H1: Dissociation of central and peripheral stress responses.

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Corticotrophin-releasing hormone (CRH) neurons are the primary neuronal population controlling the hypothalamic-pituitary-adrenal (HPA) axis and the secretion of corticosteroid stress hormones. As such, it has been assumed that corticosteroid levels in the blood serve as an accurate proxy for the level of CRH neuron activity in the brain. However, these neurons also have central projections within the brain to control behaviour. This has led to the question of whether CRH neural activity and stress hormone secretion can be dissociated under certain conditions.

Here we set out to test this by recording CRH neuronal activity in vivo with GCaMP fiber photometry under different physiological conditions. These included 1) under the resting state in the day vs the night, 2) in response to acute stress, 3) comparison between males and females and 4) comparison between virgin and lactating mice.

Under each of the conditions/physiological states, we found evidence that CRH neuron activity is regulated independently of peripheral stress hormone secretion. The mechanisms responsible for this are diverse but include the fact that peripheral stress axis output can be modified by factors that act directly on the pituitary and adrenal gland, but not CRH neurons.

Together, these studies suggest an unappreciated complexity in the HPA axis where central and peripheral stress responses can be independently regulated. We speculate that this allows CRH neurons to regulate behaviour and peripheral stress responses semi-independently.

H2: Characterizing a hypothalamic pathway connecting stress and action control circuits

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Background:Acute stress exacerbates the symptoms of neuropsychiatric disorders associated with inflexible behaviour, including substance use disorder, Tourette Syndrome (TS) and obsessive compulsive disorder (OCD). Much research around the role of stress in these disorders has focussed on stress hormones, specifically those involved in the hypothalamic pituitary adrenal (HPA) axis such as cortisol. However stress hormone changes fail to explain some aspects of stress effects on flexible behavioural control, and therapeutics based on this hypothesis have failed in clinical trials. Recently, a novel circuit was identified linking hypothalamic stress sensitive neurons that initiate the HPA axis, and a nucleus in the indirect pathway of the basal ganglia, which is involved in suppression of actions, however the functional role of this circuit is unknown. We hypothesize that synaptic activity in this pathway may mediate stress-induced exacerbation of inflexible behaviour in psychiatric disorders, and reflect a new treatment target.

Methods:To examine this circuit, cell-type and pathway specific optogenetic activation and inhibition was performed during baseline and stress sessions. A mix of male and female transgenic mice expressing cre-recombinase in corticotrophin releasing hormone (CRH) neurons were used (n=26). Optogenetics was used to examine the circuit connecting these neurons in the paraventricular nucleus (PVN) of the hypothalamus to the globus pallidus externa (GPe) in the indirect pathway, by expressing either the excitatory opsin ChR2 or inhibitory opsin eOPN3 in PVN-CRH neurons and implanting bilateral fiber optic probes above the GPe.

Results:Optogenetic activation of the PVN-CRH->GPe pathway induces grooming in mice, which is similar to what is observed following an acute stress (laser x virus interaction p=0.02). Preliminary findings suggest that inhibition of this PVN-CRH->GPe pathway suppresses repetitive behavioural responses to stress (grooming; 0.076).

Conclusions:This work adds to a growing literature demonstrating important actions of synaptic projections of PVN-CRH neurons beyond their role in the HPA axis, that have important implications for how stress contributes to the pathophysiology and symptoms of neuropsychiatric disorders. Future work identifying strategies to target these pathways may help guide the development of new treatment strategies that enhance control over symptoms when patients are exposed to stress.

H3: The effects of oxytocin receptor stimulation in the amygdala and nucleus accumbens on aversive and appetitive conditioning

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Oxytocin (OT) influences a range of social behaviors by enhancing the salience of social cues and regulating the expression of specific social behaviors (e.g., maternal care versus defensive aggression). I previously showed that stimulating OT receptors in the basolateral amygdala of rats also enhanced the salience of fear conditioned stimuli: relative to rats given vehicle infusions, rats infused with TGOT showed greater discrimination between a cue predictive of danger, and one that signaled safety. In the present series of experiments, the effects of OT receptor activation in the basolateral amygdala on stimulus processing were examined further using conditioning protocols that consist of changes in stimulus-outcome contingencies (i.e., extinction and reversal), and with stimuli paired with aversive (i.e., foot shock) and appetitive (i.e., sucrose) outcomes. It was revealed that the effects of OT receptor stimulation in the BLA diverge for aversive and appetitive learning – enhancing the former but not the latter. Finally, using an OT receptor-cre mouse, I show that OT receptors in the nucleus accumbens core are involved in appetitive learning.

H4: Altered function of arcuate leptin receptor expressing neuropeptide Y neurons depending on energy balance

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Objective: One of leptin's main targets in the hypothalamus are neuropeptide Y (NPY) neurons, with selective deletion of leptin receptors (*Lepr*) specifically in *Npy* neurons resulting in major alterations of energy partitioning between fat and bone mass. However, the specific action of these *Npy+/Lepr+* neurons compared to *Npy*-negative *Lepr* (*Npy-/Lepr+*) neurons in regard to energy homeostasis regulation is unknown.

Methods: Specific AAV viral vectors were generated using DREADD and INTRSECT technology and used in male $Lepr^{Cre/+}$ and $Lepr^{Cre/+};Npy^{Flp/+}$ mice to assess the effect of activating either all *Lepr* neurons or specifically *Npy+/Lepr+* or *Npy-/Lepr+* neurons only on feeding, energy homeostasis control, and body composition.

Results: Selective stimulation of *Npy+/Lepr+* neurons led to an immediate decrease in respiratory quotient followed by a delayed increase in food intake in standard chow fed, but interestingly not in high fat diet (HFD) fed mice. In addition, stimulation of *Npy+/Lepr+* neurons led to a robust increase in brown adipose tissue thermogenesis and improved glucose tolerance. These effects were not observed in standard chow fed mice when *Npy-/Lepr+* expressing neurons were specifically activated, suggesting the effects of leptin on these parameters are driven by NPY. However, under HFD condition when leptin levels are elevated, the stimulation of the *Npy-/Lepr+* neurons increased food intake, physical activity and energy expenditure. Interestingly, chronic stimulation of *Npy*-positive *Lepr* neurons was able to increase bone mass independently of bodyweight, whilst chronic stimulation of the *NPY-/Lepr+* neurons resulted in increased bodyweight and fat mass with proportionate increases in bone mass.

Conclusions: Together, these data indicate that leptin signalling through *Npy*-positive *Lepr*-expressing neurons controls energy partitioning via stimulation of thermogenesis, energy expenditure, and the use of fat as a fuel source. However, under prolonged HFD, leptin resistance may occur and actions of leptin signalling through *Npy*-negative *Lepr* hypothalamic neurons may exacerbate excess food intake.

H5: Investigating the role of CRH neurons in the selection of defensive behaviours.

