Abstract Book

7th International Melanocortin Meeting

15-17 September 2025

Clare College, University of Cambridge

Cambridge, UK

Oral Communications

Symposium 5: Melanocortin Receptor Structure and Pharmacology
Tuesday 16 September 2025, 11:00 – 12:30

OC1

Matching Melanocortin-Receptor Selectivity With Activity and Route of Administration in Disease Applications

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Melanocortin peptide agonists are potential therapies for a wide range of diseases, such as metabolic (eg, diabetes) and inflammatory diseases (eg, dry eye disease and inflammatory bowel disease). The melanocortin receptor (MCR) selectivity profile of the agonist influences the indication, with selective MC1R and MC4R agonists relevant for inflammatory bowel disease and obesity, respectively. Highly efficacious therapies that minimize adverse effects can be achieved by customization of melanocortin receptor selectivity and consideration of the route of administration.

Melanocortin peptides delivered by routes of administration that have the potential for systemic distribution (eg, oral and subcutaneous) should only have activity at the receptors necessary for efficacy. The selective MC1R peptide agonist PL8177 is being developed as an oral treatment for inflammatory bowel disease. PL8177 does not activate MC2R, MC3R, or MC5R and is 1,000-fold more potent for MC1R than MC4R. PL8177 acts on the surface of the colon lumen, and in a phase 2 study, showed substantial efficacy without systemic exposure. Palatin is developing long-acting selective MC4R peptide agonists (MC4R potency >1000X MC1R potency) for treatment of obesity, with efficacy at nanomolar concentrations.

Receptor selectivity is of less importance for melanocortin peptides delivered locally, as there is low risk of systemic exposure and undesirable effects. PL9643 is a pan agonist of MC1R, MC3R, MC4R, and MC5R that activates these receptors at sub-nanomolar concentrations and is currently in phase 3 clinical studies as a topical treatment for dry eye disease. Local delivery to the retina through intravitreal implant requires compounds with high potency due to the limited achievable concentrations. PL9654 activates MC1R at sub-nanomolar concentrations and has demonstrated efficacy in multiple retinal disease models.

OC2

Molecular insights into the potentiation of melanocortin and ghrelin receptor signalling by the MRAP2 accessory protein

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The melanocortin-2 receptor accessory protein-2 (MRAP2) is a single transmembrane protein that interacts with melanocortin receptor 4 (MC4R) and the ghrelin receptor (GHSR) to potentiate their signalling, and human mutations in MRAP2 cause obesity, with hyperglycaemia and hypertension. Previous studies have been unable to consistently show whether MRAP2 affects MC3R activity, and there are unanswered questions regarding how MRAP2 forms heterodimers with GPCRs, the structural regions involved in facilitating GPCR signalling and how human mutations in MRAP2 affect receptor signalling and trafficking. Here we used single-molecule pull-down (SiMPull) to confirm that MRAP2 directly interacts with MC4R, GHSR and MC3R. Analysis of fluorescent photobleaching steps showed that MRAP2 forms monomers or occasionally homodimers at cell surfaces, while the three GPCRs are predominantly monomeric, although MC4R and GHSR have a higher propensity to form oligomers. When co-transfected, MRAP2 readily forms heterodimers with the three GPCRs, most commonly with a 1:1 or 2:1 stoichiometry. Two-colour SiMPull also revealed that dimerisation with MRAP2 disrupts oligomerisation of all three GPCRs. Structural homology models of MRAP2 with the GPCRs identified putative interactions at the transmembrane interface and alanine mutagenesis of five MRAP2 residues significantly reduced MRAP2 effects on GPCR signalling. Moreover, these models revealed a putative intracellular α-helix in the MRAP2 cytoplasmic region that we hypothesised may affect G protein coupling and/or β-arrestin recruitment. Generation of MRAP2 proteins with either truncation of the whole of the cytoplasmic region or chimaeric proteins with either the transmembrane or cytoplasmic region substituted with the MRAP1 protein, demonstrated that the MRAP2 cytoplasmic region is critical for potentiation of signalling and impairment of trafficking of the three GPCRs. Finally, we showed genetic variants in MRAP2 that have been identified in individuals that are overweight or obese prevent MRAP2's enhancement of signalling by MC4R, MC3R and GHSR. Thus, these studies reveal new insights into the molecular mechanisms by which MRAP2 regulates GPCR function and provide further evidence for the crucial role of MRAP2 in energy homeostasis.

Symposium 7: Selected Talks from Abstracts
Wednesday 17 September 2025, 11:00 – 12:00

OC3

Caudal hypothalamic POMC neurons link energy status to reproductive function via melanocortin signaling

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Reproduction is an energy-demanding process highly dependent on nutrient availability and regulated by complex neuroendocrine circuits, including those processing metabolic information regarding body energy depots. However, although some efforts have been paid to unveil the central mechanisms involved in the connection between energy status and reproductive function, neuronal scaffolding participating in these circuits remains yet to be clarified. In this context, POMC neurons, located in the hypothalamic arcuate nucleus, are key components of circuits controlling energy homeostasis, with a dominant anorexigenic (satiety) effect. However, while activation of POMC neurons occurs in conditions of nutrient availability/energy excess, required for proper reproductive function, the predominant role of this neuronal population in the reproductive axis remains ill-defined.

OBJECTIVE: The aim of this study was to explore the effect of the activation of arcuate POMC neurons on the reproductive axis *in vivo* by using a chemogenetic approach.

METHODS: POMC-Cre mice were injected stereotaxically with adeno-associated virus carrying vectors for Cre-dependent expression of the activator DREADD (*Designer Receptors Exclusively Activated by Designer Drugs*) hM3Dq. POMC neurons were activated by intracerebroventricular injection of clozapine-n-oxide (CNO), as pharmacological ligand of hM3Dq. Subsequently, LH release was evaluated, as surrogate marker of the activity of the reproductive axis. Double immunohistochemistry was performed for histological validation and quantification of the chemogenetically activated POMC neurons across the entire rostro-caudal extension of the arcuate nucleus.

RESULTS: Chemogenetic activation of arcuate POMC neurons, by acute CNO administration, resulted in a sustained increase of LH secretion. Neuroanatomical analyses suggested a gradient, rostral to caudal, in the effect elicited by POMC activation on LH secretion, with caudal POMC neurons having a major contribution to this effect. On the other hand, LH response to POMC neuronal activation was partially blunted by the intracerebroventricular administration of the melanocortin receptor 3/4 antagonist SHU9119.

CONCLUSIONS: Our findings provide the first evidence of a rostro-caudal gradient in POMC neuron activation of the reproductive axis mediated, at least partially, by melanocortin signaling. In this context, our data disclose a stimulatory role of POMC neurons that coordinates fertility with energy availability.

OC4

Developmental reprogramming of the melanocortin neurons regulates energy homeostasis

Baijie Xu, Li Li, Xiameng Chen, Rong Wan, Amanda Almeida, Steven Wyler, Swati FNU, Chen Liu

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Central melanocortin neurons play a critical role in regulating energy balance in mammals, with hypothalamic POMC and AgRP neurons driving satiety and hunger, respectively. Despite their well-documented roles in adulthood, the developmental plasticity of these neurons and its impact on long-term metabolic health remain poorly understood.

Using single-cell multiomics, we traced *Pomc*-lineage (PL) neurons in the adult mouse hypothalamus and identified 13 distinct subpopulations. Notably, only 29.8% of these neurons retained *Pomc* expression, while 54% of AgRP neurons were found to originate from *Pomc* precursors.

Our analysis revealed the homeodomain transcription factor *Otp* as a critical determinant of the developmental switch from POMC to AgRP cell fate. Loss of *Otp* in *Pomc* precursors ($Otp^{Pomc\ KO}$ mice) significantly reduced the number of POMC-derived AgRP neurons while expanding a specific POMC neuron cluster. These changes resulted in increased *Pomc* and decreased *Agrp* expression in the adult hypothalamus, reduced food intake, and resistance to high-fat diet-induced weight gain, particularly in females. This sex-dimorphic effect was linked to increased estrogen receptor alpha (ERα) expression and enhanced anorexigenic responses to estrogen in $Otp^{Pomc\ KO}$ mice.

Our findings reveal unexpected developmental plasticity within the central melanocortin system that governs the balance between antagonistic feeding neuron populations. This study establishes a novel connection between developmental programming, sex hormones, and adult susceptibility to diet-induced obesity.

OC5

Development and function of the PVT MC3R neurons and implications for responses to nutritional challenges

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Energy homeostasis is precisely controlled by the brain. Seminal research has established the role of the melanocortin system, specifically within the hypothalamus for the maintenance of energy balance. However, when challenges to the organism are introduced, either through reduced food availability or ample food choice, the brain must regulate the response not only whether to eat or not, but how to prioritize eating behaviors. Recent work has implicated a region dorsal to the hypothalamus, the paraventricular nucleus of the thalamus (PVT) for roles in integrating internal and external environment information to elicit physiological and behavioral responses. Here we show that the melanocortin 3 receptor, (MC3R) is robustly expressed in the PVT, the region is highly innervated by POMC and AgRP neuropeptide projections and that these neurons functionally respond to different environmental perturbations – stemming from fasting or HFD feeding to early developmental overnutrition. Mapping the network of MC3R neurons using genetically tagged MC3R synaptic labeling, monosynaptic rabies tracing and retrograde AAV technologies, uncovered distinct connections of the PVT. Assessment of behavioral responses showed distinct effects mediated by PVT MC3R neuronal activity in response to feeding and influencing feeding-related behaviors. Further we could show that maternal overnutrition alone is sufficient to alter physiological responses to setmelanotide, a melanocortin agonist, in adulthood, both in whole animal physiology and activation of neuronal circuits. The PVT MC3R neurons represent a novel nexus of behavioral control and could indicate a unique brain region involved in integrating feeding-related behavior in various challenging environments.

