MedSci Abstracts

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M1. Using genetic manipulations to understand the sex-specific role of RFRP-3 in the regulation of reproduction

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In 2000, gonadotrophin-inhibitory hormone was discovered in birds and shown to inhibit gonadotrophin secretion. The mammalian ortholog was concurrently discovered in humans and rats and termed RFamide-related peptide-3 (RFRP-3). We have recently shown, by administering RFRP-3 centrally, that the effects of RFRP-3 on gonadotrophin secretion are sex- and cycle stage-dependent in mice and hamsters. In order to further our understanding of the ways in which RFRP neurons modulate GnRH function, we have developed two novel transgenic mouse lines. Using a Cre-loxP conditional transgenic method, we knocked the receptor for RFRP-3 (GPR147) out of GnRH neurons and analysed puberty onset in these mice. The absence of GPR147 on GnRH neurons induced a significant advancement in male puberty, but resulted in significant delay in first estrus in female mice. However, no deficits in reproductive cycles or male and female adult fertility were noted. In parallel, we have been using a chemogenetic approach to elucidate the effect of activation of RFRP neurons on LH secretion. In male mice expressing the hM3Dq DREADD in RFRP neurons, 1 mg/kg clozapine-N-oxide (CNO, the DREADD ligand) had no effect on circulating LH levels. An additional dose of 10mg/kg of CNO had no effect on LH secretion. Further studies will aim at characterising the effect of CNO administration in females, notably at the time of the preovulatory LH surge. Taken together, these new tools provide us with the possibility to advance our understanding of the functions of the RFRP neuronal system in mice.

M2. Suppression of the response to restraint stress in the lactating mouse

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Suppression of the hypothalamic-pituitary-adrenal (HPA) axis is a well-characterised maternal adaptation which limits the exposure of the offspring to maternal stress hormones. The hormone prolactin may play a role in mediating this suppression as concentrations are elevated during lactation and prolactin has known anti-stress actions[1]. This study investigated the HPA axis response to a 30 min restraint stress in diestrous and lactating mice. An additional cohort of lactating mice treated with bromocriptine (D2 dopamine receptor agonist), to block endogenous prolactin secretion, were included to investigate the role of prolactin in the regulation of the stress response during lactation.

In situ hybridisation revealed an increase in *Crh* mRNA expression in the hypothalamic paraventricular nucleus (PVN) of diestrous mice in response to stress. In lactating mice, the basal levels of *Crh* mRNA were suppressed in comparison to diestrus, as was the stress-induced increase in *Crh* mRNA expression. Blocking prolactin secretion with bromocriptine did not reverse the lactation-induced suppression of *Crh* mRNA expression under either basal or stressed conditions. Consistent with the hypothalamic response, adrenal corticosterone (CORT) secretion, as measured by an enzyme-linked immunosorbent assay (ELISA), increased in response to restraint stress in diestrous mice, however this response was absent in lactation. Blocking prolactin secretion had no effect on either basal or stress-induced CORT secretion during lactation.

In addition to its anti-stress actions, pituitary prolactin secretion is also stress-sensitive[2]. In the present study, restraint stress increased pituitary prolactin secretion in diestrous mice. While basal prolactin concentrations were higher in lactating mice, the stress-induced increase in prolactin secretion was absent.

This study showed that the HPA response to stress is suppressed during lactation in the mouse. However, as acutely blocking prolactin secretion did not reverse this effect, the mechanism regulating this suppression remains unknown.

1. Torner, L., N. Toschi., A. Pohlinger, R. Landgraf and I.D. Neumann (2001). *Anxiolytic and anti-stress effects of brain prolactin: improved efficacy of antisense targeting of the prolactin receptor by molecular modelling.* The Journal of Neuroscience. 21: 3207-3214.
2. Kirk, S. E., Xie, T. Y., Stern, F. J., Grattan, D. R., & Bunn, S. J. (2017). *Restraint stress increases prolactin-mediated phosphorylation of signal transducer and activator of transcription 5 in the hypothalamus and adrenal cortex in the male mouse.* Journal of Neuroendocrinology. 29(6).

M3. Central prolactin receptor activation contributes to the hyperphagia of lactation

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Pregnancy and lactation are metabolically challenging states where uninhibited increases in energy intake are required to meet the demands of the growing conceptus and then the subsequent demands of milk production. The mother undergoes numerous adaptations to cope with these metabolically demanding states, including increased food intake. One candidate for mediating these adaptive changes is the hormone prolactin. Central prolactin action, particularly in the paraventricular nucleus of the hypothalamus (PVN), has been shown to stimulate appetite in non-pregnant rodents, and prolactin or its homologue placental lactogen are elevated throughout pregnancy and lactation. The aim of this study was to examine the role of central prolactin action in the changes in food intake and body weight during pregnancy and lactation. Transgenic mice with either a forebrain-specific deletion or a PVN-specific deletion of the prolactin receptor were monitored during pregnancy and lactation for food intake and body weight changes. The lack of prolactin receptor in either forebrain neurons or specifically in the PVN did not alter food intake or body weight gain during pregnancy. Pregnancy-induced leptin insensitively was also assessed in forebrain Prlr KO mice, and both control and forebrain Prlr KO mice showed a pregnancy-specific attenuation of the satiety effect of leptin and similar leptin-induced pSTAT3 in the arcuate nucleus. During lactation, forebrain Prlr KO mice had reduced food intake and their litters gained less weight compared to control mice. PVN Prlr KO mice had slightly lower food intake than control mice during lactation, but no effect was observed on litter weight gain. In conclusion, these results suggest that prolactin action in forebrain neurons is not required for changes in food intake during pregnancy nor the development of pregnancy-induced leptin insensitivity, yet contributes to the further increases in food intake observed during lactation.

M4. Adaptation of tuberoinfundibular dopaminergic neurons to estrous cycle and lactation

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The hypothalamic tuberoinfundibular dopaminergic (TIDA) neurons play a key role in regulating prolactin secretion and are known to exhibit remarkable plasticity during lactation, switching from dopamine to enkephalin release. This study investigates if this functional plasticity is accompanied by morphological changes. The arcuate nuclei of adult female, tyrosine hydroxylase (TH):Cre, rats were unilaterally injected with Cre-dependent AAV expressing Brainbow markers. Two weeks later Brainbow expression was compared between reproductive stages. The effect of 17β-estradiol (E_2) on ovarectomized animals was examined in a separate cohort. Immunohistochemistry revealed that approximately 90% of Brainbow-expressing cells were TH-positive with about 50% of the TIDA neurons transfected. This moderate transfection efficiency facilitated morphological examination of individual TIDA neurons. Neuronal spine density was measured at the cell soma and proximal dendrite (30-60 µm) of 5 animals at each stage (total of 73 - 93 cells per group). Dendritic spine density remained constant, but soma density decreased towards estrous, falling from 0.088 ± 0.006 spines/µm at diestrus to 0.059 ± 0.004 by estrous (*P<0.001*). A role for estrogens was suggested by the finding that E2 treatment of ovarectomized animals caused a decline in somatic spine density (from 0.140 ± 0.011 spines/µm to 0.099 ± 0.012, *P<0.05*). Interestingly, somatic, but not dendritic, spine density increased during lactation, to become significantly higher than at any other stage. These data revealed that TIDA neurons undergo morphological plasticity across the reproductive cycle. Intriguingly, the rise in somatic spine density during lactation is suggestive of increased TIDA neuronal activity at this time.

M5. Characterisation of the acute effects of prolactin upon prolactin receptor-expressing neurones in the mouse hypothalamus

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The anterior pituitary hormone, prolactin, a fundamental regulator of lactation, plays a role in many other physiological processes including maternal behaviour, reproduction, immune response and even energy balance. Indeed, prolactin receptors (Prlr) are widely distributed throughout the brain, further attesting to its pleiotropic nature. Previous research has identified key areas upon which prolactin exerts transcriptional effects, yet its acute modulation of electrical properties of Prlr-expressing neurones remains to be elucidated. To identify and probe the function of these Prlr cells, we utilised a transgenic mouse line in which Cre recombinase is specifically expressed in the coding region of the prolactin long form receptor gene (*Prlr*^{Cre}). This mouse line was crossed with a Cre-dependent calcium indicator (GCaMP6s) transgenic mouse, allowing us to visually monitor the electrical activity of Prlr-expressing neurones in *ex vivo* 200μm brain slice preparations. Here we survey hypothalamic regions implicated in prolactin's diverse physiological functions such as the arcuate nucleus of the hypothalamus (ARC) and the medial preoptic area (MPOA). We observe that in both males and females, bath application of prolactin is able to induce electrical changes in a subset of Prlr-expressing cells that reside in the ARC (~30% responders) and in the MPOA (~10% responders). The effects detected range from rapid or sustained increases in intracellular calcium to slower increases in calcium transients, hinting at a heterogeneous nature of these Prlr-expressing populations. These results enhance our understanding of the neural circuits influenced by prolactin and provide a potential mechanism of prolactin's actions in the mouse brain.