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Threats in the external environment elicit defensive behaviours to prioritise safety. While this is crucial for survival, it may necessitate sacrificing conflicting behaviours, such as foraging. Hypothalamic corticotropin-releasing hormone (CRH) are activated by threats and coordinate the stress response. Emerging evidence suggest CRH neurons are also implicated in generating defensive behaviours. Therefore, the aim of this research is to investigate the role of CRH neurons in selecting defensive behaviours using a novel foraging task. Here, mice can voluntarily leave their home area and explore a novel, and therefore potentially dangerous, foraging area to find chocolate pellets. This creates a conflict between the motivations to remain in safety (defensive behaviour) or foraging for food. We hypothesised that CRH neuron activation would increase defensive behaviours. To test this, we transduced the excitatory designer receptor (hM3Dq) exclusively in CRH neurons using a viral vector. Activation of hM3Dq-expressing CRH neurons with its selective agonist, descloroclozapine (DCZ), increased the time spent in the home area compared to control mice without CRH neuron activation.

To determine whether CRH neuron activation can influence the selection of defensive behaviours over competing motivations (a need for food), we administered DCZ to fasted mice during foraging. Initially, both groups of fasted mice, with and without CRH neuron activation, exhibited similar defensive behaviours as they entered the novel foraging area to obtain food. However, over time, we observed a gradual increase in defensive behaviours in DCZ-treated mice. This suggests that while CRH neuron activation is sufficient to cause defensive behaviours, increased competing motivations (hunger) can overcome this effect. We propose that as hunger motivation diminishes with food intake, defensive behaviours generated by CRH neuron activation regain priority.

H6: Acute stress regulates Agrp neuronal activity

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Hunger is a complex physiological drive that affects both the mood and motivation of an organism to promote food consumption and restore energy balance. Agouti-related peptide (Agrp) neurons in the hypothalamus sense hunger and promote feeding, however when food is unavailable, Agrp neurons promote adaptive behaviours by reducing anxiety and increasing food-seeking behaviour. Thus Agrp neurons respond to environmental stimuli that convey information relevant to food seeking and food detection. Indeed, recent discoveries show that food detection and consumption suppresses Agrp neural activity assessed by fibre photometry. However, when foraging within an environment, food is not the only potential environmental stimulus to be encountered; other such stimuli include acute stressors signalling threat, fear or danger. This study aimed to investigate the effects of stressors on Agrp neural activity and whether optogenetic control of Agrp neurons can simulate the stressful event.

To do this, we combined fibre photometry with various stress paradigms. We recorded Agrp neuronal responses using GCaMP7s in fed and fasted mice during restraint stress, novel environment/object exposure and elevated zero maze. In both, fed and fasted mice, Agrp activity dropped when exposed to stress but less compared to food.

Our experiments show that Agrp neurons are transiently inhibited by acute stressors but rebound immediately once the stressful event has passed. With this insight, we demonstrated that mice learn to avoid the Y-maze arm paired with optogenetically suppressed Agrp activity.

Together our results suggest that a transient decrease in Agrp neural activity encodes a broader “stop foraging” signal that has differential outcomes for food consumption based on the presence of stressful stimuli. Future studies using single cell resolution calcium imaging (Inscopix) aim to reveal the identity and pathway of stress responsive Agrp neurons.

H7: Whole-brain activity mapping of stress in the zebrafish

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Stress responses are evolutionarily conserved and allow organisms to survive in challenging environments. When an organism perceives a potential threat, this leads to the activation of stress circuits in the brain and body to regulate stress responsiveness. To date, no studies have surveyed changes in neural activity at a cellular level across the entire brain following stress hormone elevations due to the sheer volume and complexity of mammalian brains. Zebrafish have a neuroendocrine stress axis homologous to humans whereby stress leads to the release of cortisol and increased locomotion. Zebrafish larvae are transparent and conducive to whole-brain imaging. We performed whole-brain imaging by using immunohistochemical tagging of recently active neurons with MAP kinase markers (MAP-map) and real-time calcium imaging with GCaMP6s (a genetically coded brain activity indicator) in stressed and cortisol-treated zebrafish larvae. MAP-map after repeated agarose embedding stress showed the activation of NPO, an area equivalent to the centre for human stress responses in the hypothalamus. However, we found little evidence of acclimatisation through repeated embedding; larvae that experienced 15 min of embedding had less NPO and whole-brain activation than those embedded three times across three days. In GCaMP6s imaging experiments, we observe a ~20 min ramping down of neural activity and sparse activation of NPO to a stable baseline after agarose embedding, corroborating our MAP-map findings. We conclude that repeated stress in zebrafish larvae only marginally decreased whole-brain responsiveness to the stressor, and the whole-brain activity stabilises within a 30 min time window. Using these temporal insights on how a stressor modifies brain activities, we are currently examining how whole-brain activities are different by directly applying cortisol to the larvae.

H8: Controlling stress at the terminal

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Corticotropin-releasing hormone (CRH) neurons are an essential population of neurons which control stress responses. Activation of CRH neurons leads to the release of CRH peptide from nerve terminals in the median eminence. This in turn drives the release of adrenocorticotropic hormone (ACTH) from the pituitary and the secretion of corticosterone from the adrenal gland. Two key signalling molecules which control the excitability of CRH neurons are noradrenaline (NA) and corticosterone. It has been previously shown that NA can excite CRH neuron cell bodies and corticosterone can inhibit them. However, the effect of these signalling molecules on CRH nerve terminal function has not been determined.

To investigate this, we performed GCaMP6f Ca^{2+} imaging of CRH nerve terminals in brain slices from mice. We electrically stimulated the median eminence to activate the CRH nerve terminals and recorded both baseline and action potential evoked Ca^{2+} responses. We found that corticosterone (1µM) had no effect on basal or evoked Ca^{2+} responses in CRH terminals (Two-way ANOVA, P=0.69, $F_{(1,328)}$=0.16). However, 20 µM NA significantly inhibited CRH terminal Ca^{2+} responses (Two-way ANOVA, P<0.0001, $F_{(1,120)}$=158.5). This inhibitory effect was blocked by α2-adrenergic receptor antagonist yohimbine (20µM) (Two-way ANOVA, P<0.0001, $F_{(1,31)}$=49.08, Yoh vs NA). This suggests circulating NA may inhibit CRH release from terminals in contrast to the excitatory effect NA has at the soma of these same neurons. To investigate if NA induced inhibition of terminal activity leads to a decrease in CRH peptide release we are currently performing experiments using CRH sniffer cells. These FRET based sniffer cells respond to CRH peptide, we will record fluorescent responses to stimulation in the presence and absence of NA.

These findings show how signalling molecules such as NA and CORT may have differing actions at the cell body versus the terminal, having important implications for how CRH and other neurons are controlled.