OC6

A Novel Monogenic Agouti-Like Obesity Trait: Insights from Mice and Men

Kathrin Landgraf[1,2,3], Eric Wenzel[1,2,3], Martha Hanschkow[1,2], Robert Stein[1,2,3], Julia Schuller[2], Oscar Karges[1], Luisa Rajcsanyi[4], Melanie Schirmer[3,5], Thomas Reinehr[6], Winfried Rief[7], Wieland Kiess[1], Anja Hilbert[8], Alexander Jais[2], Kerstin Krause[9], Matthias Blüher[2,9], Anke Hinney[4], Martin Wabitsch[3,5], Nora Klöting[2], Antje Körner[1,2,3]

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Background: We recently identified a novel monogenic obesity trait characterized by a genomic rearrangement resulting in ectopic expression of agouti-signalling protein (*ASIP*) in a girl with severe early onset obesity, red hair, tall stature, and insulin resistance (Kempf&Landgraf et al., 2022). ASIP is a natural inverse agonist of the MC1R (melanocortin 1 receptor) in the skin and can act at other MCRs, e.g. the MC4R, with lower affinity *in vitro*.

Aim: We hypothesized that due to the type of mutation many patients are undetected and set out to screen for new patients and characterize the clinical phenotype. To gain mechanistic insights, we established a humanized transgenic mouse model mimicking the patient mutation and thereby identify therapeutic strategies for treatment of affected patients.

Results: We identified altogether 23 patients with the identical genomic rearrangement, who consistently exhibited extreme obesity from early childhood and a height SDS > 50th percentile. Red(dish) hair was common (particularly during childhood). Hyperphagia was more inconsistently reported. Interestingly, resting metabolic rates adjusted for lean mass were significantly lower compared to age-matched peers. Concordantly, we found lower mitochondrial respiration in primary SVF cells and iPSC-derived hypothalamic-like neurons of the *ASIP* mutation index patient.

Similar to the patients, heterozygous knock-in mice (ki/wt) developed early and sustained obesity and increased body length compared to wild-type control mice (wt/wt) with an increase in fat mass but not so much lean mass which was accompanied by glucose intolerance and insulin resistance. Metabolic phenotyping revealed a modest increase in food intake, decreased locomotor activity but no alterations in energy expenditure. Treatment with the MC4R agonist setmelanotide led to a reduction in the bodyweight of ki/wt mice and was associated with an inhibition of food intake.

Conclusions: The identification of 23 patients carrying the *ASIP* mutation indicates a relatively high frequency of the new monogenic obesity trait with the implication to rethink screening algorithms. The patient phenotypes mimic the agouti-mouse phenotype. Our findings from the humanized

mouse model indicate that the obesity phenotype of our patients is caused by ectopic ASIP and might be treatable with the MC4R agonist setmelanotide.

Poster Presentations

P1

Hypothalamic Alpha-Melanocyte-Stimulating Hormone Regulates Inflammation

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Background:

Obesity is linked to inflammation and worse survival after infection, although the mechanistic links remain unclear. The melanocortin system is comprised of multiple pro-opiomelanocortin (POMC)-derived peptides that are produced primarily in the brain and pituitary gland. One POMC-derived peptide, α-melanocyte-stimulating hormone (α-MSH), has long been assumed to be the principal melanocortin peptide involved in regulating body weight and inflammation. We hypothesized that centrally signaling hypothalamic α-MSH, but not peripherally circulating pituitary-derived α-MSH, critically mitigates inflammation during endotoxemia. To test our hypothesis, we developed a novel constitutive α-MSH deletion model (*αMSH*^{KO} mice) and a Cre-dependent α-MSH deletion model (*αMSH*$^{fl/fl}$ mice).

Methods:

Male wild-type and *αMSH*^{KO} mice (8-14 weeks old) received intraperitoneal (i.p.) injections of saline or lipopolysaccharide (LPS, 0.1-8.0 mg/kg). For rescue experiments, i.p. saline or melanotan II (MTII, 0.1 to 1 mg/kg), a melanocortin receptor agonist, was co-administered to mice with LPS. We used telemetry to measure core body temperature and multiplex immunoassay to measure plasma cytokines and leptin levels. To generate pituitary-specific deletion of α-MSH, we bred *αMSH*$^{fl/fl}$ mice with *Tbx19-Cre* mice. *Tbx19* is expressed by POMC-expressing cells of the pituitary but not by POMC neurons of the hypothalamus. Pituitary-specific deletion of α-MSH was validated by mass spectrometry.

Results:

Compared to wild-type controls, the *αMSH*^{KO} mice displayed exaggerated inflammatory and thermoregulatory responses to LPS. Low-dose LPS (0.1 mg/kg) induced significantly higher plasma levels of IL-1β, IL-6, MIP-1α and MIP-1β in *αMSH*^{KO} mice compared with WT mice. In *αMSH*^{KO} mice, MTII attenuated the LPS-induced elevations in IL-1β and MIP-1α. Notably, MTII attenuated the prolonged hypothermic response induced by LPS in *αMSH*^{KO} mice. Next, we administered LPS (0.1 mg/kg) to *Tbx19-Cre:: αMSH*$^{fl/fl}$ mice and their Cre-negative littermates and observed no differences in cytokine levels.

Conclusions:

Our findings indicate that hypothalamic α-MSH is a crucial regulator of inflammatory responses to LPS, while pituitary-derived α-MSH is dispensable in this context.

P2

Engineering genetically encoded tools for spatiotemporal control of endogenous melanocortin-4 receptor

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Obesity poses a significant health challenge, particularly in the United States, where over 40% of adults and 20% of children are affected. The peptide hormone α-melanocyte-stimulating hormone (α-MSH) regulates energy balance and feeding behavior by activating the melanocortin-4 receptor (MC4R). Loss-of-function mutations in MC4R are the most prevalent genetic cause of obesity, emphasizing the need to understand the complex neuronal circuitry involving melanocortin receptors. Traditional methods for studying this pathway often have poor spatiotemporal control or rely on artificial neuronal activation, failing to mimic the natural signaling environment. We addressed these limitations by engineering a cell surface-tethered protein switch to control the activity of α-MSH and therefore perturb endogenous activity of the melanocortin receptors, including the MC4R. These tools were engineered and validated in mammalian cell cultures using a live-cell luciferase-based assay. *In vivo* implementation in mouse models demonstrated significant behavioral changes as a result of both overexpressed and endogenous MC4R activation. Our findings indicate that this approach can be used to further study MC4R function within the melanocortin system, specifically its role in regulating feeding behavior and energy homeostasis. This offers promising new directions for understanding the tissue-specific actions of the melanocortin receptors.

P3

The metabolic and inflammatory phenotypic changes resulting from the agonism of the ABCA1 transporter in the brain of mice

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Proopiomelanocortin (POMC) neurons in the mediobasal hypothalamus act as first-order sensors, responding to hormonal and nutrient signals to promote reduced food intake and increased energy expenditure. Apolipoprotein-E (ApoE) is known to activate POMC neurons, contributing to reduced food intake. In this project we used the peptide CS-6253 (CS), an agonist of the cholesterol transporter ABCA1 in the central nervous system as an approach aimed at increasing the expression of ApoE, an effect observed in previous in vitro studies. We hypothesized that the CS would lead to an increased ApoE expression in the hypothalamus that could activate POMC neurons leading to reduced food intake and body mass. We administrated the CS continually using miniosmotic pumps connected to an intracerebroventricular canula in male mice to ensure the central effects of the peptide. Mice were fed chow or a high fat diet (HFD) for 6 weeks before surgery to implant the canula and throughout the treatment. Body mass and food intake were analyzed for 14 days and at the end of the protocol tissue was collected for gene expression analysis. Body mass was significantly lower in male mice treated with CS in the first days in HFD group. There were no differences in food intake for both chow and HFD groups. At the moment of euthanasia, fasting glucose and adiposity differed between diet groups, only, with no effect of the CS treatment. In the gene expression analysis, CS increased inflammatory markers in the hypothalamus and decreased the Cart gene expression, which is related to appetite, energy balance, maintenance of body weight, reward and addiction. In the liver and white adipose tissue, CS treatment modulated the expression of Fasn gene in the chow group, only. We conclude that the brain activation of ABCA1 may acutely affect energy balance; however, the effect fades away after prolonged treatment. At this moment, we are conducting experiments aimed at providing a deeper understanding of the acute effects of CS-6253. CEUA number: 6586-1/2024. Funding: FAPESP 2023/16530-0

P4

BMP signalling regulates diversity of POMC neurons in a human stem cell model of the tuberal hypothalamus

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The arcuate nucleus (ARC) and the ventromedial hypothalamus (VMH) of the tuberal hypothalamus are central regulators of food intake and energy balance. Despite its small size, the tuberal hypothalamus contains high neuronal diversity and a complex developmental origin that is not fully understood until today. In this study, we fine-tuned the differentiation of human pluripotent stem cells (hPSC) towards the ARC and VMH lineage guided by single-cell RNA sequencing (scRNA-Seq) datasets from human fetal and adult hypothalamus (Herb *et al.*, 2023, Braun *et al.*, 2023, Tadross *et al.*, 2024).

With our ARC protocol, we were able to generate multiple key appetite-regulating cell types including agouti related peptide (AGRP), pro-opiomelanocortin (POMC) and prepronociceptin (PNOC) neurons as well as tanycytes, glial cells in the ARC (Mueller, Abay-Nørgaard, Häninnen *et al.*, 2024). When we compared our *in vitro* derived cells to a fetal human hypothalamic scRNA-Seq dataset, our cells mapped almost exclusively to ARC and tanycytes over other hypothalamic nuclei. After 50-70 days of differentiation, our ARC culture released AGRP as measured by ELISA and functionally responded to relevant hormones like leptin or GLP-1 through calcium signalling.