M6. Developing tissue simulant to mimic the dielectric behaviour of human blood for multi-frequency bioimpedance analysis

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Phantoms, or physical simulants of biological specimens are useful for research and development due to the unavailability of real tissues. One such example is human blood, which is a precious resource in healthcare and is not normally available for research purposes, unless it is no longer fit for human use. This work focusses on the considerations for developing a tissue mimicking material, specifically to exhibit the dielectric properties of human blood tissue, for bioimpedance analysis. The behavior for the tissue simulant was identified based on the conductivity and permittivity values within the β dispersion frequency range (1 kHz – 2 MHz). Propylene Glycol, Ethanol and Glycerol presented themselves as suitable candidates as they exhibit a similar dielectric response as that of blood. The study identified their individual dielectric parameters, followed up with experiments on their mixtures to approximate the conductivity and permittivity of human blood. A mixture of 80% propylene glycol and 20% 4 M saline solution was found to replicate the properties of blood within acceptable tolerances in the β dispersion frequency range. The methodology adopted provides a very economical, reproducible and robust means for investigating the dielectric response of tissues across several research and training platforms, and more importantly bioimpedance applications.

M7. Development of Conductive Polymer Composites for Electrocardiography Sensing

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Preparation and mechanical testing of thin-polymer electrodes was done to efficiently measure the biomedical signals while providing a conformal skin-contact. Thin electrode films using Polydimethylsiloxane (PDMS) with different Young's moduli were developed. The Young's modulus was evaluated for each film by conducting tension-relaxation tests. The PDMS electrodes were made conductive by mixing them with Multi-Walled Carbon Nano Tubes (MWCNTs) and Graphene Nano Powder (GNP) using an ultrasonic mixer. The CNTs and GNP were wetted in N-N Dimethylformamide (DMF) prior to their dispersion in PDMS. This was done to avoid them clumping in PDMS and to ensure more uniform dispersion. The conductance of the PDMS/MWCNT and PDMS/GNP electrodes were measured using an impedance spectrometer and were compared with that of standard silver-silver chloride (Ag/AgCl) and dry silver (Ag) electrodes.

M8. Estimating reference values of aortic pulse wave velocity for the New Zealand population

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Pulse wave velocity (PWV) is a gold-standard measure of arterial stiffness (AS) and has been acknowledged as an independent diagnostic marker of stroke and cardiovascular (CV) risk. New Zealand (NZ) has a high prevalence of people with cardiovascular disease (CVD) with ethnic disparities. However, the use of PWV for routine clinical assessment of CVD risk is not practiced due to a lack of reference values and official recommendations for the NZ population. In this research, aortic carotid-femoral PWV values were estimated (N=92/120) using Doppler ultrasound for a 'reference value population' (RVP, n=26) with CVD risk factors but free from diabetes, high cholesterol, any known heart disease or on medications for these conditions. Whereas, participants with normal BP without any CVD risk factors constituted the 'normal value population' (NPV, n=66). The screened participants were grouped by age (18-30, 30-60, > 60 years) and blood pressure (BP) (normal, elevated, stage 1, stage 2) categories. Peripheral and central systolic/diastolic BP, pulse rate, and augmentation index (AI) were measured by USCOM BP+ monitor.

The data collected so far shows that PWV shares a positive correlation with age (R^2=0.4) and blood pressure (R^2=0.2). The mean aortic PWV were significantly lower in the NVP (5.1 ± 0.92 m/s) compared to RVP (6.3 ± 1.26 m/s) ($p<0.001$). The mean PWV values for the three age categories were 4.8, 5.6, and 6.7 m/s respectively with higher values in the RVP. Effect of age, sex, body mass index (BMI), AI, mean BP, smoking, alcohol consumption, diabetes, dyslipidaemia, and hypertension on PWV was assessed. Multiple regression analysis showed a significant contribution to the prediction of the Mean PWV with age (β=0.5, p=<.001), and BMI (β=0.1, p=.03).

The preliminary results show PWV could have value in a proactive approach to CVD risk assessment that would help in attaining long-term community health goals of the NZ government. The final outcome will assist in establishing the normal and reference values of PWV in the NZ population.

M9. Effect of Positive Pressure Oscillations on Cultured Human Epithelial Cells

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Lung supportive devices (LSD) are widely used for respiratory ventilation and therapy to help providing breathing support for patients with various lung diseases including Obstructive Sleep Apnea. These devices deliver continuous air to the patient through a nasal or a facial mask. However, the use of these devices normally results in dryness in the upper airways. Various methods have been developed to improve the continuous positive pressure features using pressure oscillations (PO), which can reduce the patient requirements such as reduction in the titration pressure and humidification requirements.

Thus, the objective of this research is to investigate the differences between continuous positive pressure and positive pressure oscillation on the upper airways humidification. Human nasal (RPMI2650) and airway (Calu-3) epithelial cells grown in air-liquid interface (ALI) on permeable supports are used as a respiratory model. Trans-epithelial electrical resistance (TEER), apparent permeability (P_{app}) and mucus secretion rate are measured before and after each experiment to evaluate the cell layer integrity, permeability and functionality. Significant differences are found between the continuous positive pressure and positive pressure oscillation experiments.

M10. An investigation into the effects of pressure oscillations on airway smooth muscle in chronic asthma.

Roos K.L.T.

The hyperconstriction of airway smooth muscle (ASM) is the main driving mechanism during an asthmatic attack. The airway lumen is reduced, resistance to airflow increases, and normal breathing becomes more difficult. The tissue contraction can be temporarily relieved by using bronchodilator drugs which induce relaxation of the constricted airways. With one of the highest prevalence rates in the world, New Zealand's costs for asthma treatments total an estimated NZD$825 million per year.

While widely used in asthma therapies, pharmacological treatments vary in their effectiveness from one subject to another, as do the side effects of long-term usage. Studies have shown that application of mechanical oscillations which are equivalent to the physiological patterns of normal breathing and deep inspirations in healthy airways can induce airway relaxation. This type of relaxation response is not observed in asthmatics.

Utilizing length oscillations (arising from positive pressure) in association with breathing patterns provides non-pharmacological options for augmenting treatment of the ASM hyperconstriction which is present in many respiratory diseases such as asthma. There is currently little known about the effects of applying superimposed pressure oscillations in combination with breathing patterns to healthy and asthmatic airways during an asthmatic attack.

Results from *in vivo* studies of a chronic murine asthmatic model indicate that the use of superimposed pressure oscillations (SIPO) over normal breathing patterns facilitates relaxation during an induced asthmatic attack in healthy and asthmatic subjects. Oscillation patterns, physiological pressure equivalents, and their effects on key respiratory parameters are presented. Comparisons of healthy and asthmatic lung resistance (R_L) and dynamic compliance (C_{dyn}) values are used as assessments of the changes in airway responses to applied mechanical pressure oscillations. Additionally, a standard respiratory constant is used to normalize acute and chronic asthmatic models' data. Use of the constant assists in modeling the effects of SIPO by transforming R_L and C_{dyn} data into Work and Power equivalents for use in interpreting ASM mechanics.

M11. Rested-state contraction of cardiac muscle: beyond the contribution from the sarcoplasmic reticulum

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When the contraction of cardiac muscle is resumed after a period of rest, the force associated with the first twitch is large in its magnitude. The subsequent twitches follow a complex profile prior to settling to a new steady-state magnitude. This phenomenon is known as the 'rested-state contraction'. Its physiological interest resides in the consistent findings that the magnitude of the first twitch is dependent on the resting duration during which the sarcoplasmic reticulum (SR) continues to be loaded with Ca^{2+}. Thus, when contraction is resumed, a large amount of stored SR Ca^{2+} is released, enhancing the first twitch force. We performed experiments on isolated rat ventricular trabeculae where force and Ca^{2+} were measured simultaneously using our cardiomyometer. Analysis of the resultant force-Ca^{2+} loop revealed that the loop changes during the 'rested-state contraction', indicative of a 'dynamic' myofilament Ca^{2+}-sensitivity. While it is known that the SR plays a role in the 'rested-state contraction', our findings demonstrate the hitherto-unrecognised synergistic contribution from the myofilament sensitivity to Ca^{2+}.