H9: Feeding Neurons Integrate Metabolic and Reproductive States in Mice

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Balance between metabolic and reproductive processes is important for survival, particularly in mammals that gestate their young. How the nervous system coordinates this balance is an active area of study. We demonstrate that somatostatin (SST) neurons of the tuberal hypothalamus alter feeding in a manner sensitive to metabolic and reproductive states in mice. Whereas chemogenetic activation of SST neurons increased food intake across sexes, ablation decreased food intake only in female mice during proestrus. This ablation effect was only apparent in animals with low body mass. Fat transplantation and bioinformatics analysis of SST neuronal transcriptomes revealed white adipose as a key modulator of these effects. These studies indicate that SST hypothalamic neurons integrate metabolic and reproductive cues by responding to varying levels of circulating estrogens to modulate feeding differentially based on energy stores. Thus, gonadal steroid modulation of neuronal circuits can be context-dependent and gated by metabolic status.

H10: Inhibin deficiencies promote adiposity in female mice

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Ovarian inhibins (α/β dimers), members of the Transforming Growth Factor – beta superfamily, are classically known for their abilities to constrain follicle stimulating hormone (FSH) production at the pituitary. Roles for inhibins beyond the pituitary are poorly understood owing to knockout models developing pathological increases in activins (β/β dimers) triggering gonadal tumour growth and lethal body wasting. We recently generated *Inha*$^{R233A/R233A}$ mice which produce bioinactive inhibin, whilst maintaining physiological concentrations of activins. Lack of negative feedback by inhibin in *Inha*$^{R233A/R233A}$ mice resulted in a 2-3 fold elevation in serum FSH levels and a subsequent enhancement in ovulation rates. Importantly, *Inha*$^{R233A/R233A}$ mice do not develop gonadal tumours or associated onset of cachexia. Intriguingly, female *Inha*$^{R233A/R233A}$ mice display increased body weight compared to wildtype controls which was attributed to accumulation of white adipose tissue. We report the first evidence to suggest that loss of inhibin function may alter adiposity specifically in females. Future investigations will aim to determine the mechanisms by which withdrawal of inhibin activity influences adiposity.

H11: Rewarding Mum: understanding hormonal regulation of reward behaviour in mothers

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Mammalian mothers heavily invest in caring for new-born offspring. To facilitate this investment, parturition is accompanied by increased motivation to provide maternal care. Our work focuses on how hormones act on neural circuitry to promote postpartum maternal behaviour. We have previously shown that prolactin action through prolactin receptor (Prlr) expression in the medial preoptic area of the hypothalamus (MPOA), is critical for the onset of postpartum maternal behaviour and survival of offspring. We hypothesise that prolactin action in the MPOA leads to activation of reward circuitry to promote care-giving behaviour. Here, we have investigated the role of a prolactin-sensitive neural projection from the MPOA to the ventral tegmental area (VTA) in promoting reward aspects of maternal behaviour.

H12: Multiple metabolic cues for fine-tuning puberty onset

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The onset of puberty requires exquisite coordination of genes, hormones and brain circuitry, and in humans it's timing varies enormously. A complex interplay between nutritional status and reproductive function exists, such that puberty is delayed in situations of prolonged low energy availability. A range of hormonal signals produced by peripheral organs supply information about metabolic and developmental status to the gonadotrophin-releasing hormone (GnRH) neurons that control reproduction and their network of afferent inputs. An increasing level of body adiposity, signalled to brain neurons via the fat-derived hormone leptin, is recognised as a major factor controlling puberty onset (1). The leptin signaling pathways by which leptin exerts its modulatory effects on puberty and reproductive function remain poorly understood, and furthermore no population of neurons has been shown to be the sole mediator of this role of leptin. However, leptin actions via hypothalamic agouti-related peptide (AgRP) (2,3) and pituitary adenylate-cyclase-activating polypeptide (PACAP) (4) neurons have been shown to be partially responsible for puberty onset and adult fertility. In contrast, targets of the pancreatic hormone insulin for reproductive modulation may involve astrocytes (5) rather than brain neurons (6). Other hormonal candidates for heralding adolescent development to the GnRH neuronal network include insulin-like growth factor from the liver (7) and irisin from muscle (8). Muscle development seems to correlate more closely to pubertal age than adiposity in sheep and cattle, and possibly to menarchal age in girls. A role for muscle as a cue for puberty onset could help explain, for example, why Māori and Pacific young people (who have relatively low levels of body fat at a given BMI) exhibit earlier puberty than those of European descent. This example shows the importance of obtaining a more complete understanding of the multifaceted regulation of puberty onset across a range of body fat and muscle compositions.

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H13: Developing a permanent sterilization strategy for mammalian pest species using a cell-targeting approach

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Many mammalian pest species, including rats, wallabies, rabbits and the brushtail possum were introduced into New Zealand through human settlement, threatening the country's biodiversity. For example, by infecting livestock with diseases like bovine tuberculosis and preying on native birds, the possum has significantly reduced native fauna. Poisoning and trapping are conventional methods of control that have worked well on smaller scales. However, these techniques have not been nearly as successful on populations covering larger regions which would be essential in preventing further loss of local species. Public acceptance of these methods and their consequences on the environment raises questions regarding their effects on non-target animals and waterway contamination.

Research into the development of a strategy to address these issues is expanding. The ultimate humane predator control strategy would ideally be permanent, ineffective against native birds and without surgical intervention. An avenue being explored is disrupting the reproduction process. This has been studied through immunocontraception, in which vaccines have been developed to elicit immune responses against reproductive proteins that could cause infertility. However, some of the essential requirements for an effective control strategy remain absent from this strategy.

The requirements for the ultimate control strategy would be included in an alternative strategy we are developing. We aim to permanently ablate a group of cells associated with fertility with the cytotoxin, SN38. We developed a targeting molecule that is coupled to SN38 and is taken up in a cell-specific manner via a receptor that is relatively specific to this group of cells. This approach has the advantage of eluding any negative effects on birds because the target receptor is absent, thereby preventing the cytotoxin from affecting our native birds. By destroying this group of neurons, we hypothesize it will permanently sterilize possums and other mammalian predators and cause them to become infertile.

HH14: Spironolactone modulation of hyperandrogenism: a potential treatment for polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder affecting women of reproductive age and is the leading cause of female infertility. A PCOS diagnosis requires the presence of two of the syndrome's three cardinal symptoms: menstrual irregularity, hyperandrogenism, and polycystic ovarian morphology. 43-47% of women with PCOS also experience metabolic syndrome which has been shown to increase PCOS severity. Current research suggests hyperandrogenism is underlying the PCOS etiology, however, all available treatments are symptom-focused rather than addressing underlying causes. Spironolactone, an anti-androgenic drug has demonstrated its efficacy in managing some hyperandrogenemia symptoms, particularly hirsutism. However, full extent of spironolactone's impact on the reproductive and metabolic aspects of PCOS remain unclear.