By manipulating the key morphogen BMP, we recapitulated developmental processes recently discovered in the chick and shifted patterning of the *in vitro* derived cells towards the ventromedial hypothalamus (VMH) (Chinnaiya *et al.*, 2023). While both protocols gave rise to POMC neurons, scRNA-Seq analysis revealed two types of POMC neurons depending on BMP exposure, one ARC-specific expressing *NR5A2*/*TBX3* and one expressing *NR5A1*/*SOX14*/*GPR149*. Comparing their transcriptomic profiles to human reference data showed that *POMC*/*NR5A1*/*SOX14* cells are more similar to fetal and adult VMH. In summary, this study provides insight into developmental processes in the human tuberal hypothalamus and enables access to well characterised human ARC and VMH cultures that provide a novel platform for obesity drug screening like GLP1R agonists or disease modelling to better understand obesity.

P5

A Polyendocrine Puzzle: Unravelling Schmidt Syndrome (Autoimmune Polyendocrine Syndrome Type II) Presenting In Crisis

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Background:
Schmidt syndrome is a rare autoimmune disorder characterised by the coexistence of at least two of the following: Addison's disease, autoimmune thyroid disease (Graves' or hypothyroidism), and type 1 diabetes mellitus (T1DM).[1] The estimated prevalence is 1.4–4.5 per 100,000, more commonly affecting middle-aged women.[2]

Case Presentation:
We report a 34-year-old British Caucasian woman presenting with fatigue, salt craving, and progressive skin pigmentation. She had a known history of Graves' disease, now on levothyroxine, and a family history of Addison's disease in her father. On examination, she appeared lean, had skin hyperpigmentation and was mildly hypotensive. Laboratory tests revealed significant hyponatremia (Na^+ 122 mmol/L), borderline hyperkalemia (K^+ 5.3 mmol/L). Investigations revealed a low 9am cortisol (146 nmol/L), markedly elevated ACTH (>2000 ng/L), positive adrenal cortex antibodies, and a positive short Synacthen test confirming primary adrenal insufficiency. Notably, T1DM was excluded and coeliac screen was negative.

Management:

While awaiting confirmation, the patient was empirically treated for Addison's disease with intravenous hydrocortisone. Endocrinologist's opinion was sought. As her condition stabilised, she transitioned to oral hydrocortisone, and fludrocortisone was started. She received an emergency IM(Intramuscular) injection kit and was educated on the sick day rule. She is now routinely followed up, adherent to treatment, well-informed, and feels supported by her care team.

Conclusion:

APS type-2 presents notable diagnostic challenges due to its vague and non-specific symptoms.[3] Patients face a 2.5-fold higher risk of adrenal crisis compared to those with Addison's disease alone.[1] Given the potential for sudden deterioration triggered by various stressors, timely diagnosis and appropriate hormone replacement are vital for survival.[2] A holistic management approach is crucial for preventing both under- and overtreatment.[1] As the condition is rare, patients are often unaware of its lifelong nature and must be supported to integrate its management into daily life effectively.

References:

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2. Resende E, et al. Autoimmune polyglandular syndrome type 2 with AIRE mutation. *Hormones (Athens)*. 2014;13(3):470–6.
3. Bain A, et al. Addison's in hypothyroidism: APS type 2. *BMJ Case Rep*. 2015;2015:bcr2015210506.

P6

Beta 3 Adrenergic Receptor Regulation of Leptin Within Adipose Tissue

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Background: Leptin, synthesized in adipose tissue, is a critical controller of energy balance. However excessive leptin production can result in leptin resistance and disrupted energy balance. Evidence suggests beta 3 adrenergic receptors (β3-AR), play a crucial role in preventing this and maintaining leptin within a homeostatic range as global deletion of β3-AR greatly increases circulating leptin levels. However, it is unclear if this regulatory role of β3-AR is controlled by globally expressed β3-AR or specifically those located within adipose tissue, the site of leptin synthesis. We investigated this, using a novel mouse model allowing for expression of β3-AR in adipocytes only.

Methods: Male and female mice with a transcriptional block (TB) of the gene encoding β3-AR, Adrb3, were bred resulting in a global prenatal knockout (Adrb3TB). Mice were crossed with an adiponectin-Cre mouse, resulting in re-expression of Adrb3 exclusively within adipocytes (Adrb3TB; Adipo-Cre). To investigate adipocyte β3-AR regulation of leptin production, a β3-AR agonist (CL316,243; 1mg/kg) or vehicle control (saline) was administered to 8-week-old Adrb3TB and Adrb3TB; Adipo-Cre mice as well as WT littermates (n=4) 2 hours prior to sacrifice. Plasma leptin and gonadal white adipose tissue (gWAT) leptin gene expression were assessed.

Results: Saline treated male Adrb3TB mice displayed a significant increase in plasma leptin compared to WT littermates (p=0.008) whilst females displayed a trended increase (p=0.10). Saline treated male Adrb3TB;Adipo-Cre mice did not differ from WT or Adrb3TB mice, whilst female saline treated Adrb3TB;Adipo-Cre mice had significantly lower levels than Adrb3TB mice (p=0.03). CL316,243 treatment significantly decreased male and female WT leptin levels (p=0.04 and 0.030) whilst causing a trended decrease in male and female Adrb3TB;Adipo-Cre mice (p=0.09 and 0.11). There was no effect in Adrb3TB mice. gWAT leptin gene expression was no different between groups in saline treated mice but CL316,243 caused decreases in male and female WT (p=0.03 and 0.04) and Adrb3TB;Adipo-Cre mice (p=0.04 and 0.12). There was no effect in Adrb3TB mice.

Conclusions: These findings indicate that adipocyte β3-AR are very important for regulating leptin production. However, further investigation, involving additional flox and cre models, is required to determine the extent of their control.

P7

Food choice modulating relaxin/insulin-like family peptide receptor 4 (Rxfp4) expressing neurons in the ventromedial hypothalamus as a new target for the melanocortin system.

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The ventromedial hypothalamus (VMH) is described as deeply involved in the regulation of the energy balance. It contains glutamatergic neurons that inhibit feeding and promote glycemia control and populations that are sensitive to glucose.

Recently, our group has identified a neuronal population in the ventro-lateral VMH expressing the receptor RXFP4 whose ligand is the colonic hormone InsL5. RXFP4 is a Gi coupled receptor which, when activated by InsL5, decreases the level of intracellular cAMP. Furthermore, infusion of InsL5 in the vlVMH increases the intake of highly palatable food. Consistent with this orexigenic action of InsL5, which inhibits Rxfp4 neurons, chemo-activation of this population decreases motivation for highly palatable food rewards. Finally, investigation of the transcriptome of Rxfp4^{vlVMH} neurons reveals that they express the melanocortin receptor MC4R, knowingly involved in the control of energy homeostasis.

Our goal was to further characterize the role of the RXFP4 neuronal population of the VMH and to determine if and how this population connects with the hypothalamic melanocortin systems, including AgRP and POMC populations of the arcuate nucleus.

Using life cell imaging in brain slices, we show that RXFP4^{vlVMH} neurons increase their intracellular cAMP in response to MC4R agonists and those responses are downregulated during the fasted compared to the fed state. We demonstrate that innervation from AgRP+ and POMC+ fibers are present in close proximity to RXFP4^{vlVMH} neurons, without making synaptic contacts, as rabies tracing from Rxfp4^{vlVMH} neurons failed to label AgRP and POMC neurons in the arcuate nucleus of the hypothalamus. However, we show that synaptic plasticity around RXFP4^{vlVMH} neurons occurs during change of nutritional status with an increase of glutamatergic inputs at fed state vs fasted state. Mirroring those data, we observe that RXFP4^{vlVMH} neurons present more calcium oscillations in the fed state compared to fast, consistent with an increase of RXFP4^{vlVMH} neuronal activity. Finally, we demonstrate using the activation marker cFos that refeeding activate the RXFP4 population.

Together these results show that the RXFP4^{vlVMH} population is modulated by the nutritional status, in part through the melanocortin system, identifying a new pathway by which the melanocortin system might affect food choices.

P8
Assessing PKA activity in primary cilia of MC4R neurons *in vivo*

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The primary cilium is a critical organelle for intercellular signaling extending from the cell body of many cell types, including most neurons. We have demonstrated that the Melanocortin 4 receptor (MC4R) localizes to the neuronal primary cilium, and that MC4R's capacity to control food intake and body weight requires adenylate cyclase (ADCY) activity at the primary cilia of paraventricular nucleus MC4R (PVN^{MC4R}) neurons [PMID:33938449]. ADCY leads to production of cAMP. The primary cilia pool of cAMP conveys different information than the cytoplasmic pool, including by local activation of PKA [PMID:33932338]. There are currently no tools available to measure the level of ciliary PKA activity *in vivo*.

To assess the activation status of ciliary PKA in MC4R neurons, we developed an Adeno-associated virus (AAV) delivered Cre-dependent ciliary targeted PKA activity reporter. The reporter consists of a chimeric protein that includes a peptide phosphorylated by PKA (VASP(Ser157)), a monomeric red fluorescent protein (RFP) and a ciliary localization signal. This reporter is delivered by stereotaxic injection of an AAV and expressed following Cre-mediated recombination. Levels of ciliary PKA activity are quantified in brain section by the ratio of VASP phosphorylation, detected by a phospho-VASP(Ser175) specific antibody, to the total amount of targeted reporter protein, detected by an RFP antibody.

Using this ciliary PKA sensor, we detect strong ciliary PKA activity at the primary cilia of PVN^{MC4R} neurons in *ad libitum*-fed mice. Genetic Crispri mediated downregulation demonstrates that MC4R and the neuronal cilia specific ADCY3 are both required for this ciliary PKA activity. The ciliary PKA activity in PVN^{MC4R} neurons is also downregulated in leptin-deficient mice and following calorie restriction, conditions in which the balance of MC4R ligands results in MC4R inhibition.