M12. Characterising the role and therapeutic potential of novel peptide DWORF in heart disease.

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Background: Heart disease is a leading cause of death and disability in New Zealand, and new treatment options are needed. A recent breakthrough in genetic research has led to the discovery of a new class of proteins, one of which is called DWORF. This small protein is found almost exclusively in the heart and is believed to cause increased contraction of the heart muscle by regulating the cellular mechanisms responsible. Levels of DWORF are decreased in the hearts of people who have had heart attacks, suggesting it might play a role in the development of heart disease and may have potential as a treatment for restoring contraction of damaged hearts. However, the effect on the heart of direct DWORF administration is unknown. Aim: To investigate the effect of DWORF in normal and ischaemic hearts. Methods: In Langendorff isolated perfused rat hearts, cardiac function was measured following DWORF administration in (a) normal hearts and (b) hearts subjected to 40 minutes ischaemia followed by reperfusion (which mimics the reopening of coronary arteries after a heart attack). Results: DWORF administration in the normal rat heart induces significant and dose-dependent increases in perfusion pressure, an indication of coronary blood flow. Further, in hearts subjected to injury by ischemia/reperfusion, we found that DWORF administered after ischemia and at the onset of re-oxygenation not only increases coronary pressure, but also improves heart contractility (as shown by increases in derivative of maximal pressure over time).– actions likely to prove beneficial in patients suffering from a heart attack and reduced cardiac function. Conclusion: The actions of DWORF to increase coronary blood flow and improve contractility following ischemia/reperfusion injury in the isolated rat heart suggests the peptide may have therapeutic potential in patients suffering from a heart attack and reduced cardiac function.

M13. Characterisation of periventricular nucleus kisspeptin neuron projections to oxytocin neurons in pregnancy and lactation

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Oxytocin induces uterine contractions during birth. Oxytocin is secreted from the posterior pituitary gland by hypothalamic paraventricular nucleus (PVN) and supraoptic nucleus (SON) neurons. The trigger for increased oxytocin secretion is largely unknown. However, we have shown that kisspeptin increases the firing rate of oxytocin neurons in late-pregnant rat but not in non-pregnant rats. Here, we tested whether kisspeptin fibre density changes around oxytocin neurons in pregnant mice and determined the origin of these kisspeptin fibres. To determine whether kisspeptin projections to oxytocin neurons are changed in pregnancy, kisspeptin and oxytocin double-label immunohistochemistry was performed on brain slices from non-pregnant (n = 7), day 7, 14 and 19 (n = 8/group) pregnant and day 7 lactating (n = 7) mice. Sections were photographed on a confocal microscope and the fraction of kisspeptin-positive voxels in each area was analysed using FIJI. PVN kisspeptin fibre density was similar in non-pregnant, day 7 and day 19 pregnant mice but was lower on day 14 of pregnancy and day 7 of lactation than in non-pregnant mice ($P < 0.05$). A similar pattern of kisspeptin fibre density was evident in the SON. Close appositions of kisspeptin fibres with oxytocin cell bodies and dendrites are currently being analysed. To determine the origin of the kisspeptin projection to oxytocin neurons, retrograde label was injected into the PVN of non-pregnant and pregnant mice. Retrograde label was only found in the periventricular nucleus (PeN), with a higher proportion of retrogradely-labelled PeN kisspeptin neurons in day 18 pregnant mice than in non-pregnant mice. We conclude that the PeN kisspeptin projection to oxytocin neurons is more prominent at the end of pregnancy.

M14. Mapping O-linked glycosylation in the brain during pregnancy

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During pregnancy, maternal metabolism changes to support the developing fetus and to prepare for lactation. Among the many maternal changes that occur is an increase in circulating glucose levels, which in many cases cannot be fully offset by increased insulin production, leading to hyperglycemia and even gestational diabetes. Glucose can affect cell function by O-linked N-acetylglucosamine (O-GlcNAc) modification of proteins, but it is unknown whether this process plays a physiological role in the central regulation of metabolism in pregnancy. Food intake and energy balance are regulated by various neuron populations in hypothalamic arcuate nucleus, paraventricular nucleus and ventromedial hypothalamus. We are currently performing immunohistochemistry for O-GlcNAc modification in sections of hypothalamus from virgin and late-pregnant rats to determine whether, and where, O-GlcNAc modification occurs and will report the regional distribution of O-GlcNAc modifications in the hypothalamus.

M15. Prolactin Acutely Influences Running Wheel Activity But Not Ambulatory Activity in Female Mice

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Prolactin is implicated in a broad range of functions in the brain. In particular, prolactin plays a role in energy homeostasis through stimulating food intake and bodyweight gain. We have recently observed a link between prolactin and running wheel activity; male mice with prolactin-receptors deleted from RIP-cre cells (beta cells and subset of neurons) have increased running wheel activity[1], suggesting that prolactin may suppress activity, facilitating a positive energy balance. The aim of this study was to further investigate the effect of prolactin on physical activity to determine if 1) prolactin can acutely influence running wheel behaviour and 2) if this effect is specific to running wheel activity or generalized for other forms of physical activity. Female C57B6/J mice received prolactin (i.p.; 5mg/kg) or saline then running wheel activity was measured for 12 hours. Prolactin treatment significantly decreased dark phase running wheel distance compared to vehicle treatment, especially within the first 4 hours. Using a second cohort of female mice, it was found that prolactin treatment induced no significant changes in locomotor activity (distance traveled) compared to saline when assessed through open field and elevated plus maze testing. Additionally, in experiments investigating home-cage activity, neither distance traveled or total beam breaks differed across treatments indicating no effect of prolactin on home-cage activity either. Dual-label immunofluroescence studies were conducted for cFos and GFP using a transgenic mouse line specifically designed to express GFP in neurons expressing the prolactin receptor. It was found that prolactin signalling in the medial division of the BnST is not implicated in the suppression of wheel running. Overall, these data describe a novel suppressive role for prolactin specifically on running wheel activity, as opposed to general activity, and begun to characterise regions and populations through which prolactin does not act to exert these effects.

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M16. Delay in puberty onset after neonatal underfeeding can be reversed by silencing AgRP neurons in male mice

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Agouti-related peptide (AgRP) is co-expressed with neuropeptide-Y and gamma-aminobutyric acid in arcuate neurons. The activity of these neurons is modulated by metabolic hormones such as leptin, ghrelin, and insulin and therefore act to convey metabolic cues to the Hypothalamic-Pituitary-Gonadal axis. However the effect of AgRP neuronal modulation on reproductive function and puberty onset has yet to be clearly elucidated. Using 'Designer Receptors Exclusively Activated by Designer Drugs' technology, we selectively silenced AgRP neurons non-invasively by administering the synthetic ligand CNO into drinking water of male mice from postnatal day 26 to 30 (~2 mg/day/mouse). The age at preputial separation (an anatomical marker of puberty) was significantly advanced (by 2.1 days) in AgRP neuron-inhibited mice compared to controls ($p<0.05$). We wondered whether this effect could counteract the delay in puberty onset observed under neonatal underfeeding. Pups mice were fostering within 3 days after birth into large litters (12 pups) to create underfeeding conditions, or into normal litters (6 pups), and received the same treatments as described above. As expected, the age at preputial separation was delayed in controls from large litters compared to controls from normal litters ($p<0.05$), but this effect was reversed in AgRP neuron-inhibited mice, with a similar age at preputial separation compared to mice from normal litters. Additional studies are underway to look at the effects on puberty onset in females. Whether this effect occurred in response to AgRP itself or one of the other secreted products of these neurons also remains to be determined.

M17. Hyperuricemia driving pancreatic β-cell death through Rictor, a subunit of mTORC2

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Hyperuricemia is characterized by a raised plasma concentration of the purine metabolite, uric acid. Large epidemiological studies have identified a link between hyperuricemia and type 2 diabetes mellitus, however, the molecular mechanism is unknown. It has been reported pancreatic β-cells have reduced mass and insulin secretion capacity under hyperuricemic conditions. One proposed mechanism is based on the mechanistic target of rapamycin (mTOR) complex, specifically the second complex, mTORC2. Reduced mTORC2 activity under nutrient overload and diabetic conditions has been correlated to decreased pancreatic β-cell mass and insulin secretion. Knocking down mTORC2 subunit Rictor in rat islets has produced significant reduction in pancreatic β-cell mass and insulin secretion. This has resulted in my hypothesis that Rictor could be modulated under hyperuricemic conditions, resulting in a reduction of mTORC2 activity, therefore, contributing to the development of type 2 diabetes mellitus. This hypothesis will be examined through analysis of expression, phosphorylation and ubiquitination of Rictor in MIN6 (mouse) and 1.1B4 (human) pancreatic β-cells. These cells will be exposed to the conditions of normouricemia (300 μM), hyperuricemia (500 μM), in the presence of urate transporter Glut9 inhibitor benzbromarone (100 μM), and in the presence of an mTOR complex inhibitor, PP242. Rictor expression and phosphorylation will be quantified through western blotting with Rictor and phospho-Rictor antibodies, respectively. Rictor ubiquitination will be analyzed through a pulldown experiment, then quantified through western blotting with an ubiquitin antibody. Apoptosis and MTT assays will be performed to identify any functional change to the cellular apoptosis and proliferation rates in each treatment group.