With this in mind, the present study aimed to establish a suitable spironolactone dose for mice. Male mice were used in this experiment as they have clear androgen-dependent traits to measure, such as seminal vesicle size. 24 C57BL/6 adult male mice were given either DMSO as a control, or 25mg/kg, 50 mg/kg, or 100mg/kg of spironolactone daily for two weeks orally in Nutella (n=6 per group). Bodyweight measurements were taken throughout. An ITT was conducted prior to perfusion, and blood samples were taken for testosterone ELISA assays. Along with seminal vesicles, other features such as testis weight and abdominal fat were dissected and measured after perfusion. We also obtained dorsal skin samples to assess skin thickness in the hopes of identifying other androgen dependent traits that may be present in females for future validation.

For each of the endpoints described above, data analysis revealed that spironolactone treatment had no significant effect at any dose, suggesting ineffective androgen receptor blockade. Consequently, rather than transitioning to a female mouse model of PCOS we have initiated a follow-up experiment in male mice, with continuous administration of spironolactone and an extended treatment duration.

H15: A cytotoxic cell-targeting approach to induce sterility in predator species of Aotearoa

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Aotearoa faces a pressing issue with pests, and the solution lies in a humane approach that specifically targets cells to disrupt their reproductive capabilities. Each year, our cherished taonga (treasured) species are endangered by the escalating populations of possums, rats and stoats. These predators pose a threat to delicate wildlife, ravage their habitats, and deplete precious resources. Additionally, possums can transmit a deadly virus to agricultural animals, while rabbits and wallabies graze on pastoral land, resulting in significant annual economic losses.

The current approach to pest management relies on the widespread use of 1080 poison, which is effective lethally but causes accidental harm to non-target species. Furthermore, the suffering endured by exposed animals can be considered inhumane. To address these concerns, a more environmentally friendly and ethically conscious strategy focuses on impeding animal reproduction, specifically by induing sterility in the target species. This ongoing project aims to achieve this objective by evaluating the potential of two novel cytotoxic drugs, saporin and D-KLAKLAK2, conjugated to a mutual targeting molecule.

The cytotoxins are hypothesized to selectively target reproductive-regulating cells, leveraging the affinity of the targeting molecule to these cells. Once internalized, the cytotoxins induce apoptosis, effectively shutting down the animals' reproductive capacity. The initial set of experiments primarily revolved around assessing the targetting capability of the cytotoxins in mouse brain samples using fluorescent immunohistochemistry. The subsequent set of experiments will focus on evaluating the reproductive inhibition effect by monitoring the absence of regular estrous cyclicity in female mice that received intracerebral injections of the cytotoxins. Brain samples from these female mice will also be collected to detect apoptosis signals and analyse cell numbers, comparing them to control groups using general immunohistochemical methods.

H16: Genetic and Optic Dissection of the Hypothalamic Function in Metabolism

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The long-term goal of Dr. Kong's research is to bring together molecular, cellular, and system approaches to decipher the synaptic plasticity and neural circuits that are subject to metabolic regulation. Toward this goal, the following three questions are asked in his lab: 1) How do neurons in the brain translate their intrinsic firing properties to physiological functions and adaptive behaviors surrounding metabolic homeostasis? 2) How do metabolism-related cues, including internal nutritional signals, blood-borne hormones, and neuropeptides, modulate circuit neurons and shape their firing outputs? 3) What are the molecular contexts underlying such regulations and how do their dysfunctions contribute to human disorders such as obesity and diabetes? Dr. Kong's lab is employing multidisciplinary approaches, including genetically engineered mice, recombinant viral vectors, optogenetic and pharmacogenetic techniques, patch-clamp electrophysiology, 2- photon laser scanning microscopy combined with 2-photon laser uncaging methods, and CRISPR, to interrogate these questions.

H17: Targeted stimulation of Npff neurons induces a torpor-like state in mice

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Background and Objectives: Neuropeptide FF (NPFF) belongs to the evolutionarily conserved RF-amide peptides family, with NPFF receptor 2 (NPFFR2) considered to be its cognate receptor. Although best known for its modulatory role in pain response, increasing evidence suggest that the NPFF system also plays important roles in other physiological processes including energy and glucose metabolism. In keeping with this, strongest NPFF expression is found in the sub postrema area immediate next to the circumventricular organ area postrema, and its expression alters according to energy status. NPFF-expressing neurons co-express various receptors for peripheral factors involved in energy and glucose homeostasis and are mostly glutamatergic. In this study, we investigated the role of brainstem NPFF neurons in the regulation of energy homeostasis by examining the impact of chemogenic stimulation of these neurons on energy homeostasis and thermogenic response.

Methods: Male $Npff^{cre/+}$ mice at 10 weeks of age were bilaterally injected with an AAV-DIO-hM3Dq-mCherry vector into the dorsal-lateral part of the solidary nucleus. Mice were then examined for energy homeostasis in metabolic cages (Promethion system), body temperature and brown adipose tissue (BAT) thermogenesis by infrared imaging upon the stimulation of brainstem NPFF neurons by i.p. injection of clozapine N-oxide (CNO,1.5 mg/kg bw) or saline injection as control.

Results: Brainstem stimulation of NPFF neurons by CNO led to significant reduction in energy expenditure, that occurred within 30 min upon CNO injection and lasted ~10 hours. This hypometabolism following NPFF neuronal stimulation was associated with significant reduction in respiratory exchange ratio, - indicative of a shift towards lipid as fuel source, reduction in locomotion due to increased time being still, and reductions in food and water intake. Moreover, rectal temperature and BAT thermogenesis were also significantly decreased, indicating hypothermia is induced following NPFF neuronal stimulation. These effects of NPFF neuronal stimulation were seen at both 22^{o}C and 28^{o}C ambient temperatures. Importantly, the increases in energy expenditure and BAT thermogenesis expected from decreasing ambient temperature from 28^{o}C to 22^{o}C were also seen with CNO injection, indicating a functional thermoregulatory system in NPFF stimulation-induced hypometabolic and hypothermic state, - a hall mark of torpor. Lending further support, mice injected with CNO at 4^{o}C while remained hypothermic compared to saline injection, exhibited sufficient BAT thermogenesis to remain at a stable body temperature.

Conclusion: These results show that stimulation of NPFF neurons induces a torpor-like state associated with the regulated hypometabolism and hypothermia. Targeting these neurons may have therapeutic potential for the induction of surgical hypothermia.