In summary, our data demonstrate that following coupling to Gs, MC4R activates neuronal cilia specific ADCY3 and a pool of locally active PKA. Further studies will be aimed at identifying the targets of this local PKA pool in PVN^{MC4R} neurons.

More generally, as many neuropeptides signal through ciliary localized Gs coupled GPCRs, our reporter will allow broad insight into neuropeptide activity throughout the central nervous system.

P9

In vitro-derived human hypothalamic neurons and tanycytes to investigate hormonal and pharmacological regulation of appetite

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The arcuate nucleus (ARC) and ventromedial hypothalamus (VMH), containing high cellular heterogeneity, are key regulators of food intake and energy balance. Specifically, ARC neurons such as AGRP and POMC mediate body weight by sensing metabolic signals, while NR5A1-expressing neurons in the VMH are critical for glucose sensing. Novel appetite-suppressing drugs like semaglutide and liraglutide are hypothesized to act on these populations, the mechanism of which is only partially resolved.

In this study, we use our novel protocol to differentiate human pluripotent stem cells towards key appetite-regulating cell types, including AGRP, POMC, PNOC, NR5A1 neurons, and tanycytes, to establish an in vitro platform for investigating cellular responses to metabolic hormones and candidate drugs. To validate the transcriptomic identity of our in vitro ARC and VMH cultures, we performed single-cell RNA sequencing (scRNA-seq) and compared the data to human hypothalamic reference datasets, confirming strong similarity to their human counterparts. Functional maturation was assessed with ELISA assay on the culture medium, confirming increased AGRP secretion over time.

Next, we used our cultures to conduct a stimulation experiment using scRNA-seq and calcium imaging to demonstrate the potential of this system for studying cellular responses upon compound administration in a human model. To investigate activated molecular pathways, we treated our mature ARC cultures with FGF1, a growth factor known to induce transcriptional changes in tanycytes, followed by scRNA-seq. This experiment revealed early response gene activation (FOS, IER2, and EGR1) specifically in tanycytes. Furthermore, to investigate cellular activity changes, calcium imaging showed immediate responses to key appetite-regulating hormones, such as leptin and GLP1. Finally, to facilitate scalable functional screening of in vitro cultures, we introduce a calcium imaging pipeline that combines automated pipetting and imaging with a novel deep learning-based analysis tool, designed to quantify cellular responses to stimuli. In summary, we developed a human stem cell-derived model containing ARC and VMH subtypes, validated its molecular and functional identity, and demonstrated its application for stimulation experiments of metabolic hormones and drug candidates.

P10

Attractin-Like Protein 1 is a critical interactor of MC4R and potentiates its signaling and function

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The melanocortin 4 receptor (MC4R) plays a critical role in the central control of energy homeostasis and its disruption causes severe early onset obesity. The activity of MC4R is tightly regulated by agonists, inverse agonist and accessory proteins like MRAP2. In this study we demonstrate the critical role of the single transmembrane protein Attractin-like protein 1 (ATRNL1) in promoting MC4R signaling and its anorexigenic function. We show that ATRNL1 interacts with MC4R and potentiates its response to agonists in vitro. We also find that expression of ATRNL1 is essential for the activation of MC4R neurons in rodents and that deletion of ATRNL1 in MC4R neurons result in the loss of anorexigenic effect of MC4R agonists. Deletion of ATRNL1 in the paraventricular nucleus of the hypothalamus causes increased food intake and obesity. We identified several mutations in ATRNL1 gene of obese patients and pharmacological studies demonstrate that several of those mutations result in a loss of ATRNL1 function that likely contribute the obesity phenotype. Cumulatively, these findings establish ATRNL1 as an important regulator of energy homeostasis and suggest that modulators of the MC4R / ATRNL1 complex may be effective in the treatment of obesity.

P11

Re-expression of GPR45 rescues knockout phenotype but does not confer protection in wild-type mice

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In the last 50 years, obesity rates have risen across the globe spurring increased interest in the underlying genetics of this disease. Over the last decade, GPCRs, particularly orphan GPCRs, have increasingly emerged as key players that regulate energy metabolism. Accordingly, murine knockout of the neuronally expressed orphan GPCR *Gpr45* is associated with monogenic obesity, though the mechanism by which a deficiency in Gpr45 causes obesity remains unknown. Similar to prior findings, we discovered our in-house *Gpr45*$^{-/-}$ mice are significantly heavier and have increased fat mass compared to their wildtype counterparts. We further determined that *Gpr45*$^{-/-}$ mice exhibit significant metabolic dysfunction, including but not limited to hypoactivity, hyperphagia, and reduced bone mass. Upon investigating the gene expression changes in the *Gpr45*$^{-/-}$ mice, we discovered that genes directly adjacent to *Gpr45* (*Tgfbrap1, Ashwin*) are upregulated in all tissues of *Gpr45*$^{-/-}$ mice. To investigate this unique locus, we conducted H3K27ac-Hi-ChiP sequencing on the hypothalamus of *Gpr45*$^{-/-}$ mice and discovered that the knockout of *Gpr45* causes physical changes to the epigenomic landscape of the *Gpr45* gene body and surrounding locus, likely leading to upregulation of *Tgfbrap1/Ashwin*. These results suggested that Gpr45 may not be the sole contributor to this extreme obesity phenotype. Therefore, we treated wildtype and *Gpr45*$^{-/-}$ mice with an AAV/PHP.eB-CAG-m-GPR45/Ctrl and discovered that replacing Gpr45 led to a rapid and significant decrease in the weight, particularly fat mass, of both male and female *Gpr45*$^{-/-}$ mice, with a concurrent reduction in food intake, a normalization of bone mass, improvement in glucose homeostasis and insulin sensitivity. Interestingly, locomotor activity was not rescued by exogenous Gpr45 expression. Importantly, the expression of *Tgfbrap1/Ashwin* did not change after Gpr45 was re-expressed in the *Gpr45*$^{-/-}$ mice, suggesting that Gpr45 was responsible for rescuing the obesity phenotype. We also found overexpression of GPR45 in WT mice did not induce any major metabolic changes, suggesting GPR45 is necessary in homeostatic regulation of body weight and metabolic function, but overexpression would not likely be a viable therapy for obesity.

P12

Cross-species studies implicate the melanocortin 3 receptor more strongly in the control of pubertal development than energy balance

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Hypothalamic neurons expressing either POMC or AGRP sense nutritional state directly and indirectly and transmit the neuropeptide signals to other brain centres through the melanocortin 3 and 4 receptors. MC4R is primarily concerned with the control of appetite and energy expenditure while MC3R is more closely related to the control of linear growth and the timing of puberty. The role of MC3R in the long-term control of energy balance and body composition is less clear, particularly in humans. We have undertaken studies in humans, domestic dogs and mice with the goal of clarifying the relative impact of MC3R deficiency on energy balance, growth and sexual development. By studying three consanguineously enriched human populations, we identified nine individuals who are homozygous for functionally null MC3R variants. The BMI of the homozygous MC3R variant carriers was not significantly different from that of age, sex and demographically matched controls, with only 3 of 9 having a Body Mass Index (BMI) >30kg/m2.

We detected a canine MC3R missense variant (M320I) which is common in Labrador retrievers and showed that this significantly impairs receptor signalling. Dogs homozygous for M320I were lighter and showed delayed pubertal development but were not significantly more obese than wild-type nor heterozygous dogs.

Finally, we studied growth and pubertal trajectories of 43 children carrying rare loss of function MC3R variants and found that male carriers had significantly delayed peak height velocity and genital development but had no evidence for excess body fat.

Our results support MC3R having a conserved role across mammals in controlling growth and pubertal timing and indicate that while MC3R deficiency may influence body composition to favour fat over lean mass, complete loss of MC3R in humans does not result in a penetrant obesity syndrome.

P13

Conditional deletion of melanocortin 3 receptor in kisspeptin cells in a murine model

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The melanocortin-3 receptor (MC3R) regulates the timing of sexual maturation, the rate of linear growth and the growth of lean mass, all known to be energy-sensitive processes. MC3R knockout ($Mc3r^{-/-}$) mice display defective regulation of reproductive development as indicated by delayed onset of puberty. Male $Mc3r^{-/-}$ mice had a 2-day delayed puberty compared to their wildtype counterparts whereas female mice similarly had delayed first estrus, dysregulation of estrus cyclicity, and spent more time in proestrus-estrus. Most striking is the observation that MC3RKO mice are resistant to fasting-induced suppression of the HPG axis. Importantly, the role of the MC3R in reproductive development has been demonstrated in both heterozygous and homozygous loss of MC3R function in humans, with one homozygous patient not reaching puberty until 20 years of age. To further elucidate the role of MC3R in modulating both development of the reproductive axis, and its regulation by nutritional state, our lab has created the first MC3R floxed mouse to delete MC3R in adult animals as well as specific neuronal populations known to be involved in reproduction. Following deletion of MC3R in kisspeptin neurons, there was no effect on time to puberty in males or females. This suggests that there could be an afferent MC3R neuron upstream of kisspeptin that relays nutritional information to modulate reproductive maturation. We did find that males and females developed an obesogenic phenotype with increased body weight and fat mass and decreased lean mass. We also found a decrease in testosterone levels in male KissMC3RKO's compared to WT littermates and an early cessation of cycling in female KissMC3RKO's compared to WT littermates. We are also currently testing the role of MC3R in kisspeptin cells in transmitting information on nutritional state to the HPG axis. Overall, these data expand upon the developing understanding of a role for the MC3R in modulation of energy homeostasis by reproductive state.

P14

Species divergence in the human leptin-melanocortin system revealed by large-scale single-cell and spatial hypothalamic transcriptomics

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The hypothalamic melanocortin system comprising pro-opiomelanocortin (POMC) and agouti-related peptide (AgRP) neuron signalling via melanocortin-3 and -4 receptors (MC3R, MC4R), is fundamental to energy homoeostasis. Yet the cellular blueprint of this pathway in the human hypothalamus remains unresolved.