M18. Sexually dimorphic corticosteroid negative feedback in hypothalamic CRH neurons

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The hypothalamic pituitary adrenal (HPA) axis is controlled by corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus (PVN) of the hypothalamus. In response to stress, noradrenaline is released into the PVN which causes the activation of these neurons and the downstream release of adrenal corticosteroids. Released corticosteroids can then feedback onto the HPA axis to shut off its activity by activating glucocorticoid receptors (GRs). Previous work has shown that males and females have different patterns of HPA axis activity across the day, different acute stress responses and different expression levels of GRs in the brain. However, it is currently unknown if the excitability of CRH neurons or the effectiveness of negative feedback on excitability of CRH neurons differs between the sexes. We have examined this by performing GCaMP6f calcium imaging of CRH neurons in PVN slices to assess their responses to noradrenaline and corticosterone treatment. Application of noradrenaline (10μM) excited a proportion of CRH neurons, inducing a bursting pattern of activity. Our preliminary data suggests that the proportion of CRH neurons activated by noradrenaline is not different between males (27.9 ± 4.2%, n=9) and females (21.1 ± 3.8%, n=12, P>0.05). To assess differences in negative feedback, mice were treated with 25μg/ml corticosterone in their drinking water for 14 days. The proportion of CRH neurons activated by noradrenaline was significantly reduced in male mice treated with corticosterone (9.7 ± 1.6%, n=9, P=0.004 compared to control male response). However, no effect of corticosterone was observed in female mice (29.7 ± 3.5%, n=9, P>0.05 compared to control female response). Together these data give insights into possible differences in HPA axis regulation between the sexes.

M19. Uric acid as a modulator of endothelial sodium channels in the vasculature

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Cardiovascular disease, the number one cause of death worldwide, is often preceded by hypertension and development of endothelial dysfunction in the blood vessels. High sodium and uric acid blood serum concentrations have been associated with decreased vasodilator nitric oxide (NO) production in the vasculature, as well as hypertension and an increased cardiovascular disease risk. Recently, the epithelial sodium channel (ENaC) has been shown to act as a shear stress sensor in blood vessels, its activity resulting in a down regulation of NO production in resistance arteries. In the kidney tubules, increased uric acid concentrations has also been shown to increase all ENaC subunit expression. Studies have not yet linked uric acid concentrations to the subunit expression of ENaC in the vessels. Using human umbilical vein endothelial cells and Western Blotting techniques we will investigate how exposure to increased sodium and uric acid concentrations impact ENaC subunit expression, and if this effect can be abolished by blocking uric acid influx with GLUT9. To mimic the shear stress of blood flow growing vessels are under *in vivo*, this experiment will be performed using cells in both static and perfused systems. Should a link between uric acid and ENaC subunit expression be identified, uric acid transporters in the future may act as therapeutic targets in preventing the development of hypertension.

M20. Is pancreatic β-cell death under hyperuricemic conditions facilitated by the mTOR-Raptor complex?

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Uric acid is a compound primarily produced from the breakdown of purines from the diet. Serum uric acid (UA) levels exceeding 350µM are considered hyperuricemic putting individuals with hyperuricemia at greater risk of developing hypertension, gout or diabetes mellitus (DM). A main characteristic of DM is the loss of pancreatic β-cell mass.

The mechanistic target of rapamycin (mTOR) is a central regulator of cell growth, proliferation and survival through regulation of cell autophagy. mTOR-complex 1 comprises the mTOR core unit, and its activity is tightly controlled by nutrients such as glucose and amino acids as well as by its main regulatory subunit Raptor. The binding of Raptor to mTOR leads to an increase in protein synthesis and a reduction of autophagy thereby regulating β-cell mass. If β-cells are exposed to hyperuricemic conditions for three days they show significant apoptosis. Therefore, the aim of my project is to determine the involvement of the mTOR-Raptor complex in β-cell death under hyperuricemic conditions.

I hypothesise that a change in Raptor expression or phosphorylation is causing an overdrive in β-cell autophagy leading to β-cell death. 1.1B4 and MIN6 cell lines will be treated with 300µM UA, 500µM UA, 500µM UA + 100µM Benzbromarone, 500µM UA + PP242. Raptor expression, phosphorylation and β-cell autophagy will be measured via western blot analysis. An MTS assay kit will determine β-cell proliferation, and Raptor ubiquitination will be analysed with a ubiquitin pull-down assay to determine if changes in raptor expression are caused by changes in Raptor ubiquitination.

M21. Mechanosensitivity of transient receptor potential vanilloid channels

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Vasopressin is synthesised by hypothalamic magnocellular neurons and is secreted from the posterior pituitary gland in response to increased plasma osmolality to maintain body fluid balance by promoting renal water retention. Vasopressin neurons are directly osmosensitive via mechanosensitive transient receptor potential vanilloid (TRPV) channels that are inactivated by membrane stretch caused by reduced osmolality. Mechanosensitivity is conferred by ΔN-TRPV1 but most TRPV channels express more than one type of TRPV subunit. Vasopressin neurons also express TRPV2 and 4 but the subunit composition of the TRPV channels expressed by vasopressin neurons is unknown, as is the potential contribution of any changes in subunit composition to vasopressin neuron function. Here, we test the hypothesis that a change in TRPV channel composition alters the mechanosensitivity of ΔN-TRPV1-containing channels. Initial experiments are being conducted in *Xenopus* oocytes. Homomeric channels are being expressed (by injection of 0.2 ng cRNA/oocyte) or heteromeric channels using a combinations of two subunits (ΔN-TRPV1+TRPV2; TRPV2 + TRPV4; 0.1ng cRNA/oocyte and subunit) or three subunits (ΔN-TRPV1+TRPV2+TRPV4; 0.07ng cRNA/oocyte and subunit). cRNA will be injected into *Xenopus* oocytes and incubated for 24 h – 48hrs. Results from two electrode voltage clamp electrophysiology (with membrane current recorded at -60 mV and) will be presented.

M22. The emerging role of the antioxidant uric acid in the pancreatic β-cell viability

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Introduction: Uric acid (UA) is known as the main antioxidant in the blood. However, elevated serum uric acid (SUA) called hyperuricemia and causing gout has been linked to diabetes mellitus (DM)1. Loss of viable pancreatic β-cells is a hallmark of both development and progression of DM. We aimed to determine how elevated SUA levels can induce a loss of cell viably through inhibition of the mTOR cell survival pathway.
Material and methods: We employed the human pancreatic β-cell line 1.1B4. Western blot techniques, MTT and Caspase 3/7 assays, dynamic cell proliferation monitoring, and co-immunoprecipitation were used to determine the effects of hyperuricemia on cell viability.
Results: Exposure of 1.1B4 cells to hyperuricemic conditions resulted in significant reductions in pancreatic β-cell viability by both reduced metabolic activity and increased autophagy. This could be attributed to a direct inhibition of the mTOR complex 1 by the negative regulator DEPTOR. Additionally, initial experiments indicate that hyperuricemia alters DEPTOR ubiquitination.
Conclusion: Hyperuricemic conditions resembling elevated SUA levels resulted in significant decreases in pancreatic β-cell viability possibly initiated by changes in DEPTOR ubiquitination providing a causal basis for the connection between elevated SUA and diabetes mellitus.