H18: A dahlia flower extract has anti-diabetic properties by improving insulin function in the brain

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Hypothalamic inflammation appears to play an important role in dysregulation of whole-body glucose homeostasis (1). Butein, a rare chalcone found in the toxic plant *Toxicodendron vernicifluum,* has been shown to regulate glucose homeostasis via inhibition of the inflammatory IKKβ/NF-κB pathway in the brain (1). Here we investigated whether the non-poisonous plant *Dahlia pinnata* could be a source of butein as a potential treatment for type 2 diabetes (T2D). In mice fed high-fat diet (HFD) to induce glucose intolerance, an oral *D. pinnata* petal extract improved glucose tolerance at doses of 10 and 3.3mg/kg body weight. Surprisingly, this effect was not mediated by butein alone but by butein combined with the closely related flavonoids, sulfuretin and/or isoliquiritigenin. Mechanistically, the extract improved systemic insulin tolerance. Inhibition of phosphatidylinositol 3-kinase to block insulin signalling in the brain abrogated the glucoregulatory effect of the orally administered extract. The extract reinstated central insulin signalling and normalised astrogliosis in the hypothalamus of HFD-fed mice. Using NF-κB reporter zebrafish to determine IKKβ/NF-κB activity, a potent anti-inflammatory action of the extract was found. A randomised controlled cross-over clinical trial on participants with prediabetes or T2D confirmed the safety and efficacy of the extract in humans. In conclusion, we identified an extract from *D. pinnata* flower petals as a novel treatment option for T2D, potentially targeting central regulation of glucose homeostasis as a root cause of the disease.

1. Benzler J, Ganjam GK, Pretz D Oellkrug R, Koch CE, Legler K, Stoehr S Culmsee C, Williams LM, Tups A (2015). Central inhibition of IKKβ/NF-κB signalling attenuates high fat diet-induced obesity and glucose intolerance. Diabetes. Jun;64(6)

H19: Neuronal deletion of STAT3, but not ERK2, causes obesity and delayed puberty onset in mice.

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Leptin, an adipose-derived hormone, is important for regulating reproduction. The canonical Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) pathway is the most well-characterised leptin receptor (LepR) signalling pathway. Neural STAT3 deletion is known to cause obesity, however its reproductive influence is less-understood.

Previous data suggests STAT3 signalling may be unnecessary for puberty timing and reproduction [1]. Since these experiments used a weakly expressing Cre line, LepR-Cre, this warranted re-evaluation with a strongly expressing Cre line. This experiment aimed to investigate whether STAT3 knockout from brain neurons would unveil the necessity of STAT3 in reproduction. We also tested the role of extracellular signal-regulated kinase 2 (ERK2/MAPK1), an alternative leptin signalling pathway.

Transgenic mice with neuronal STAT3 or ERK2 knockout (n=7-11/group) were created using the Cre-loxP system (Cam-Kinase IIα-Cre). Puberty onset was observed post-weaning by examining genitalia. Reproductive cyclicity (females) and reproductive organ weights (both sexes) were measured in adults. Metabolic effects were evaluated through body and abdominal fat weight and fasting glucose levels. Brain tissue was analysed to assess STAT3 and ERK2 cellular response to leptin.

STAT3 KO mice showed significantly increased bodyweight and abdominal adiposity compared to controls. Males had a significant delay in preputial separation (5-days), while females exhibited significant delays in vaginal opening (7-days), first estrus (9-days), and showed pronounced acyclicity (all $p<0.01$). STAT3 KO mice had elevated fasting glucose levels and regressed reproductive organs. In contrast, mice with ERK2 knockout had normal bodyweight, and unchanged puberty onset, estrous cyclicity, and reproductive organ weight compared to controls.

These data have prompted re-evaluation of previous conclusions that STAT3 is not necessary for normal reproduction, since these results highlight its importance in these processes while ERK2 signalling appears less critical. Future experiments will target STAT3 knockout to specific neuronal populations known to be important for reproduction.

1. Singireddy, A., et al., *Neither Signal Transducer and Activator of Transcription 3 (STAT3) or STAT5 Signaling Pathways Are Required for Leptin's Effects on Fertility in Mice.* Endocrinology, 2013. **154**.

H20: Phox2b medullary centres motorise feeding

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It has long been known that orofacial movements for feeding can be triggered, coordinated, and often rhythmically organised at the level of the brainstem, without input from higher centres. Here, we show that pan visceral homeobox gene Phox2b demarcates many of the medullary pre-motor structures that organise and pattern these behaviours. Using trans-synaptic viral tracing from lingual, supra-hyoid and masticatory muscles, we show that these genetically defined nuclei, located within intermediate reticular formation (IRt) and the supra (SupV) and peri trigeminal (PeriV) regions of the medulla, are directly pre-motor to all jaw-opening, closing and tongue muscles, and hardwire their unified contraction through axon collaterals. Optogenetic stimulation of these Phox2b pre-motor populations recruits orofacial muscles in a coherent fashion, driving coordinated mouth opening and closing, and protrusion and retraction of the tongue, furthermore, photometry based population activity recordings show they are active during natural feeding behaviours like lapping, chewing and biting. Lastly, rabies based retrograde tracing identifies these populations as pre-motor relays for a diverse range of executive brain areas, including the motor cortex, colliculus and cerebellum. Our observations demonstrate that medullary Phox2b pre-motor neurons provide excitatory drive to the orofacial muscles and may constitute the central pattern generating circuits that coordinate stereotyped feeding behaviours.

H21: The role of the parabrachial nucleus in regulating fluid intake.

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Recent evidence suggests that the parabrachial nucleus (PBN) comprises several different subdivisions involved in regulating fluid intake, including the dorsolateral (for fluid satiation), the external lateral (for satiation of food and caloric fluids) and the central lateral (for regulating sweet-tasting or palatable fluids).

We have focused on characterising the role of the dorsolateral PBN, which contains neurons expressing the oxytocin receptor (OxtrPBN neurons). Chemogenetic activation of OxtrPBN neurons robustly suppresses rapid initial intake of most solutions after overnight dehydration, including water, sucrose, ethanol, saccharin, saline, but only slightly decreased intake of highly caloric solutions, such as Ensure. OxtrPBN activation also suppressed cumulative, longer-term (2 h) intake of lower caloric, less palatable solutions, but not highly caloric, palatable solutions. These results suggest that OxtrPBN neurons predominantly control initial fluid-satiation responses after rehydration, but not longer-term intake of highly caloric, palatable solutions.

To characterise the neurocircuitry regulating fluid satiation, we examined the brain regions activated by $Oxtr^{PBN}$ neurons, using Fos as a proxy marker for neuronal activation; and compared this to brain regions activated by physiological-induced fluid satiation. We demonstrated that both models of fluid satiation activated similar brain regions, suggesting that the chemogenetic model of $Oxtr^{PBN}$ neuron is a good representation of physiological fluid satiation.

We also examined the interaction between the PBN and hypothalamic regions of the brain in controlling fluid intake. We demonstrated that the hypothalamic agouti-related peptide (AgRP) inhibited OxtrPBN firing in a concentration-related manner, suggesting a complex interaction between the PBN and hypothalamic regions in regulating fluid intake.