We combine single-nucleus RNA sequencing of 400,000 cells with spatial transcriptomics to generate a comprehensive spatio-cellular map of the human hypothalamus: 'HYPOMAP'. Our data illuminate the transcriptomic identity and spatial localisation of key leptin-melanocortin pathway components within the adult human hypothalamus, including POMC, AgRP, and melanocortin receptor neurons. Human-mouse comparison incorporated receptor-level concordance mapping and smFISH validation. Genetic relevance was assessed with CELLECT-MAGMA enrichment of BMI genome-wide association data and rare-variant burden testing.

Three arcuate clusters displayed high POMC expression. Canonical POMC/PRDM12 neurons which co-expressed *LEPR* and *GLP1R* (mutually exclusive in mice); a periventricular POMC/CALCR cluster, and a divergent POMC/ANKRD30A cluster that lacked a murine counterpart, revealing human-specific heterogeneity with therapeutic implications. AgRP/NPY neurons formed a conserved cluster, but *AGRP* transcripts were additionally present in GHRH and AVP-SIM1 paraventricular neurons—the latter not previously reported in humans or mice. *MC4R* expression was diffuse rather than nucleus-restricted, peaking in paraventricular TRH/CRH neurons, lateral pre-optic cholinergic and HMX3-positive medial pre-optic neurons that lack Mc4r in mice. *MC3R* was enriched in arcuate GHRH and KISS1 neurons and in conserved ventromedial and periventricular populations, suggesting receptor-specific circuit segregation.

Finally, we find significant expression enrichment of BMI GWAS genes in mid-hypothalamic neurons. This enrichment was driven by 426 genes, six of which were cross-validated using exome-sequencing data from the UK Biobank study; rare deleterious variants in MC4R, POMC, CALCR, and PCSK1 conferred expected adiposity effects, whereas BSN and newly implicated CORO1A emerged as additional effectors.

HYPOMAP furnishes a high-resolution cellular and spatial framework of the human melanocortin system, exposing species-divergent POMC subtypes, extrinsic *AGRP* expression and expanded MC4R

topography. These findings refine mechanistic understanding of human energy balance and prioritise targets for next-generation MC4R agonists, GLP1R/CALCR co-agonists and allied anti-obesity therapeutics, underscoring the indispensability of human-centred atlases for translational neuroendocrinology. The dataset is openly available to accelerate global scientific collaboration and innovation.

P15

Characterising the transcriptional signature of obesity in Pomc neurons – a maladaptive change in identity?

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The melanocortin system is a central regulator of energy homeostasis, with hypothalamic Pomc neurons playing a critical role in appetite control and metabolic balance. These neurons are remarkably diverse forming a heterogeneous and highly plastic population, varying in both identity and secretory output. In obesity, a significant fraction of Pomc neurons lose both their function and peptidergic identity. However, the molecular basis for these identity shifts, and their consequences in the pathogenesis of obesity, remain unexplored.

To address this, we developed a novel transgenic mouse model to trace Pomc neurons across the course of diet-induced obesity. Using a Cre-lox system, we permanently tagged Pomc neurons at conception. Mice from both sexes were placed on a high-fat diet protocol for four and eight months. To isolate the tagged cells, we developed and validated an antigen-based nuclei extraction protocol using fluorescence imaging. These nuclei were subsequently profiled using single-nucleus RNA sequencing.

We observed successful tagging in all experimental groups, along with significant weight gain in both sexes following dietary intervention. The sequences were aligned to a custom mouse reference with the transgenic gene to validate our targeted nuclei extraction. After stringent quality control to remove debris and doublets, we obtained transcriptional data from 60,000 nuclei across experimental conditions. Notably, we identified discrete neuronal clusters with altered gene expression, including signatures consistent with disrupted neuropeptidergic identity.

This study offers a first step toward understanding drives the reconfiguration of the transcriptional identity of POMC neurons. Through delineation of the underlying activity-dependent processes, this work lays the groundwork for the identification of molecular targets that may enable restoration of both, neuronal function and cellular identity.

P16

Activation of NAc in response to dietary and genetic changes specifically focused on the role of MC3R neurons

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Recently, the paraventricular thalamic nucleus (PVT) has been implicated in coordinating metabolic signals in the whole animal and thus might be functioning to integrate the melanocortin system signalling into the whole animal behaviour. Furthermore, the nucleus accumbens (NAc) has a neural interface between motivation and action in the feeding behaviour and is known to be functionally connected to the PVT and modulated by melanocortin system activation. In this study, we aimed to understand the neuronal connections between these brain regions, responsible for motivation and decision making in eating behaviour in the melanocortin system. We analysed neuronal responses to food intake in these regions using innovative methods in the mouse brain.

Our experiments established fundamental insights into PVT-NAc neuronal connections. Using unique transgenic and viral approaches, we could show that MC3R neurons project to the NAc, with a significant proportion of their innervation originating from the PVT. Notably, about 35% of these projections are MC3R-positive, indicating a strong MC3R innervation on NAc directly from the PVT compared to other brain regions.

Additionally, we explored how MC3R activation directly in the PVT modulates feeding behaviour, particularly in response to high-fat diet (HFD) and sucrose consumption. By stimulating PVT MC3R neurons, we observed a direct impact on food intake behaviour. Changes within the NAc after PVT stimulation were measured using neuronal activation marker (cFos and pCREB). To probe further the development of this connection, we performed these studies in a model of maternal overnutrition known to disrupt melanocortin system development and function. Our findings indicate that maternal overnutrition disrupts PVT-melanocortin signalling of energy homeostasis, suggesting long-term neurodevelopmental consequences in feeding regulation. Overall the PVT-MC3R à NAc network may aid in control of feeding behaviour warranting further investigation.

P17

Sex-differential effects of MC3R rs143321797 on nutrient partitioning in humans

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Understanding the central regulation of energy homeostasis is critical for developing effective treatments for obesity and related metabolic disorders. A conserved circuitry involved in the regulation of food intake and metabolic processes is the melanocortin pathway. The melanocortin-3 receptor (MC3R), a key component of the central melanocortin system, has been shown in rodent models to regulate glucose homeostasis, body composition, and linear growth, and to modulate feeding behaviour in response to both anorexigenic and orexigenic signals. However, the physiological relevance of MC3R in humans—particularly its sex-specific functions—remains poorly defined due to the rarity of loss-of-function (LoF) genetic variants. To address this, we analysed data from the UK Biobank (UKBB), a large-scale genome-wide association study comprising over 500,000 individuals, to investigate the phenotypic consequences of the rare MC3R LoF variant rs143321797 (p.F45S). This study represents the first sex-stratified analysis of this variant in humans. Our findings support MC3R's involvement in adult stature and the timing of sexual maturation. We further observed that while the F45S variant was not associated with increased risk for metabolic disease, there is a sex-specific influence on nutrient partitioning patterns. These findings refine our understanding of MC3R function in human physiology and emphasise the importance of considering sex-specific mechanisms when developing MC3R-targeted therapies.

P18

Brain sensing of metabolic state regulates circulating monocytes

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Changes in energy availability alter the dynamics of circulating immune cells. The existing view is that these effects are due to altered nutrient levels affecting peripheral tissue metabolism. Here, using mice and genetic approaches to manipulate the activity of distinct molecularly defined neurons, we show that the brain's perception of hunger and satiety alone is sufficient to drive these immune changes. Hunger-promoting Agouti-related peptide (AgRP) neurons in the hypothalamus were both sufficient and necessary to reduce circulating Ly6C^{Hi} classical monocytes during fasting. Mechanistically, these neurons suppressed hepatic mammalian target of rapamycin signaling via sympathetic regulation, decreasing circulating chemokine ligand 2 and monocyte numbers. AgRP neuron–induced corticosterone release and glucocorticoid receptor activation played a permissive role in this process. These changes in monocyte dynamics can occur independently of actual nutrient levels, revealing an unexpected brain-mediated control of peripheral immunity in response to perceived variation in energy state.

P19

The individual risk in obesity development is associated with differential signaling profiles of genetic MC4R variants

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Introduction: The melanocortin 4 receptor (MC4R), a G protein-coupled receptor (GPCR), is a critical regulator of body weight within the hypothalamus and is embedded in the leptin-melanocortin signaling pathway. Heterozygous *MC4R* gene mutations have been identified as potent genetic risk factors for the development of obesity. Ligands for the MC4R can activate a set of G protein subtypes such as Gs and Gq, whereby it was recognized in recent years that specific activation of Gq and concomitant downstream signaling has strong impact on body weight regulation. While many functional, pharmacological and structural information regarding MC4R-ligand complexes is already available, the precise mechanisms and specific details in the interplay between *MC4R* variants and different endogenous or synthetic ligands remains elusive.

Methods: We analysed *in vitro* the signaling capacity of 20 individual *MC4R* variants, which have been identified in a cohort of children with severe obesity, regarding Gs, Gq/11, ERK, G12/13 and β-arrestin2 recruitment in combination with the stimulation by different MC4R ligands (a-MSH, b-MSH, setmelanotide) in HEK293 cells. Additionally, MC4R variants were further characterized by analysis of the cryo-electron microscopic (cyro-EM) structure.

Results: We observed a ligand and genetic variant dependent differential (bias) signaling of the MC4R. The "obesity-protective" *MC4R* variant V103I was associated with an increase of Gq signaling after stimulation with a-MSH. Contrary to complete loss-of-function variants such as Y80C, D90N and S127L, the S77L or T178M variants showed a reduction of non-Gs signaling cascades, while Gs signaling was not altered. In addition, the diverse MC4R variant signaling profiles were dependent on the used ligand-subtype. Moreover, a structural analyses of the mutant distribution at a 3-dimensional MC4R model revealed, that variants with a specific impact on receptor functions can be partially clustered at certain parts in the global receptor structure.