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M23. Selective activation of arcuate nucleus GABA neurons promotes luteinizing hormone secretion in mice

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GABAergic neurons originating in the arcuate nucleus (ARN) densely contact gonadotropin-releasing hormone (GnRH) neurons, cells in the brain critical for fertility. Plastic changes in this circuit are evident in a mouse model of polycystic ovary syndrome (PCOS), a syndrome associated with elevated GnRH neuron activity and luteinizing hormone (LH) secretion. To investigate the biological relevance of this circuitry, the present study aimed to determine the impact of selective activation of arcuate GABA (ARN GABA) neurons on LH secretion using *in vivo* optogenetic activation in healthy and prenatally-androgenized PCOS-like mice. Cre-dependent expression of channelrhodopsin-2 E123T accelerated variant (ChETA) was targeted to ARN GABA neurons in vesicular GABA transporter (VGAT)-Cre mice. Cell-attached voltage-clamp recordings showed that ChETA-transduced ARN GABA neurons respond to blue light pulses with high spike fidelity (100%) up to 50 Hz (N = 19 GABA neurons). *In vivo* studies in anesthetized VGAT-Cre$^{+/-}$ male (N = 6), diestrous female (N = 10) and PCOS-like (N = 5) mice revealed that 20-Hz light stimulation evoked robust LH release in male and diestrus female mice, lasting over 60 minutes after stimulation ($P < 0.05$). Interestingly, 20 Hz-optogenetic activation of ARN GABA neurons in PCOS-like mice induced smaller changes in LH levels when compared to male and diestrous female groups ($P < 0.05$). However, PCOS-like mice also showed a significantly lower LH secretion in response to a GnRH challenge, indicating a smaller releasable pituitary pool of LH. These data indicate that ARN GABA neurons are a functionally relevant input to GnRH neurons, capable of stimulating GnRH neuron activity and LH secretion. Activation of this circuit drives similar LH release in healthy males and females and a diminished LH response in PCOS-like mice, reflecting a decreased releasable pool of pituitary LH due to high endogenous GnRH pulse frequency in the PCOS-like condition.

M24. Impaired sexual behaviour in a mouse model of PCOS

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Polycystic ovary syndrome (PCOS) is the most common infertility disorder in women worldwide. In addition to infertility, recent epidemiologic studies indicate that PCOS is associated with decreased sexual desire, increased sexual dissatisfaction and gender dysphoria. Prenatally androgenized (PNA) animal model of PCOS exhibit an adult hyperandrogenism, impaired sensitivity to progesterone signalling in the brain and alterations in the gonadotropin-releasing hormone (GnRH) neuronal network linked to reproductive dysfunction. However, the impact of prenatal androgen excess and the development of PCOS features on sexual behaviour remains unclear. This study aimed to *determine whether the PNA mouse model of PCOS exhibits typical female sexual behaviour and mate preference.* To model PCOS, female dams received injections of dihydrotestosterone, a non-aromatisable androgen (PNA n=8), or oil vehicle (VEH n=5) daily from gestational day 16 to 18. Adult female offspring were ovariectomized and implanted with a silastic capsule of estradiol to examine lordosis behaviour. PNA females exhibited an overall reduction of the lordosis quotient compared to VEH females ($p<0.01$). These data suggest that increased androgen signalling during the perinatal period impaired sexual differentiation of the brain and behaviour in addition to other PCOS features. Ongoing experiments are 1) determining if the partner preference of female PNA mice is different from VEH and 2) investigating activated brain areas following sexual behaviour assessment to determine the specific neuronal targets potentially disrupted in PCOS-like females.

M25. Soluble Klotho Changes Epithelial Sodium Channel Activity

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Epithelial sodium channels (ENaC) are widely expressed across epithelia of many organs. In the kidney, these proteins function to allow the influx of sodium ions and subsequently indirect flow of fluid into the cells. Therefore, making these channels an integral part of blood pressure regulation.

Klotho is a single transmembrane protein originally identified in aging studies. Post translational modifications of this protein produces a soluble form that has humoral factors. This soluble form shares homology with glycosidases which act to cleave off the sialic acid from N-glycans of certain proteins. This modulation to N-glycans decreases the retrieval rate of channels from the membrane causing a high expression of channels such as calcium (TRPV) channels.

Neuraminidase is a glycosidase acting similarly to klotho and cleaving off sialic acid from N-glycans. Preliminary electrophysiological recordings from the Fronius lab show instead of a retention of ENaC and an increase in ENaC-mediated current, a decrease in sodium current after neuraminidase treatment. This suggests two opposing models for the mechanism of action for the soluble form of klotho.

Thus, the aim of my project is to determine the effect of klotho on ENaC activity. This is important as klotho could be a modulator of ENaC activity and ultimately blood pressure. I hypothesize that klotho will change the activity of ENaC.

To study this, I will be using Xenopus oocytes to co-express ENaC and klotho and using the two-electrode voltage clamp setup for functional recordings and western blotting for klotho expression analysis.

M26. Therapeutic modulation of microRNA-320 to prevent diabetic cardiomyopathy using Locked Nucleic Acid oligonucleotides

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Diabetic cardiomyopathy (DCM) is a chronic complication in individuals with diabetes which is characterized by ventricular dilation and hypertrophy, diastolic dysfunction, decreased or preserved systolic function and reduced ejection fraction eventually resulting in heart failure. Despite being well characterized, the fundamental mechanisms leading to DCM as well as its prevention or treatment are still elusive. Recent studies identified the involvement of small non-coding RNA molecules such as microRNAs (miRNA) playing a key role in the aetiology of DCM. Therefore, miRNAs associated with DCM represents a new class of mechanistic targets, which may yield marked benefits compared to other therapeutic approaches. Among several miRNAs, miRNA-320, expressed abundantly in cardiomyocytes and microvascular endothelial cells, has been demonstrated to exhibit a pro-apoptotic role by targeting pro-survival insulin growth factor-1 and insulin growth factor-1 receptor proteins.
We hypothesized that, modulation of miRNA-320 is crucial for the prevention of DCM through the inhibition of apoptosis. In this study, AC-16 ventricular cardiomyocytes were treated with high (30 mM) and normal glucose (5.5 mM) for 24 to 120 hours. At each time point, total RNA and protein was collected and miRNA-320 expression was estimated by revers-transcriptase polymerase chain reaction and protein expression was estimated by western blot analysis. In addition, apoptosis was measured by Caspase3/7 assay at each time point mentioned above. Results so far demonstrated high glucose significantly increased the expression of miRNA-320 ($P<0.01$ vs. normal glucose treated cells) while markedly reducing the expression of its target proteins insulin growth factor-1 and insulin growth factor-1 receptor ($P<0.05$ vs. normal glucose treated cells). Importantly, this was associated with increased cell death in high glucose treated cells. Taken together, our results demonstrate that high glucose activates pro-apoptotic miR-320 and that therapeutic modulation of miRNA-320 using synthetic anti-miRs may be novel approach for the treatment of DCM.

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M27. Elucidating the effect of prenatal androgen excess on male reproductive function and metabolism

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Elevated maternal androgens are associated with the development of polycystic ovary syndrome (PCOS), a prevalent female neuroendocrine disorder[1]. Emerging clinical research has postulated that male offspring exposed to elevated maternal androgens exhibit a male form of PCOS. It has been observed that the male relatives of women with PCOS manifest similar reproductive and metabolic abnormalities[2,3]. Therefore this project aimed to understand the impact of prenatal androgen excess on male reproductive and metabolic biology.

Pregnant mouse dams were subcutaneously injected with either dihydrotestosterone (250µg) (prenatally androgenized mice (PNAM) offspring, n=20) or a control oil vehicle (200µL, VEH control, n=15) on gestational days 16, 17 and 18. Pubertal onset and fertility was analyzed by determining the age of the first successful mating resulting in conception and daily anogenital distance was measured (the distance between the glans penis and anus) from postnatal day (PND) 35 onwards. Body weight was recorded every 5 days from PND35 and then every 10 days from PND55. Testes and seminal vesicles weight was measured.

PNAM were not significantly different to controls in terms of body weight, age of first successful mating, and seminal vesicles weight. PNAM anogenital distance, normalized to body weight, was significantly shorter at postnatal day 40 in comparison to controls (P=0.0036). Therefore, PNAM mice manifest a more female-like anogenital distance peripubertally, suggestive of decreased circulating androgens. However, testosterone levels were not significantly different between PNAM and VEH control mice in adulthood. Although, testes weight was significantly lower in PNAM mice in comparison to controls (P = 0.0152). This study has illustrated males exposed to elevated prenatal androgens manifest altered external genitalia and gonadal weight suggestive of developmental impairments, but remain fertile and exhibit no overt metabolic phenotype. It remains to be determined whether elevated maternal androgens impact brain circuits associated with male gonadal function.

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M28. Dose dependent central insulin resistance in response to high fat diet

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Hypothalamic dysregulation of insulin signaling has been shown to be a predisposition to developing type 2 diabetes and obesity. A key region involved in the regulation of body weight and glucose metabolism is the arcuate nucleus (ARC) of the hypothalamus. Therefore, the aim of the current study was to investigate whether central insulin resistance occurred in response to a high fat diet (HFD) and whether insulin responses within the ARC were dose-dependent.