H22: The role of hypothalamic NPY in salt-induced hypertension and energy expenditure

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Chronic high salt intake is one of the leading causes of cardiovascular diseases particularly hypertension. The hypothalamic arcuate nucleus (Arc) contains NPY neurons that can sense and respond to nutrition levels in the body, regulating appetite and energy homeostasis. However, it remains unclear whether these neurons play a role in the development of salt-induced hypertension. Recently, we have demonstrated that wild type mice given NaCl salt water developed hypertension which was associated with a marked downregulation of Arc NPY expression as demonstrated in NPY-GFP reporter mice as well as by *in situ* hybridisation analysis. Furthermore, salt intake activates neurons in the hypothalamic paraventricular nucleus (PVN) where mRNA expression of brain-derived neurotrophic factor (BDNF) and vasopressin was found to be upregulated, leading to elevated serum vasopressin levels. Additionally, our research revealed that salt intake induced thermogenesis in adipose tissue, leading to increased energy expenditure. This process is likely mediated by the Arc NPY neurons. Together, our studies highlight the important role of Arc NPY in integrating nutrition and electrolyte signals, enabling the coordination of fluid balance and energy homeostasis. These findings provide new insights into the mechanisms underlying salt-induced hypertension and its potential connections to metabolic regulation.

H23: The role of baroreflex regulation of vasopressin neuron activity in the development of hypertension

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Vasopressin is a potent vasoconstrictor but circulating vasopressin levels can be paradoxically elevated in hypertension. It is unknown when vasopressin levels increase in hypertension and whether increased circulating vasopressin contributes to the development of hypertension. Vasopressin secretion is triggered by action potential firing in hypothalamic vasopressin neurons, which is normally inhibited by baroreflex activation of inhibitory GABAergic inputs to vasopressin neurons. We hypothesised that blunted baroreflex inhibition of vasopressin neuron activity would increase blood pressure to contribute to the development of hypertension. Conscious transgenic Cyp1a1-Ren2 rats with inducible angiotensin-dependent hypertension were made moderately hypertensive over seven days. Subcutaneous administration of the vasopressin V1 receptor antagonist, dGly[Phaa1,d-tyr(et), Lys, Arg]vasopressin did not affect blood pressure in non-hypertensive Cyp1a1-Ren2 rats but prevented the increase in blood pressure between days 3 and 7 during the induction of hypertension in hypertensive rats. Under urethane anaesthesia, vasopressin neuron firing rate was higher in hypertensive rats than in non-hypertensive rats on day 7 and intravenous administration of the α1-adrenoreceptor agonist, phenylephrine, caused baroreflex inhibition of vasopressin neurons in non-hypertensive rats, but not in hypertensive rats. In patch-clamp recordings from vasopressin neurons in brain slices, the GABA-A receptor antagonist, bicuculline, excited vasopressin neurons from non-hypertensive rats and the GABA-A receptor agonist, muscimol, excited vasopressin neurons from hypertensive rats. Taken together, our results suggest that increased vasopressin secretion contributes to the development of hypertension and is driven by reduced baroreflex inhibition of vasopressin neurons via a switch in GABA inhibition to excitation.

H24: Do prolactin-sensitive neurons regulate the expression of stress responses in female mice?

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Pregnancy and lactation are periods of significant physiological adaptation to ensure a mother can meet the demands of a growing fetus and new-born offspring. One important adaptation is attenuation of the stress response, with pregnancy and lactation being periods of stress hypo-responsiveness. The mechanism underling attenuation of the stress response is unclear. Corticotropin-releasing hormone (CRH) neurons in the hypothalamic paraventricular nucleus (PVN), govern the stress response through regulating corticosteroid release. Elsewhere in the hypothalamus, we have previously shown that prolactin acting through the prolactin receptor (Prlr) in the medial preoptic area (MPOA) is required for maternal care-giving behaviour. We have recently found a GABAergic inhibitory neuronal current from MPOA Prlr-expressing neurons projecting to the PVN, exclusively in lactating mice (*unpublished data*). We hypothesise that a prolactin-sensitive MPOA to PVN neural circuit regulates the stress response in female mice.

We aimed to firstly, establish testing paradigms that can reliably distinguish differences in stress responses between virgin female and lactating mice. We used a white noise-induced corticosteroid test and a modified pup retrieval test where female mice must enter a novel environment to access pups. Virgin female mice took approximately 10x longer to enter a novel environment and approach pups, indicating a suppressed stress response in lactating mice. Secondly, we aimed to determine whether Prlr-expressing MPOA-PVN neurons regulate the stress response. Two groups of virgin Prlr-Cre mice received bilateral injections of an AAV encoding a light-activated receptor channel Chrimson (AAV-Syn-FLEX-rc[ChrimsonR-tdTomato]) into the MPOA and a fibre optic implanted above the PVN. Animals were tested in the same behavioural paradigms as Aim 1 with one group receiving light stimulation and the control group, no light. If this projection regulates the stress response, we predict that stimulated virgin females will show reduced noise-induced corticosteroid release and increased pup care-giving behaviour compared to controls.

H25: Brain metabolic circuits in cancer cachexia

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Cachexia is a progressive loss of body weight, accompanied by loss of appetite, which affects as many as 80% of cancer patients. The current focus of anti-cachexia research is blockade of tumour-derived circulating factors at the level of fat and muscle. However, given that the brain is the master regulator of metabolic control, targeting the brain to alter metabolic outcomes in cachexia is an attractive idea. Ghrelin is a peptide hormone which rises in response to fasting, drives eating behaviour, decreased energy expenditure and growth hormone release. The primary target neurons of ghrelin are the Agouti related peptide/neuropeptide Y-containing neurons in the arcuate nucleus of the hypothalamus (AgRP neurons). Therapies using ghrelin analogues have been trialled as a way to target the brain to treat cachexia.

We used a mouse model of pancreatic ductal adenocarcinoma (PDAC) to assess ghrelin action *in vivo*. After the onset of PDAC-induced anorexia, PDAC-carrying mice ate significantly less than control mice in response to injected ghrelin, indicating ghrelin loss of ghrelin sensitivity in cancer cachexia even before noticeable wasting has occurred.

To circumvent the observed ghrelin resistance, we used targeted chemogenetics (DREADDs) to chronically artificially activate AgRP neurons during cancer cachexia in PDAC-bearing mice. AgRP neuronal activation rescued fat and skeletal muscle mass loss, decreased brown fat thermogenesis and slightly but significantly increasing locomotor activity in PDAC mice. We measured circulating levels of the pro-cachexia factors, activin A and B. PDAC-bearing mice with or without AgRP neuronal activation showed a similar, significant elevation in activin A and B levels, compared to non-PDAC bearing mice. Importantly, AgRP neuronal activation protected mice from the wasting effects of elevated activins, as the levels seen in this model are sufficient to drive significant wasting.

This provides the first evidence that direct activation of AgRP neurons drives muscle and fat retention in cancer cachexia, and opens the door to therapies that exploit central mechanisms for energy balance control.