Conclusion: Our findings emphasize the critical role of differential (bias) signaling for MC4R function.Structural- combined with *in vitro* functional data allowed to gain further insights into the regulation of the MC4R, which can be relevant to optimize MC4R agonists as a treatment option for patients with certain forms of obesity.

P20

Structure of the Ion Channel Kir7.1 and Implications for its Function in MC4R Signaling

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Hereditary defects in the function of ion channel Kir7.1 in the retinal pigment epithelium are associated with the ocular diseases retinitis pigmentosa, Leber congenital amaurosis, and snowflake vitreal degeneration. Studies also suggest that Kir7.1 may be regulated by a GPCR, the melanocortin-4 receptor, in certain hypothalamic neurons. This study presents the first structures of human Kir7.1 in various conformational states, determined by cryo-EM, providing crucial insights into its gating mechanism and pharmacological modulation. Data showing the structure with blocker Kir7.1 bound reveal that ML418 acts as a potent blocker of the channel by binding in the inner vestibule beneath the selectivity filter. We show that channel blockade in vivo activates MC4R neurons in the paraventricular nucleus of the hypothalamus (PVH), inhibiting food intake and inducing weight loss. These effects were found to be dependent on Kir7.1 expression in MC4R neurons, as confirmed through experiments using mice with selective deletion of Kcnj13 (the gene encoding Kir7.1) from MC4R-expressing cells. Expression and pharmacological characterization of an in tandem construct of MC4R and Kir7.1 suggests that the fusion protein exhibits ion channel regulation by GPCR (MC4R) ligands. This research elucidates a unique signaling mechanism in hypothalamic energy balance regulation, and also suggests that structural and functional characterization of the MC4R-Kir7.1 signaling complex may provide a path to biased signaling control of MC4R action.

P21

Syndecan-3 modulates MC4R signaling

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Syndecans are a family of heparan sulfate proteoglycans, proposed to function as co-receptors for GPCR proteins. Overexpression of syndecan-1 in the mouse brain leads to hyperphagia and obesity, whereas syndecan-3 knockout mice show resistance to high-fat diet induced obesity. SDC3 is known to stimulate feeding behavior in mice by enhancing AgRP binding to MC4R. However, AgRP-independent mechanisms for SDC3-mediated MC4R signaling have not yet been investigated. In this study, we explored whether SDC3 affects downstream MC4R signaling independently of AgRP.

Analysis of human hypothalamic single-cell RNA sequencing data showed neuronal co-expression of MC4R and SDC3 at a ratio of 1:1. We therefore assessed the impact of SDC3 at this ratio on α-MSH-induced MC4R cAMP responsiveness in HEK293 cells, which lack AgRP. At a 1:1 ratio with MC4R, SDC3 suppressed Emax by 24±1.5% ($P<0.0001$), but had no significant effect on EC50. A previous mouse study showed that fasting causes hypothalamic SDC3 protein levels to increase >4-fold. To mimic this hunger state, we investigated the effect of SDC3 on α-MSH-induced cAMP production using an MC4R:SDC3 ratio of 1:5. At this ratio we observed stronger suppression of Emax (36±1.5%; $P<0.0001$), and an increase in EC50 (from 8±0.5 nM to 21±5.4 nM; $P<0.05$). Next, we investigated whether SDC3 modulates MC4R expression on the cell surface. We found that SDC3 had no significant effect at a ratio of 1:1, but a ratio of 1:5 markedly suppressed MC4R cell surface levels by 51±4.2% ($P<0.0001$), potentially explaining the reduction in cAMP production. Similarly, total expression levels of MC4R were not suppressed at a ratio of 1:1, but were suppressed by 33±5.8% ($P<0.05$) at a ratio of 1:5.

In conclusion, SDC3 suppresses downstream MC4R signaling independently of AgRP. The lack of effect on cell surface MC4R levels at a 1:1 ratio suggests that SDC3 can modulate intracellular signaling. The dose-dependency of this effect could be paralleled in vivo upon fasting when hypothalamic SDC3 levels are increased.

P22

Melanocortin-4 receptor agonist antibody with an improved safety profile to treat obesity

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The melanocortin-4 receptor (MC4R), plays a crucial role in regulating hunger and satiety signals. Located in the hypothalamus, MC4R integrates both central and peripheral signals to modulate appetite. Despite intense drug discovery and development efforts since the 1990s, nearly all peptide and small molecule MC4R agonists have failed pre-clinically or in phase 1/2 trials due to both on-target and off-target safety issues. Notably, treatment-emergent blood pressure and heart rate elevation were observed most likely due to activation of MC4R in the central nervous system (CNS). Additionally, the lack of selectivity over melanocortin-1 receptor (MC1R) is associated with skin tanning. Recently, setmelanotide (Imcivree®, Rhythm pharmaceuticals), an MC4R agonistic peptide, has been approved for the treatment of rare genetic obesity disorders. While generally well tolerated, setmelanotide lacks selectivity over MC1R and hence causes skin tanning, which is treatment limiting. To mitigate these challenges, targeting MC4R with an antibody is anticipated to de-risk tanning due to high target selectivity and to reduce the unwanted cardiovascular effects by avoiding CNS exposure. We have successfully generated highly potent and highly selective MC4R agonistic antibodies, binding in the orthosteric pocket of MC4R and mimicking the interaction of the endogenous peptide ligand. We have demonstrated efficacy in a diet-induced obese mouse model and have observed superior selectivity profile over setmelanotide, with no skin tanning.

P23

Genomic and Epigenomic Characterization of Hypothalamic MC4R Neurons

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The Melanocortin 4 receptor (Mc4r) locus is a major genetic contributor to the polygenic predisposition to obesity. Previous literature has highlighted the importance of MC4R-expressing neurons in the hypothalamus for long-term control of feeding behavior and body weight. Yet, we still lack a molecular understanding of the genetic and epigenetic mechanisms at play in this neuronal population. A major challenge in studying these neurons stems from the rarity of the population and low abundance of *Mc4r* transcripts, which limits the detections and analyses by the conventional single cell sequencing methods.

To overcome the limitations, we employed the Nuclear tagging and Translating Ribosome Affinity Purification (NuTRAP) system in *Mc4r-Cre* mice. This tool enables the genetic labelling and isolation of MC4R neurons nuclei by fluorescence-activated nuclei sorting (FANS), as well as actively translating ribosomes-bound mRNA by immunoprecipitation, specifically from MC4R neurons. Using such tool, we established the transcriptome, translatome, and chromatin accessibility profiles of hypothalamic MC4R neurons in *ad libitum-fed* mice. Notably, whole transcriptome analysis revealed that this population is mainly characterized by the expression of *Mc4r*, with no other gene showing a comparable degree of enrichment. This suggests the absence of a radically distinct transcriptional landscape in this hypothalamic population, and favors the hypothesis that their functional specificity derives mainly from the expression of MC4R. Last, we performed single-nucleus multiome sequencing on MC4R nuclei sorted from the whole hypothalamus. This revealed that the transcriptional heterogeneity of MC4R nuclei mainly reflects their anatomical localization, rather than a homogeneous, specific transcriptional identity.

Collectively, our work provides a detailed view of the transcriptional and epigenomic landscape of MC4R neurons. Furthermore, it establishes a functional framework for interpreting the contributions of obesity-related single nucleotide polymorphisms (SNPs) at the regulatory elements in MC4R neurons.

P24

Chronic Inhibition and Activation of Ventral Tegmental Area Melanocortin-3 Receptor Neurons

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The hypothalamic melanocortin system, involving the central melanocortin receptors (MC3R and MC4R), is an essential component in the neural regulation of food intake and body weight. MC3Rs are highly expressed in the ventral tegmental area (VTA), which is the center of Mesolimbic Dopamine circuit (MLDa). The MLDa circuit is the primary neural circuit controlling reward behavior, but it also plays a role in multiple different aspects of feeding. We previously showed that acute inhibition and excitation of VTA neurons expressing MC3Rs (VTA MC3R neurons) caused a sex- and activity-dependent decrease in food intake. The effects of long-term changes in VTA MC3R neurons activity remain unknown, however.

In these studies, we tested whether chronic inhibition or excitation of VTA MC3R neurons affected food intake or body weight. Inhibitory (hM4Di) or excitatory (hM3Dq or rM3DGs) Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) were expressed in VTA MC3R neurons of male and female mice. Mice were treated with clozapine N-oxide (CNO) in their drinking water for 8 days to chronically inhibit or excite VTA MC3R neurons, and food intake and body weight were measured daily. Similar to our previous data with acute inhibition or activation of VTA MC3R neurons, chronic inhibition and activation shows sex- and activity-dependent effects on both food intake and body weight. In addition, we also assessed whether inhibition or excitation of VTA MC3R neurons affects sucrose self-administration under fixed-ratio and progressive-ratio schedules.

These findings help to advance our understanding of the role of MC3R signaling in the VTA in the regulation of feeding and body weight. Further exploration will delve into how VTA MC3R neurons activity control food intake and body weight, particularly on high-fat diets.

P25

A new platform for CRISPRi gene downregulation in specific neurons implicates MC4R neurons in ciliopathy-associated obesity

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To enable more rapid investigation of individual gene contributions to the function of specific neuronal populations, we developed a modular *in vivo* CRISPRi system that enables gene downregulation with simultaneous cell-type, spatial, and temporal specificity. This system utilizes a Cre-dependent dCas9-KRAB (lsl-CRISPRi) allele for cell-type specificity and an AAV-delivered sgRNA with Cre-dependent fluorescent marker providing regional and temporal control and enabling identification of CRISPRi-treated neurons. Because of our long-standing interest in body weight regulation through Melanocortin 4 receptor (MC4R)–expressing neurons in the paraventricular nucleus (PVN) of the hypothalamus, we validated this approach by downregulating *Mc4r* in PVN^{MC4R} neurons. We confirmed decreased *Mc4r* transcription in the $Mc4r^{Cre}$ lsl-CRISPRi mice receiving the *Mc4r*-targeted sgRNA and observed significant weight gain compared to littermates receiving a non-targeting control sgRNA, phenocopying PVN-specific loss of *Mc4r.*

MC4R localizes to the primary cilium and its ciliary localization is essential for its role in regulating body weight. Some ciliopathies, human syndromic conditions caused by mutations in genes required for primary cilia structure or function, manifest with obesity. We hypothesized that obesity-associated ciliopathy genes impair the anorexigenic function of PVN^{MC4R} neurons.