Sixty-seven male mice were fed either a HFD (60% kcal from fat) for four weeks, until obesity and glucose intolerance were fully established, or were maintained on a low fat diet (10% kcal from fat) (LFD). Mice were then injected intraperitoneally with decreasing insulin concentrations of 1, 0.2, 0.04, 0.008 milligrams per kilogram of body weight (mg/kg bw) or vehicle, 15 minutes prior to transcranial perfusion (n=6-7/treatment/diet). Insulin signaling was measured at the level of AKT, an important insulin target downstream of the receptor, by counting the number of phosphorylated-AKT (S473) immunoreactive cells.

A linear dose relationship was observed, with increasing insulin concentration corresponding with an increased number of pAKT-immunoreactive cells in both LFD and HFD fed mice. While at the higher doses of 1 and 0.2 mg/kg bw, and the lowest dose of 0.008 mg/kg bw, the number of pAKT-immunoreactive cells within the ARC were not significantly different between LFD and HFD, HFD led to a marked impairment in the insulin response at 0.04 mg/kg bw relative to mice on LFD. These results suggest that the transport mechanism of insulin into the brain may follow a linear relationship. Furthermore, HFD-induced central insulin resistance within the ARC appears only to be prevalent when insulin is administered at relatively low doses.

M29. The Role of the Cystine/Glutamate Antiporter in Glutamate Metabolism in the Mouse Retina

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The Cystine/Glutamate Antiporter (CGAP) facilitates the uptake of extracellular cystine and export of intracellular glutamate. CGAP has been localised to the outer plexiform layer (OPL) of the rat retina[1], indicating that CGAP may play a role in glutamate neurotransmission and signalling.

The generation of a CGAP knockout (KO) mouse revealed that KO mice develop retinal spots at earlier ages than wild-type (WT) mice, reminiscent of drusen deposits seen in human age-related retinal disorders. Since drusen are associated with accumulation of metabolic waste, we hypothesise that CGAP may play a role in glutamate metabolism, and that removal of CGAP may lead to the accumulation of metabolic waste, formation of drusen-like spots, and advanced retinal aging.

To test this, retinas from 6 week and 9 month old WT and KO mice were collected, and lactate dehydrogenase (LDH) activity, since lactate is the primary product of retinal glucose metabolism, and ATP concentrations were measured. Glutamate/glutamine levels were also measured using silver-intensified immunogold labelling.

At 6 weeks of age, KO retinas exhibited a significant decrease in LDH activity compared to age-matched WT retinas, but not at 9 months, when drusen-like deposits were observed. Interestingly, no differences in ATP levels were observed between genotype at either age. At both ages, significant increases in glutamate immunoreactivity were observed in the OPL and photoreceptors, and a significant decrease in glutamine immunoreactivity was observed in the Müller cells, of the KO retinas compared to age-matched WT retinas. These results suggest that CGAP removal disrupts lactate metabolism, and alters the glutamate-glutamine balance in the retina, well before the appearance of deposits. This indicates that metabolic dysfunction may increase the susceptibility of the retina to drusen formation in later life. Further research will increase our understanding of retinal disorders, and will assist in the development of novel treatments.

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M30. The inotropic response to prostaglandin F2α in a rat model of right ventricular hypertrophy

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Right heart disease arises as a result of increased right ventricular (RV) afterload. Over time, increased workload leads to hypertrophy of the RV wall, which later progresses to failure. Heart failure is characterized by an inability to generate sufficient force to perfuse the body. Previous findings have shown prostaglandin F2α (PGF2α) has a positively inotropic effect on rat trabeculae[1]. Our aim was to determine whether PGF2α could improve contractility in isolated hearts from a rat model of RV hypertrophy.

RV hypertrophy was induced in 300g male rats by injection of 60mgKg^{-1} of monocrotaline (MCT, n=10). Control (CON, n=11) rats were injected with saline. Four weeks post injection, *in vivo* electrocardiogram recordings were made prior to euthanasia. Hearts were removed and Langendorff-perfused in sinus rhythm. Measurement of left ventricular (LV) pressure and the electrocardiogram were made and the response to a single bolus of 1μM PGF2α and 0.1μM isoproterenol (ISO) was determined.

PGF2α increased LV pressure in CON and in 60% MCT hearts, with the peak response 60s post application. However, 40% of MCT hearts developed arrhythmias during the time of peak response. The time to reach peak pressure in response to PGF2α was 0.069 ± 0.004s in CON and 0.071 ± 0.005s in MCT. In contrast, the time to peak LV pressure in response to ISO was much shorter for both rat groups, although the % increase in response to ISO was reduced in MCT relative to CON.

In conclusion, we confirmed PGF2α was positively inotropic for healthy hearts, but generated arrhythmias in 40% of hearts with RV hypertrophy. The slower time to reach maximum response in comparison to β-adrenergic stimulation suggests a different signalling pathway is activated by PGF2α. However, it cannot be concluded that PGF2α is a suitable alternative to β-adrenergic stimulation for acute treatment of failing hearts.

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M31. Chronic prolactin administration increases kisspeptin expression in virgin mice

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Oxytocin is secreted from the posterior pituitary gland by hypothalamic neurones in the supraoptic and paraventricular nuclei (SON and PVN) and is required for normal parturition. Kisspeptin fibre density surrounding oxytocin neurones increases during pregnancy and we have previously demonstrated that kisspeptin excites oxytocin neurones only in late pregnancy. Kisspeptin and oxytocin neurons express prolactin receptors and placental lactogen, which acts on prolactin receptors, is elevated in late pregnancy. Thus, we hypothesised that prolactin receptor activation might increase kisspeptin fibre expression to excite oxytocin neurones in late pregnancy. Here, we determined the effect of prolonged prolactin infusion on kisspeptin and oxytocin neurones in virgin mice.

Following subcutaneous infusion of ovine prolactin (1500 µg/day at 1µl/hr for seven days) or vehicle (0.01M $NaHCO_3$), double-label immunohistochemistry (IHC) for kisspeptin and oxytocin was carried out. There was no significant difference in the mean number of kisspeptin-labelled cells in the hypothalamic periventricular nucleus (58.6 ± 23.7 vs 49.1 ± 11.7, P = 0.20) or in oxytocin-labelled cells in the PVN (139.9 ± 22.8 vs 138.1 ± 19.6, P = 0.47) or SON (48.8 ± 8.9 vs 52.1 ± 2.5, P=0.21). Prolactin administration increased kisspeptin fibre density in the SON and perinuclear zone (PNZ) surrounding the SON upon (both $P<0.0001$, Student's t-test), however the fibre density was not different between the two groups in the PVN.

Hence, chronic prolactin receptor activation increases kisspeptin fibre expression in the mouse hypothalamus.

M32. Identifying the role of connexin-43 gap junctions as intracellular transporter of microRNAs

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MicroRNAs (miRNA) are small non-coding gene products that regulate protein expression within cells[4]. Dysregulated expression of miRNA in cardiomyocytes is related to cardiac tissue pathology[2]. Targeting transport pathways of miRNA to restore their expression in cardiomyocytes has demonstrated subsequent recovery of the damaged cardiac tissue in mouse heart[5]. However, complications in non-cardiac tissue is also reported[5]. The current known major miRNA transport route is in membrane bound vesicles which have cell surface markers for all cell types and thus, lack specificity[4,5]. My project aims to find a cardiac specific transport route for miRNA. Connexin 43 (Cx43) protein forms gap junctions between cardiomyocytes and, in low Ca^{2+}, allows movement of small molecules directly from cytoplasm of one cardiomyocyte to another[1]. Thus, this project investigates the permeability of Cx43 to miRNA.

A single cell model of *Xenopus laevis* oocytes, injected with human Cx43 mRNA, was used to solely characterise functionality of Cx43, without interference from vesicular transport pathways seen in cardiomyocytes. Expression and function of the protein was determined using *in vitro* electrophysiology. Cx43 expressing oocyte (n=7) showed a bigger change in current of 1μA upon removal of extracellular Ca^{2+} versus 0.2μA current change observed in non-Cx43 expressing oocytes (n=6). Consistent with previous studies, current for Cx43 expressing oocytes was reduced on re-application of Ca^{2+} or Lanthanum (Connexin blocker)[3]. These oocytes are being injected with miRNA and their bath solutions collected for PCR analysis to quantify miRNA transported out in presence of low Ca^{2+} or in presence of Lanthanum. This transport route will similarly be tested in cardiomyocytes using AC-16 cell line derived from human heart ventricles.