H26: The effect of vertical sleeve gastrectomy on feeding mediated by AgRP/NPY neurons

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Currently, bariatric surgeries such as the vertical sleeve gastrectomy (VSG) are the most effective long-term treatment for obesity. The efficacy of VSG is primarily mediated through a reduction in food intake. Although VSG involves removing the greater curvature of the stomach, evidence suggests that reduced feeding after surgery is *not* due to any kind of physical restriction on the amount of food that can be consumed. Instead, VSG potentially causes alterations in neuronal pathways involved in the regulation of feeding. We investigated whether VSG was associated with changes in the regulation of food intake by Agouti-Related Peptide (AgRP) neurons in the arcuate nucleus of the hypothalamus (ARC). To address this question, we used male transgenic AgRP-Cre mice that were maintained on a high-fat diet for 8-12 weeks prior to VSG or sham surgery. We selectively expressed excitatory designer hM3D(Gq) receptors in ARC AgRP neurons of VSG and sham mice via bilateral injection of a DIO-hM3D(Gq) virus into the ARC. Activation of these neurons via administration of clozapine-N-oxide increased consumption of various diets relative to a vehicle injection across counterbalanced test days. Stimulating ARC AgRP neurons increased feeding to the same degree in both VSG and sham animals. To examine whether VSG alters the basal activity of these neurons in response to feeding, we next conducted *in vivo* fibre photometry to monitor bulk calcium signalling as a proxy for neuronal activity. We selectively expressed the calcium indicator GCaMP6s in ARC AgRP neurons and implanted a fibre optic cannula above the ARC. Animals were food-deprived overnight, then presented with food after a baseline recording window. Analysis of calcium signals pre- and post-consumption indicated greater suppression of bulk ARC AgRP neuronal activity upon feeding in VSG compared with sham mice. Together, our findings demonstrate that VSG does not disrupt AgRP-mediated feeding and suggest that VSG may restore the functioning of ARC AgRP neuronal activity in response to feeding.

H27: Olfactory perception and energy metabolism

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Olfactory stimuli from food influences energy balance, preparing the body for digestion and storage of energy when food is consumed. Social olfactory cues are an additional sensory input that predicts subsequent energy use and storage, required for social interactions, and we find that opposite-sex olfactory stimuli from mates have opposing effects on energy balance to olfactory signals received from food. This talk will contrast how olfactory signals received by the main and accessory olfactory systems influence energy expenditure and body weight in mice. While main olfactory system inputs promote energy storage, opposite-sex chemostimuli detected by the accessory olfactory system cause increased metabolic rate in male mice, and reduce body weight and adipose tissue expansion when mice are fed a high fat diet. These responses are linked to detection of female chemostimuli via G-protein Gαo expressing vomeronasal sensory neurons, since males with Gαo deleted in the olfactory system show severely blunted changes in energy expenditure and body weight when exposed to female odours. These results illustrate that detection of female chemostimuli is a central regulator of energy metabolism and lipid storage in male mice, and more broadly that individual sensory sub-systems may have diverse effects on drivers of energy balance.

H28: Cognitive and metabolic effects of diet cycling in rodents

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Studies in humans and animal models show that foods high in fat and sugar (HFHS) can impair cognition, increase adiposity and alter gut microbiota composition. However, HFHS foods are rarely eaten exclusively, and typically form part of diverse diets that vary in composition over the short- and long-term. The cognitive effects of intermittent consumption of HFHS foods are less well characterised. Here we used adult male Sprague-Dawley rats to explore how the short-term memory deficits associated with continuous consumption of a 'cafeteria-style' diet (palatable HFHS foods and 10% sucrose solution, plus chow and water) were affected by (a) withdrawal of the HFHS diet; (b) models of diet cycling and time-restricted feeding; and (c) individual differences in consumption. Short-term memory was assessed via hippocampal-dependent place recognition and perirhinal cortex-dependent object recognition tests; faecal microbiota diversity was analysed via 16S rRNA gene amplicon sequencing. The HFHS diet-induced place memory impairment was ameliorated by withdrawing the HFHS diet for 11 but not 4 days. Place memory and microbiota diversity were progressively altered by HFHS diet cycling, and were still evident following time-restricted access (8h/day). Changes in cognition were associated with microbiota beta diversity but were not predicted by total energy intake nor any macronutrient. Effects of the HFHS diet on cognition were dissociable from effects on adiposity. Results demonstrate that even intermittent or limited intake of HFHS foods may lead to changes in cognitive function.

H29: Dopamine and reward: A novel mechanism contributing to the cognitive enhancing effects of psychedelics

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There is growing interest in the potential psilocybin, the psychoactive compound produced by “magic mushrooms”, to treat a range of mental health outcomes. The therapeutic actions of psilocybin are proposed to involve breaking down inflexible patterns of thought and behaviour, however the neurobiological mechanisms underlying these effects remain inadequately understood. The majority of research has focused on the serotonin (5-HT) system in mediating psychedelic effects, and while increased striatal dopamine (DA) release is elicited by psilocybin in both humans and rats, how this action relates to behaviour or cognition has not been evaluated until now.

We used fiber photometry to examine behaviourally-evoked changes in DA release elicited by psilocybin in the ventral striatum of mice (n=8 psilocybin, n=8 saline). Fluorescence emitted by a dopamine biosensor (GRAB-DA, AAV9-hSyn-DA4.3) was measured in response to eating a palatable reward (peanut butter chip), both acutely as well as 24h and 7 days after psilocybin treatment. We also examined the effects of psilocybin on dopamine release elicited by expected and unexpected rewards, and on reversal learning strategy in a probabilistic reversal learning task using home-cage operant devices.

Dopamine (DA) release in the ventral striatum in response to food rewards was significantly augmented acutely under psilocybin (F=6.62, p=.007), however, this did not persist at either the 24h or 7-day post-administration timepoints. Intriguingly, dopamine recordings during reversal learning suggest that while expected “wins” do not elicit a differential release profile after psilocybin, unexpected “losses” elicit a steep decrease in dopamine release in psilocybin treated animals, compared to controls.

These changes in DA reward signalling indicate a novel mechanism to explain the cognitive enhancing effects of psilocybin that may play a role in its therapeutic efficacy. Ongoing studies will determine the interacting roles of cortico-striatal DA and 5-HT in the encoding of flexible learning under psilocybin.