To test this, we performed an *in vivo* CRISPRi screen downregulating a panel of primary cilia genes in which mutations are known to cause obesity in humans and mice in MC4R-expressing neurons via AAV-sgRNA injections in $MC4R^{Cre}$ lsl-CRISPRi neonates. Downregulation of *Alms1*, *Arl13b*, *Bbs10*, *Inpp5e* or *Rab23* led to significant weight gain, suggesting these genes are required for body weight regulation through MC4R neurons. In contrast, targeting *Tubby* and *Ankrd26* did not cause weight gain.

We are now testing whether these effects are specific to PVN^{MC4R} neurons in adult mice and assessing whether MC4R ciliary localization is disrupted. These studies will clarify the mechanisms underlying syndromic obesity, the intracellular pathways involved in weight regulation in MC4R neurons, and the therapeutic potential of MC4R-targeted treatments in ciliopathy patients.

More broadly, this Cre-dependent CRISPRi and AAV-delivered sgRNA platform offers a powerful tool to study other obesity-associated genes and gene regulators in PVN^{MC4R} neurons or other neuronal subpopulations, including studies of epistatic interactions.

P26

Role Of The Melanocortin-3 Receptor In Regulation Of Lean Mass, Muscle Function, And Exercise-induced Hyperphagia

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Neuronal networks of the central melanocortin system have long been known to be involved in regulation of energy homeostasis, and the amount of long-term energy stored in adipose tissue. However, it has been noted that MC4R-deficient mice and humans have an increase in lean mass compared with BMI matched controls. The melanocortin-3 receptor acts as the major inhibitory/modulatory regulator of the central melanocortin MC4R-anorexigenic pathways via its presence on GABAergic agouti-related peptide (AgRP) nerve terminals. In the absence of the MC3R, both mice and humans manifest reduced lean mass and linear growth, which mirror classical symptoms of growth axis dysfunction. In this study, we sought to advance our understanding of the role of the MC3R in the regulation of lean mass. To this end, we studied the development and function of lean mass in WT and MC3RKO mice. Our results revealed that male MC3RKO mice at different ages manifest reduced tissue growth, and poor endurance capacity. While WT 5-month-old male (21-23weeks) WT mice are capable of **37min** of high intensity exercise on the treadmill, age and sex matched MC3RKO mice could only sustain **26min** of exercise. Furthermore, we show that the increased food intake after endurance or moderate intensity exercise requires the presence of MC3R. For example, 45 min of moderate intensity running on a treadmill significantly increases 3hr food intake by **96%** in WT mice, but only by **43%** in MC3RKO mice. Therefore, MC3R is critical not only in the normal growth of lean body mass, but also in the stimulatory effects of physical exercise on food intake and growth. Ghrelin has been proposed to function in mediating exercise-induced hyperphagia in the mouse, and future work will will be focused on understanding the role of MC3R in ghrelin and other potential mechanisms involved in exercise-mediated hyperphagia.

P27

Reduced melanocortin tone mediates increased feeding during pregnancy in mice

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During pregnancy mammals increase their food intake to accommodate the elevated metabolic demands associated with fetal growth and the subsequent lactational period. Importantly, both overfeeding and underfeeding of the mother during pregnancy increases the risk of the mother and her children developing obesity and diabetes later in life. However, the molecular and neural circuit mechanisms mediating increased feeding during pregnancy are largely unknown.

Here, we demonstrate that the increased feeding observed during pregnancy is driven by elevated meal size, with a similar meal frequency observed between pregnant and non-pregnant mice. Given the established role of the central melanocortin system in feeding behavior, we next utilized in situ hybridization to characterize mRNA expression of the neuron activity marker fos in arcuate agouti-related peptide (AgRP) and pro-opiomelanocortin (POMC) neurons, and downstream paraventricular hypothalamic neurons containing the melanocortin-4 receptor (MC4R). Pregnant mice exhibited increased mRNA expression of AgRP and reduced mRNA expression of POMC. Consistently, pregnant mice exhibited elevated fos expression in AgRP neurons and reduced expression of fos in both POMC and PVN MC4R neurons. Importantly, these changes were required for promoting hyperphagia during pregnancy as chemogenetic inhibition of AgRP neurons or activation of POMC neurons both reduced the feeding of pregnant mice to non-pregnant levels. Further, consistent with reduced melanocortin circuit activity, pregnant mice were hypersensitive to both peripheral and central administration of the melanocortin receptor agonist setmelanotide compared to non-pregnant mice. Finally, to identify putative molecular mechanism(s) mediating reduced melanocortin tone during pregnancy, we performed single cell resolution spatial transcriptomics of 5000 protein-coding genes (Xenium spatial transcriptomics) in the arcuate nucleus in non-pregnant female mice, pregnant mice, and mice fasted for 24 hours. Both AgRP and POMC neurons exhibited significant gene expression changes in the pregnant state, including multiple changes consistent with positive energy balance and impaired sensitivity to insulin and leptin signaling, such as increased expression of the transcription factor Fox01 in both AgRP and POMC neurons during pregnancy.

Together, these findings outline a circuit mechanism mediating increased feeding during pregnancy, providing important mechanistic insights related to metabolic diseases and conditions at the intersection of reproduction and metabolism.

P28

Harnessing melanocortin circuits to improve age-related insulin resistance

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Ageing is associated with reduced insulin sensitivity which can impair the stringent control of blood glucose concentration. Here we investigate the therapeutic potential of targeting the brain to improve systemic insulin sensitivity with ageing. The neurotransmitter 5-hydroxytryptamine (5-HT, serotonin) has been pharmacologically exploited for the treatment of migraine, obesity, mood disorders, anxiety, and other conditions. However, the role of 5-HT in glucoregulation is not as well defined. Arcuate nucleus of the hypothalamus (ARC) neurons co-expressing neuropeptide Y (NPY), agouti-related protein (AgRP), and GABA (collectively "NAG") are known to have a glucoregulatory role. Activating NAG neurons promotes systemic insulin resistance. NAG neurons express Gi-coupled 5-HT_{1B} receptors (5-HT_{1B}Rs), and 5-HT_{1B}R agonists inhibit the activity of NAG neurons. We hypothesised that NAG neurons could be targeted to improve systemic insulin sensitivity via inhibition with 5-HT_{1B}R agonists. Here we report that NAG neurons have increased basal activity and upregulated ARC 5-HT_{1B}R mRNA in middle-aged insulin-resistant mice compared to healthy young controls. Treatment with the 5-$HT_{1B/1D}$R agonist and migraine medication sumatriptan improves glycaemic control in middle-aged mice by reducing NAG neuron activity and increasing glucose clearance, insulin sensitivity, and reducing hepatic glucose production. We report that this improvement in glycaemic control is mediated through the melanocortin 4 receptor (MC4R). Finally, the addition of sumatriptan with diabetes medication glucagon-like peptide-1 receptor agonist (GLP-1R agonist) exendin-4 significantly improves the therapeutic profile of exendin-4. These findings suggest that sumatriptan improves glycaemic control in age-associated insulin resistance, is mediated through the MC4Rs, and augments the therapeutic profile of a GLP-1R agonist diabetes medication.

P29

Role of OCRL in POMC processing

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X-linked *OCRL* (Oculocerebrorenal syndrome of Lowe) encodes a ubiquitously expressed inositol phosphatase critical for vesicle trafficking, signaling, actin cytoskeleton remodeling, and primary cilia assembly. Two isoforms exist: *OCRL-001* (901 aa), enriched in brain and hypothalamus, and *OCRL-002* (893 aa), expressed broadly in other tissues. Mutations in *OCRL* underlie Lowe and Dent syndromes, characterized by renal anomalies, congenital cataracts/glaucoma, neurodevelopmental disability, and frequently, growth abnormalities, obesity, and hypogonadism.

Methods and Results: Whole exome sequencing identified a novel hemizygous *OCRL* frameshift variant (p.Lys713ArgfsTer28) in a 3-year-old boy with severe obesity (BMI 27 kg/m², Z=+5), short stature (Z=–2.2), bilateral webbed toes, central hypoventilation, neurobehavioral dysfunction, and ganglioneuroblastoma, without renal or ocular disease. The variant segregated in his half-brother (BMI Z=+2); mother, maternal grandmother, and maternal aunt are carriers, while unaffected father and maternal granduncle lack the variant. The 24 bp frameshift occurs in exon 18a (NM_000276), unique to *OCRL-001*.

Patient-derived induced pluripotent stem cells (iPSCs) and isogenic CRISPR-Cas9-corrected iPSCs were generated. Corrected iPSCs restored *OCRL-001* expression absent in mutant cells. During differentiation to glutamatergic neurons, mutant iPSCs exhibited increased transition to neural stem cells (NSCs) but impaired further differentiation, yielding fewer neurons with reduced expression of *NeuroD1*, *NRP1*, *MAP2*, *PSD95*, *TUBB3*, and *GAP43* (all $p<0.05$). NSC-related genes (*OTX2*, *SOX2*, *PAX6*, *CDH2*) were upregulated, while oligodendrocyte-related genes were decreased, suggesting altered lineage specification.