Connexin proteins and miRNAs are both expressed in tissue specific manner[1,2]. Therefore, targeting Cx43 mediated miRNA transport route would potentially minimise complications in non-cardiac tissue and regulate miRNA expression to treat cardiac tissue pathology.

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M33. Carotid chemoreceptor stimulation increases coronary blood flow in heart failure

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The carotid bodies (CBs) are a pair of chemoreceptor organs located near the bifurcation of the common carotid arteries. Activation of the CBs elicits a powerful reflex response which increases sympathetic nerve activity and arterial pressure. However, it is unclear if CB activation causes an increase in sympathetic nerve activity specifically to the heart and whether this alters coronary blood flow. We, therefore, investigated a group of control sheep and sheep with heart failure (HF). Experiments were conducted in conscious, adult female Romney sheep (n=5 in each group). Mean arterial pressure (MAP), coronary blood flow (CoBF) and calculated coronary vascular conductance (CVC) were recorded following CB stimulation with intracarotid potassium cyanide (KCN; 30µg/kg). To determine if any changes were neurally mediated, the response was repeated after muscarinic receptor blockade with Atropine sulfate (8 mg bolus followed by 24 mg/h infusion for 30 minutes).
In control animals, activation of the CBs increased MAP (115±1%) and CoBF (121±3%) but CVC was unaltered suggesting the increase in CoBF was pressure mediated. During HF, CB stimulation increased MAP (108±2%), CoBF (128±6%), and CVC (118±5%). The MAP increase was attenuated after atropine infusion in both animal groups. However, the CoBF and CVC responses to KCN were unchanged following atropine infusion.
These results suggest that CB activation causes an increase in CoBF that is pressure mediated in the control group but partly driven by coronary vasodilation in the HF group. This vasodilation in the HF group is not due to a cholinergic mechanism. Overall, activation of the CB leads to coronary vasodilation in HF which may maintain blood supply to the heart.

M34. Epithelial sodium channel in human arteries –an emerging player for blood pressure regulation

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The epithelial sodium channel (ENaC), formed by α, β, γ and δ subunits, plays a key role in blood pressure regulation via its activity in the kidney. Recent evidence indicates expression of ENaC in vascular cells and with a role in the regulation of vascular tone and thus blood pressure. Further evidence from animal models suggests that increased expression contributes to hypertension. However, it is currently unknown if ENaC is expressed in human arteries and if it contributes to hypertension. In this study we aimed to characterise the expression of ENaC subunits in human arteries. Further we aim to compare the expression in arteries from both normotensive and hypertensive patients.

Internal mammary arteries from patients undergoing coronary artery bypass graft (CABG) surgery were collected through the HeartOtago network. ENaC subunits mRNA and protein expression analyses were performed with qPCR and western blot respectively.

All four ENaC subunits were detected at mRNA and protein level in human internal mammary artery. The mRNA expression of δ subunit was significantly higher than α, β and γ ENaC (P=0.0074; P=0.0085; P=0.0081 respectively). In arteries from hypertensive patients δ ENaC mRNA expression was significantly decreased compared to the normotensive patients (P=0.0327; n=6). However, there was no significant difference in α, β and γ ENaC mRNA in hypertensive arteries compared to normotensive arteries (P=0.2744; P=0.2939; P=0.5857 respectively; n=6).

There was a downregulation of δ ENaC mRNA in internal mammary arteries of hypertensive patients suggesting its possible association in pathogenesis of human hypertension, however, an increase in number of arteries analyzed is required to confirm the association of vascular ENaC with hypertension in humans.

M35. Alpha-Melanocyte-Stimulating Hormone Switches from Inhibition to Excitation of Oxytocin Neurons in Lactation

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Magnocellular neurons in the hypothalamic supraoptic nucleus release oxytocin directly into peripheral circulation to stimulate uterine contraction during parturition and milk ejection during suckling in lactation. In preparation for parturition and lactation, oxytocin neurons undergo changes that increase their excitability. Alpha-melanocyte stimulating hormone (α-MSH) is best known for mediating the satiety actions of the hormone, leptin. However, intra-supraoptic nucleus administration of α-MSH inhibits oxytocin neuronal firing in non-pregnant rats (Sabatier *et al.*, 2003). The inhibitory effect of α-MSH reduced in mid-pregnancy (13 days' post fertilization) (Ladyman et al., 2016) but the responses were highly variable, with some neurons showing inhibition while others showed excitation, suggesting this might be the beginning of a transition from inhibition to excitation in preparation for parturition and lactation, when suppression of oxytocin neuron activity would be counter-productive for reproductive success. Here, we used in vivo electrophysiology from virgin, mid-pregnant and lactating rats to determine the effects of microdialysis administration of α-MSH into the supraoptic nucleus on oxytocin neuron firing rate. Consistent with previous reports, α-MSH inhibited oxytocin neurons in virgin rats but had no overall effect on firing rate in mid-pregnancy. Remarkably, α-MSH excited all oxytocin neurons recorded from lactating rats to date (n=4). Hence, it appears The switch of α-MSH switches from inhibition to excitation of oxytocin neurons in lactation.

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Sabatier N, Caquineau C, Dayanithi G, Bull P, Douglas AJ, Guan XM, Jiang M, Van der Ploeg L & Leng G. (2003). A*lpha-melanocyte-stimulating hormone stimulates oxytocin release from the dendrites of hypothalamic neurons while inhibiting oxytocin release from their terminals in the neurohypophysis*. *J Neurosci* **23,** 10351-10358.

M36. Central Regulation of the Diabetic Heart

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Cardiac autonomic dysfunction is a common and clinically relevant pathology of type 2 diabetes mellitus (DM). We have shown that efferent cardiac sympathetic nerve activity (CSNA) is increased in DM. However, the central pathological mechanisms underlying this increased CSNA in DM remain unknown. The hypothalamic paraventricular nucleus (PVN) comprises oxytocin (OT) and vasopressin (VP)-expressing pre-sympathetic neurons that project to the rostral ventrolateral medulla (RVLM), the final integrative site for sympathetic outflow from the brain. We hypothesised that in DM, there would be greater activation of noradrenergic neurons in the RVLM, and of OT and VP neurons in the PVN.

Dual-label immunohistochemistry for ΔFosB (chronic neuronal activation marker) and tyrosine hydroxylase (TH, a noradrenergic neuronal marker) in the RVLM was performed in 20-week old male DM (*n*=10) and non-diabetic (nDM, *n*=8) Zucker Diabetic Fatty rats. ΔFosB and the neuropeptides OT and VP were labelled in the PVN (ΔFosB/OT and ΔFosB/VP, respectively).

In DM rats, more $\Delta FosB^+$ cells were observed in the bilateral RVLM (DM 23±1 vs. nDM 16±2 cells, *p<0.01*) and PVN (DM 50±4 vs. nDM 36±4 cells, *p<0.05*). In the RVLM of DM rats, there were more $\Delta FosB^+/TH^+$ cells (DM 9±1 vs. nDM 3±0 cells, *p<0.001*) and a greater percentage of TH cells also expressed ΔFosB (DM 28±3% vs. nDM 10±1%, *p<0.001*). There were no between-group differences in the total number of cells co-expressing ΔFosB and OT ($\Delta FosB^+/OT^+$) or ΔFosB and VP ($\Delta FosB^+/VP^+$) in the PVN.

In conclusion, increased activation of noradrenergic neurons in the RVLM of DM rats might underlie the increased CSNA in DM. The greater neuronal activation observed in the PVN of DM rats cannot be attributed to differential activation of OT- or VP-expressing neurons. Further studies are needed to elucidate the phenotypes of neurons projecting to, and influencing the activation patterns of, the RVLM in DM.

M37. α-adrenergic control of the diabetic heart

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Patients with type 2 Diabetes Mellitus (DM) have a two-fold increase in cardiovascular disease, greater risk of surgical complications & require greater doses of catecholamines to avoid anaesthesia-induced hypotension during surgery. Reduced β-adrenoceptor (β-ARs) responsiveness of the diabetic heart has been observed, but the α-AR responsiveness of the diabetic heart is unknown. Unpublished data in the Lamberts lab showed that non-specific α-AR stimulation leads to significant reduction in functional cardiac parameters in diabetic rats in vivo. The aim of this study was to further understand the functional importance of α-AR stimulation in the diabetic heart.