H30: Dynamics of MPOA prolactin receptor-expressing neurons in freely behaving mice

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Pregnancy and lactation are accompanied by dynamic changes in hormones that induce significant physiological adaptations in the maternal body; for example, changes in food intake, metabolism, and maternal care. Prolactin, acting through the prolactin receptor (Prlr) is important for the activation and execution of maternal care-giving behaviour. The medial preoptic area of the hypothalamus (MPOA) forms the centre of a complex neural circuit that governs maternal behaviour, and integrates sensory and hormonal cues to induce appropriate responses to offspring. Earlier experiments in our group showed that acute deletion of Prlr in all MPOA neurons of adult female mice abolished maternal care soon after parturition. We hypothesise that Prlr signalling during pregnancy and lactation is required for pup-induced activation of MPOA Prlr-expressing neurons. To address this hypothesis, *in vivo* fibre-photometry was employed to measure changes in prolactin-sensitive neural activity during interactions with pups, through quantifying the fluctuations in intracellular calcium (and thereby fluorescence) as a marker of neuronal activity. A genetically encoded AAV-mediated Cre-dependent calcium indicator GCaMP6 was stereotaxically injected into the MPOA of Prlr-Cre mice and a fibre optic implanted to record emitted fluorescence. With fibre photometry recordings of Ca^{2+} transients in freely behaving mice, we showed robust increase in MPOA Prlr-expressing neuronal activity in females during offspring interaction. While visual cue of 3D printed pups did not change the MPOA activity, the presence of offspring in the cage elicited a robust response. We are determining the relative contribution of auditory, olfactory and somatosensory cues form pups in triggering activation of Prlr-expressing MPOA neurons. Subsequent experiments will investigate which subpopulation, defined by projection target, of MPOA neurons are responsible for showing pup-induced activation of $MPOA^{prlr+}$ neurons.

H31: The Edinger-Westphal nucleus: GHSR mediates sex differences in midbrain neuropeptide control of binge drinking

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The circuitry mediating excessive alcohol consumption includes the understudied central projecting Edinger-Westphal (EWcp); a structure dense in neuropeptide expression, including cocaine and amphetamine regulated transcript (CART). While studies have shown a critical role for this nucleus in alcohol consumption, few studies have interrogated the contributions of distinct EW populations. To examine a functional role of these cells in binge drinking we used chemogenetics to inhibit $EWcp^{CART}$ cells in male or female CART-Cre mice. Chemogenetic inhibition of $EWcp^{CART}$ cells had no effect on binge drinking, anxiety behaviour or other consummatory behaviours in male mice; however, a specific reduction in alcohol binge drinking was observed in female mice. Using RNAscope we examined the neurochemistry of EW^{CART} cells observing strong overlap with the ghrelin receptor (GHSR). Given the dense expression of GHSR on EW^{CART} cells we examined whether CART-GHSR interactions within the EWcp mediate binge drinking in female mice. Ghrelin administration increased binge drinking in female mice, which was reduced by chemogenetic inhibition of $EWcp^{CART}$ cells. Finally, we knocked down GHSR expression non-specifically in the EW of male and female mice using a shRNA and specifically from CART (CART-Cre) and glutamatergic (vGlut2-Cre) populations using Cre-dependent Ghsr-shRNA. Non-specific EW Ghsr-shRNA knockdown reduced binge drinking specifically in female, but not male mice compared to scram-ShRNA controls. Further in female mice, Ghsr-shRNA localised to EW^{CART}, but not EW^{vGlut2} cells reduced binge drinking. Together, our results suggest the EWcp is a region mediating excessive alcohol bingeing through GHSR and CART interactions in female mice. Given the recent development of GHSR1a inverse agonists to clinical trials, understanding the neural mechanism(s) underpinning how the ghrelin system mediates alcohol consumption are critical.

H32: Investigating the role of prolactin sensitive medial preoptic neurons in driving paternal behaviour in mice: first steps and surprises.

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The medial preoptic area (MPOA) of the hypothalamus plays a well-established role in the expression of parental behaviour in mice. We have recently shown that the hormone prolactin, acting via the prolactin receptor (Prlr) in the MPOA, plays a critical role in maintaining parental care in both males and females. Blocking Prlr signalling in the MPOA, resulted in female mother mice abandoning offspring and father mice failing to show normal caregiving behaviour. Prolactin-sensitive MPOA neurons display a complex network of projections to many areas of the brain previously implicated in parental behaviour. Importantly, virgin male mice do not typically exhibit paternal behaviour, either ignoring or attacking pups and transition to pup-directed caregiving only following successful mating. Our first aim was to investigate changes in the activity of Prlr-expressing MPOA neurons in response to pups between virgins and fathers. The activity of Prlr-expressing MPOA neurons was recorded by fibre photometry, using an AAV to deliver a genetically encoded Cre-dependant fluorescent calcium indicator (GCamP6) into the MPOA, paired with an optical fibre implant. We then wished to examine the roles of specific MPOA projections in maintaining paternal behaviour. For the optogenetic stimulation of the MPOA projections, virgin Prlr-Cre mice received unilateral injections of an AAV encoding a light-activated receptor channel rhodopsin (AAV-mCherry-ChR2) or a vector containing mCherry alone (controls) and fibre optic probes stereotaxically implanted in the target area.

Using a suite of behavioural testing paradigms, we report a surprising shift in a paternal behaviour induced by individually housing virgin males. We also demonstrate population wide changes in the activity of prolactin-sensitive MPOA neurons in response to various cues, including exposure to pups. Finally, we show evidence to suggest that Prlr-expressing MPOA neurons projecting to the ventral tegmental area provide a motivational cue for pup directed behaviour in males.

H33: AgRP neuron activity throughout the estrus cycle

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AgRP/NPY neurons are a key orexigenic population in the arcuate nucleus driving food intake which respond to many hormones influencing appetite. The sex hormones, estrogen and progesterone modulate appetite and vary across the estrous cycle. Estrogen is anorexigenic and can suppress the orexigenic hormone ghrelin. In this study we investigated feeding patterns and AgRP neuronal activity in mice using *in vivo* GCamp6f fibre photometry across the estrous cycle. Food intake was similar at each stage of the estrous cycle, however changes in feeding patterns were observed. In the dark phase (12h) transitioning between estrus and proestrus, mice increased the number of meals they eat (repeated measures (RM) ANOVA p = 0.0002) while decreasing the size of each meal (RM ANOVA p = 0.03) compared to other times of the estrus cylce. AgRP neuron activity was recorded in response to palatable food (15 mg peanut butter chip) and ghrelin administration (i.p 0.3mg/kg) at the start of the light cycle on proestrus and metestrus. At both stages, peanut butter supressed AgRP activity (2-way ANOVA effect of time p = <0.0001) and ghrelin increased AgRP activity (2-way ANOVA effect of time p = <0.0001). However, the AgRP neuron responses at were not different the different stages of the estrus cycle. Our results indicate a difference in feeding behaviour in the dark phase between estrus and proestrus, with no difference in AgRP neuronal soon after the end of this dark phase on proestrus compared to metestrus. Further work is required to assess AgRP neuronal activity in the dark phase when difference in feeding behaviour are observed. Additionally, as the stage of estrus cycle impacts on meal size and duration it would be of interest to investigate AgRP neuron activity in response to factors that regulate meal size such as cholecystokinin.