Differentiated arcuate-like *POMC*-expressing neurons displayed distinct neuropeptide profiles: aberrant neuronal morphology and marker distribution, reduced *PCSK1*, markedly elevated *POMC* (by transcript, immunostaining, and secreted peptide assays), while α-MSH levels were diminished. Mutant neurons showed downregulation of pathways related to vesicle transport, neurotransmitter regulation, and secretion by RNA sequencing analysis indicating inefficient POMC processing, precursor accumulation and constitutive secretion into the cell media. Live cell imaging captured these defects at a single cell level. Replication experiments are ongoing.

Conclusions: These findings implicate *OCRL*—particularly *OCRL-001*—in neuropeptide processing in hypothalamic neurons, distinct from its roles in cortical neurons. The unique exon 18a variant highlights an underappreciated function of *OCRL* in hypothalamic endocrine regulation, potentially contributing to obesity in *OCRL* -related syndromes.

P30

Molecular basis of MC4R targeting to primary cilia

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The melanocortin-4 receptor (MC4R) signals at the primary cilium of hypothalamic neurons to regulate body weight homeostasis. However, the mechanisms by which MC4R targets to cilia remain unclear. We previously demonstrated that the melanocortin receptor-associated protein 2 (MRAP2), a single-pass transmembrane protein that interacts with MC4R, promotes MC4R entry into primary cilia both *in vivo* and in a cultured cell system. Underscoring the significance of MC4R ciliary enrichment, genetic defects in MRAP2 cause obesity in mice and humans.

Given that MRAP2, but not MC4R, efficiently targets to cilia when expressed alone, we hypothesized that MRAP2 encodes the ciliary targeting signal (CTS) for the MC4R/MRAP2 complex. Leveraging a series of truncations, alanine scanning mutagenesis and chimeras, we define a novel five-amino acid CTS within the disordered cytoplasmic tail of MRAP2 that is necessary and sufficient for ciliary enrichment of MC4R/MRAP2. This MRAP2 CTS is present in all vertebrate MRAP2 homologues but absent from the close relative MRAP1, which does not localize to cilia.

We next searched for the *trans*-acting factors mediating MRAP2 ciliary entry. The $PI(4,5)P_2$-binding protein TULP3 is thought to mediate the entry of nearly all signaling receptors into cilia via a physical association with the intraflagellar transport complex-A (IFT-A), a key ciliary trafficking complex. Surprisingly, while IFT-A is required for entry of the MRAP2/MC4R complex into cilia, TULP3 is entirely dispensable. Congruently, depleting Tubby –the TULP3 homologue in neurons– from MC4R neurons does not lead to weight gain.

We then investigated Rab23, a small GTPase, as an alternative ciliary import factor due to its association with Carpenter's syndrome, which features obesity as one of its cardinal phenotypes. GTP-bound Rab23 efficiently enters cilia, and promotes ciliary entry of certain GPCRs. Strikingly, Rab23 is essential for MRAP2/MC4R ciliary entry, and Rab23 knockdown in MC4R neurons causes pronounced obesity in mice.

Our findings reveal a novel function for single-pass GPCR partners in organelle targeting and demonstrates that the obesity gene product Rab23 operates in a non-redundant manner to TULP3/Tubby for ciliary import of signaling receptors.

P31

Does Diet-Induced Obesity involve changes in the dynamics of MC4R Localization and PKA Activity at the Neuronal Primary Cilia?

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Diet-induced obesity results in elevated circulating levels of leptin, which in turn creates a state of leptin resistance. While various mechanisms have been proposed to explain leptin resistance, conflicting data persist, leaving the exact mechanism to be poorly understood. Our aim is to better understand this mechanism by investigating a distal effector of leptin signaling, MC4R, which plays a crucial role in regulating food intake.

MC4R localizes to the primary cilium and its ciliary localization is essential for its role in body weight regulation. Our preliminary studies provide strong support for the role of PKA activity in MC4R activation status and inhibition of one of MC4R's ligands leading to accumulation of MC4R at primary cilia. To this end, we developed new tools to measure the dynamic accumulation of MC4R at primary cilia and PKA levels specifically in primary cilia in vivo. We have applied these tools to demonstrate that MC4R accumulation is leptin and ligand ratio dependent, and have shown that ciliary PKA activity is dependent on MC4R activation status in response to physiological or genetic changes that affect MC4R ligands.

Here we use our newly developed molecular and imaging approaches to investigate the relationship between leptin levels, MC4R ligand levels and signaling at the cilia of MC4R neurons in the setting of diet-induced obesity. Specifically, we have used the accumulation of MC4R at primary cilia as a proxy for ligand ratio levels to determine whether the inverse agonist is not properly suppressed by leptin in DIO. Further, to determine whether MC4R signaling is decreased, we measured PKA activity at the primary cilia of MC4R neurons employing our ciliary PKA activity sensor.

These approaches allow us to test whether leptin resistance involves alterations in signaling at the primary cilia of MC4R neurons, providing deeper insight into MC4R signaling and contributing to a better understanding of leptin resistance and diet-induced obesity. This knowledge may ultimately guide the development of more effective therapeutic strategies to combat obesity.

P32

Functional coupling between MC4R and Kir7.1 contributes to antipsychotic drug-induced weight gain

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Most antipsychotic drugs (APDs) cause hyperphagia and weight gain, but the underlying neural mechanisms remain poorly understood, partly due to difficulties in replicating their metabolic effects in rodents. Here, we develop a mouse model that recapitulates metabolic syndrome induced by clozapine, a widely prescribed and highly effective APD. Our findings demonstrate that clozapine promotes obesity in mice by driving hyperphagia through an unexpected interaction between the melanocortin 4 receptor (MC4R) and the Kir7.1 potassium channel. Clozapine inhibits MC4R-expressing neurons in the paraventricular nucleus of the hypothalamus by enhancing MC4R-Kir7.1 coupling, leading to increased inward potassium currents. Importantly, this effect occurs independently of direct MC4R binding or modulation of canonical Gαs signaling. Genetic deletion of Kir7.1 in MC4R neurons or pharmacological inhibition of Kir7.1 reverses clozapine-induced weight gain without compromising its antipsychotic efficacy. These findings uncover a G-protein-independent signaling pathway underlying clozapine's metabolic effects and highlight MC4R-Kir7.1 coupling as a therapeutic target for mitigating weight gain associated with this essential medication.

P33

Usurping hypothalamic POMC neurons to control the stress axis after survival challenges

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Hypothalamic POMC-derived peptides, such as aMSH, are well known for their role in limiting food intake. However, how they mediate signals of life-threatening insults and direct them to the apex of the hypothalamic, pituitary, adrenal (HPA) axis is unclear. This is a vital role in the body's response to stressors. To determine the pathways for hypothalamic POMC neurons to activate the HPA axis, we utilised a short-term (15 minutes) mild restraint stress model in mice. After restraint stress, there was a reproducible increase in plasma ACTH (from 90 to 225ng/L), corticosterone (from 28 to 93ng/mL) and glucose (from 8.4 to 11.6mmol/L). In addition, cFOS expression (as a marker of neuronal activation) was increased in CRH neurons. We also found increased cFOS in hypothalamic POMC neurons, but no increase in *Pomc* mRNA, POMC protein or aMSH peptide levels, although this was in whole hypothalamic extracts. To investigate the effects of POMC neurons on the HPA axis, we crossed POMC-Cre mice with an activating hM3Dq expressing DREADD mouse line. Global chemogenetic activation of POMC with clozapine N-oxide (CNO) increased corticosterone 2.5 fold in blood at 15 minutes. In addition, an hM3Dq-DREADD was injected into the paraventricular hypothalamus of MC4R-Cre mice. Subsequent activation with CNO elicited a marked reduction in food intake measured at 2h and parallel increases in plasma corticosterone measured from 15 minutes to 2h.

Together these data suggest that part of the raison d'etre for hypothalamic POMC neurons is to respond to stressors, such as restraint, by acting at the apex of the HPA axis. How this integrates with the role of aMSH in regulating food intake and energy balance remains an enigma.

P34

Melanotroph Pathology in Equine PPID: Genetic Insights

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Pituitary pars intermedia dysfunction (PPID) is a neurodegenerative endocrine disorder commonly affecting aged horses, with a disease prevalence of over 20% in equids aged 15 years and older[1]. This condition is also referred to as equine Cushing's disease. PPID is characterised by hyperplastic or adenomatous growth within the intermediate lobe (IL) of the pituitary gland. The IL is a homogenous lobe composed of melanotrophs: an often-neglected pituitary endocrine cell lineage under strict proliferative and transcriptional regulation, which synthesise and secrete pro-opiomelanocortin (POMC)-derived products. Equids affected with PPID experience highly upregulated inappropriate α-MSH and ACTH pituitary secretion, contributing to clinical expression of hypertrichosis, hypertrichosis and epaxial muscle wastage amongst other visual signs. However, the drivers of melanotroph population expansion and hypersecretion in PPID remain unknown.

The majority of previous studies have focused on a failure of appropriate hypothalamic dopaminergic regulation as the cause of PPID, with a lack of research considering the possibility of it being a primary pituitary disease arising following spontaneous melanotroph mutation[2]. This study aimed to characterise alterations in melanotroph cell gene expression as PPID progresses to understand how dysregulation leads to pathology. Differential gene expression of healthy, hyperplastic and adenomatous equine pituitary tissue (N= 24) was analysed with a DESeq2 bioinformatic pipeline. Samples were from a mixed equid population- including a range of breeds, ages, sexes and seasons of collection and these factors were considered in the analysis.

We were able to distinguish distinct genetic landscapes, which correlated different extents of pituitary pathology and identified 13 differentially expressed genes commonly upregulated between hyperplastic and adenomatous pituitaries in comparison to healthy tissue. Of particular interest, we recorded upregulation of ESM1: a used marker of human pituitary tumour development and BDNF: a documented driver of amphibian melanotroph proliferation[3,4]. Further work is needed to determine how or whether these initial candidates are responsible for melanotroph expansion and IL pathology.

1. PMID: 22594955
2. PMID: 36288169
3. PMID: 22353248
4. PMID: 20888824