggesting that changes in action potential repolarisation might contribute to changes in firing properties. These data provide evidence for intrinsic plasticity of action potential firing in *Kiss1*^{ARC} neurons during the puberty process as female mice transition from being infertile to fertile.

P87: Kisspeptin cell-type and fiber projection analysis reveals its potential role on central sensorial processing and behavioral state control

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Previous chemoanatomical studies of kisspeptin (KP) distribution in rodent brain have focused on hypothalamic regulation of the hypophysial-gonadal axis. Here, we examined the chemo- and neuroanatomy of KP neurons in mouse and rat, comparing male and female to better understand the potential function(s) of extra-hypothalamic KP projections. We employed immunohistochemistry, and dual in situ hybridization (DISH) combining mRNA probes for kisspeptin with VGAT, VGLUT2, neurokinin, dynorphin, and tyrosine hydroxylase. One hundred and seven brain regions were identified that contained KP fibers and/or terminals. Eighty-one of these regions are extra-hypothalamic, including telencephalic septum, ventral striatum, amygdaloid nuclei and bed nucleus of stria terminalis, diencephalic habenular complex, mesencephalic superior and inferior colliculi, periaqueductal grey, metencephalic parabrachial nucleus and locus coeruleus, myelencephalic *nucleus tractus solitarii* (NTS), reticular formation, and gelatinous layer of spinal sensorial trigeminal nucleus. These regions are all involved in central sensorial processing and behavioral state control. KP cells with a glutamatergic (VGLUT) phenotype are located mainly in arcuate nucleus and scattered in the dorsal hypothalamic region from anterior to posterior hypothalamus. KP cells with a GABAergic (VGAT) phenotype are found in the rostral periventricular region, the medial amygdala and the NTS. These studies provides an anatomical basis for further hypothesis generation about KP's role in neuroendocrine and neuronal regulation. We emphasize the remarkable presence of KP projections in central sensory and behavioural state control structures, which suggest a wider role for KP neurotransmission in general CNS function than previously envisaged.

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P88: New vista of postnatal neurogenesis and migration in hypothalamic vasopressinergic nuclei in rat

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In early 1990s, Dick Swaab and collaborators reported the hypothalamic vasopressin and oxytocin nuclei (VON) in pig change in size and cell number during various postnatal stages and in response to changes in gonadal-reproductive status. They suggested that the VON neurons undergo postnatal neurogenesis according to physiological demands. However, these observations went largely unnoticed. Analyzing numerous sets of whole rat brain serial sections with immunohistochemistry against vasopressin (AVP-ir), we observed morphological features that suggested the postnata AVP-ir neurons could disperse from the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei. Lateral hypothalamus was observed as the main target region but they were also found in other subcortical regions, always adjacent to AVP-ir fibres that formed bundles and/or matrices. Tunnel-like structures outlined by AVP-ir fibres were observed, contained AVP-ir cell bodies. Derived from this observation, we hypothesized that PVN and SON must undergo postnatal neurogenesis to replenish the nuclei. We devised a simple experiment by repeated administration of 5-bromo-deoxyuridine (BrdU) for 15 days in control rats and with water deprivation rats (24 hr/48hr). We observed presence of BrdU-ir nuclei in PVN and SON, and this phenomenon was increased in the experimental group. The BrdU-ir nuclei were observed as large and round shaped and frequently there were paired as twin-BrdU-ir nuclei. By double-labeled immunofluorescence AVP/BrdU, we corroborated that some BrdU-ir cells were indeed become AVP-ir mature neurons. These findings are in consistence with the early studies *vide supra* that provide new insight on the current controversy on adult neurogenesis in the mammalian hypothalamus.

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P89: The GnRH pulse generator activity in mouse models of polycystic ovary syndrome (PCOS)

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Up to one in five women worldwide suffer from polycystic ovary syndrome (PCOS), a condition typified by problems with fertility, high androgen levels, and altered metabolism. The hypothalamic GnRH pulse generator is responsible for driving the pulsatile release of LH to control ovarian function. As PCOS patients show a profound increase in LH pulse frequency, it is thought that over-activity of the GnRH pulse generator may have a major role in generating the sub-fertility of women with PCOS. Recent studies involving the real-time imaging of neural activity in genetic mouse models have revealed that a population of kisspeptin neurons located in the arcuate nucleus of the hypothalamus is the long-sought after "GnRH pulse generator". With this technique, we aimed to characterize the activity pattern of the arcuate kisspeptin neurons in the two most-widely used mouse models of PCOS: prenatal androgen (PNA) and peripubertal androgen models. In the first model, PNA female mice exhibited near-acyclic estrous cycles as previously reported, but surprisingly exhibited only a slightly (~30%) increased frequency of arcuate kisspeptin neuron synchronization events (SEs) over a 24-hour recording period compared to the controls. In the second peripubertal model, female mice implanted with dihydrotestosterone capsules at 3 weeks of age exhibited significant weight gain but fewer SEs over a 24-hour recording period. In conclusion, it appears that neither of the two most-commonly used PCOS mouse models mirrors the human condition in which many PCOS women exhibit robust increases in pulsatile LH secretion.