Hearts of male Zucker Diabetic Fatty Rats aged 21 weeks (DM n= 6 and non-DM n=6) were isolated and perfused according to Langendorff. The maximum rate of contraction (dP/dt_{max}) was recorded using a pressure balloon transducer inserted into the left ventricle. Incremental dose curves of dobutamine (DOB; α- and β-ARs agonist, 10^{-10} to 10^{-6} mol/L) under phentolamine (PHEN; α-AR antagonist, 10^{-5} mol/L) or control settings were performed.

At baseline the dP/dt_{max} was lower in the hearts of the DM compared to the non-DM animals. At the highest DOB concentration the dP/dt_{max} was significantly higher in the DM hearts (Non-DM: 2785±411 vs. DM: 4666±635 mmHg/s; $P<0.05$; n=5) with no differences between the groups at lower DOB concentrations. Furthermore, α-AR blockade with PHEN did not affect dP/dt_{max} in the non-DM hearts (Non-DM: DOB 3056±431 vs. DOB+PHEN 4199±823 mmHg/s; $P>0.05$; n=6), whilst the dP/dt_{max} was reduced under α-AR blockade (DM: DOB 4666±635 vs. NDM: DOB+PHEN 2684±476 mmHg/s; $P<0.01$; n=5).

Diabetic hearts have an increased responsiveness to humoral adrenergic stimulation, which seems at least partly caused by an augmented by α-AR activity. Increased α-AR activity in the diabetic heart might compensate for reduced β-AR responsiveness.

M38. Comparing continuous and fluctuating high glucose levels on AC16 cardiomyocytes

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There is a known association between cardiovascular disease and diabetes and many studies have sought to gain insight into the effects of high glucose (hyperglycemia) on the heart[1]. *In vitro* research into the molecular mechanisms often exposes cultured cardiomyocytes to constant high glucose for a longer period, as a proxy for clinical hyperglycaemia[2]. However, blood glucose levels fluctuate between pre- and post-prandial levels[3, 4] and physiologically cardiomyocytes are not constantly exposed to high glucose levels. Consequently, the damaging effects of hyperglycemia applied in *in vitro* studies may be overestimating the effects of (patho-) physiological fluctuating high blood glucose levels.

The aim of the proposed study is therefore to compare the effects of constant and fluctuating high glucose concentrations on *in vitro* cultured cardiomyocytes. We hypothesise that the damaging effects of exposure to high glucose concentrations will be less during fluctuating compared to constant glucose levels.

We will use a human-ventricular cardiomyocyte cell line (AC16 cells). A fluctuating *in vitro* model will be designed, where glucose levels fluctuate every six hours for a 72-hour period. AC16 cells will be randomly assigned to a fluctuating or constant group. We will determine expression levels of microRNA-1, identified as an important regulator of cardiac apoptosis and hypertrophy, and of microRNA-34a, which is involved in regulating cardiac aging and senescence. We will also measure expression levels of GLUT4, an important glucose transporter in cardiomyocytes, and measure intracellular glucose concentration and caspase-3 levels , in the fluctuating and constant models, to identify any significant differences in the damaging effects of high glucose between both the models.

Successful establishment of the best *in vitro* model to mimic hyperglycaemia will provide a valuable tool and understanding for future research into the effects of hyperglycemia on the heart.

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M39. CaMKII Inhibition as a Novel Target to Control the Early Progression of Atherosclerosis

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Atherosclerosis is the major cardiovascular disease characterised by the formation of lipid-laden lesions within the inner layer of the arterial wall. Current therapies target at lowering LDL-cholesterol (LDL-C), however, even when the LDL-C target is met, there remains a residual risk for a cardiovascular event. This suggests additional therapies targeting other pathways need to be developed.

Reactive oxygen species (ROS) are highly damaging molecules that promote inflammation. It is now well recognised that sub-acute, chronic inflammation drives many of the pathogenic mechanisms underlying atherosclerotic lesion development. Calcium calmodulin-dependent protein kinase II (CaMKII) phosphorylates a number of cellular proteins, in particular, those involved in Ca^{2+} and ROS signalling such as endothelial nitric oxide synthase (eNOS). Under physiological conditions CaMKII is only briefly active through an autophosphorylation/dephosphorylation mechanism driven by calcium events. However, under conditions of elevated oxidative stress CaMKII becomes oxidised, rendering it persistently active. The role chronically active CaMKII plays throughout the progression of atherosclerosis is yet to be established.

Apolipoprotein E knockout ($ApoE^{-/-}$) mice were administered the CaMKII inhibitor KN-93 or control compound KN-92 every second day from 16- to 20-weeks of age. The brachiocephalic artery was dissected and a serial set of sections obtained for plaque analysis and immunohistochemistry. Systemic CaMKII inhibition led to a 10.9% reduction in foam cell lesion size in the brachiocephalic artery at 20-weeks ($P<0.05$). Furthermore, the reduced lesion size was associated with a significant decrease in phosphorylated and oxidised CaMKII, as well as the superoxide producing phosphorylated eNOS (P^{1177}-eNOS).

This study is the first to identify chronically active forms of CaMKII within atherosclerotic lesions. Collectively, these findings have implicated a potential mechanism contributing to atherosclerosis and spotlight post-translational modifications of CaMKII as a novel target for controlling the progression of early foam cell lesion development.

M40. Role of arcuate nucleus AgRP/NPY neurons in LH secretion and fertility

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Homeostatic processes like metabolism and reproduction are tightly regulated by neural circuits. Any disruption in energy balance affects fertility, for example, anorexic women suffer from menstrual dysfunction and subfertility. Agouti related peptide/ Neuropeptide Y (AgRP/NPY) expressing neurons in the arcuate nucleus (ARN) of the hypothalamus are known to play an important role in feeding and energy homeostasis. NPY has also been implicated to play a role in fertility, however its role remains controversial. We investigated the role of ARN AgRP/NPY neurons in the gonadotropin releasing hormone (GnRH) neuronal network regulating lutenising hormone (LH) secretion and fertility.

We used the stimulatory DREADD in AgRP-cre mice to activate ARN AgRP/NPY neurons and measured pulsatile LH secretion by ELISA, as a readout of GnRH neuronal activity. We found that activation of AgRP/NPY neurons by injection of DREADD ligand (clozapine-N-oxide), decreased post-castration LH pulse frequency, which demonstrates a role of AgRP/NPY neurons in slowing of GnRH pulse generation.

These results led us to investigate AgRP/NPY neuron activation in a prenatally androgenized model of PCOS, known to exhibit high LH pulsatility. AgRP/NPY neuron activation in these mice also decreased LH pulsatility, indicating a potential therapeutic target.

Furthermore, we found that optogenetic stimulation of only AgRP/NPY terminals surrounding the GnRH neurons at the rostral preoptic area decreased LH pulse frequency, suggesting a functional direct role of AgRP/NPY neurons in the GnRH neuronal network.

Overall, our findings clearly identify a key role for AgRP/NPY neurons in LH secretion and fertility, thereby expanding its importance beyond its well-known role in energy homeostasis.

M41 Transient loss of very low and low frequency fetal heart rate variability after severe asphyxia in near-term fetal sheep

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Asphyxia is a major cause of neurodevelopmental disability, but many infants still do not benefit from therapeutic hypothermia, likely because injury often evolves before birth. We evaluated the utility of changes in fetal heart rate variability (FHRV) after asphyxia as a biomarker of the stage and severity of evolving injury.

Chronically instrumented fetal sheep at 0.85 gestational age underwent either umbilical cord occlusion for 10 to 19 minutes (UCO, n=22) or sham UCO (n=5). Fetuses exposed to UCO were divided into those that developed status epilepticus during recovery (severe asphyxia, n=8), those with only discrete seizures (moderate asphyxia, n=8), and those without seizures (mild asphyxia, n=6). FHR and FHRV indices were assessed until 72 hours after UCO.

After UCO FHRV power was highly suppressed. High frequency power recovered rapidly in all groups, whereas recovery of very low and low frequency FHRV was related to severity; the mild group returned to control values by 3 h, moderate by 6 h and severe by 12 h. Conversely, sample entropy was markedly elevated after UCO ($p<0.05$) and showed a relationship between severity of asphyxia and timing of recovery. From 18-72h severe asphyxia was associated with secondary suppression of low and high frequency FHRV ($p<0.05$).

This study confirms that suppression of heart rate variability is consistently associated with acute hypoxia-ischemia, but does not indicate the severity of injury within the first 3 h. The striking and persistent increase in sample entropy after severe asphyxia suggests chaotic regulation of heart rate that may be related to brainstem injury.

M42. Vasopressin is an early biomarker of neural injury after hypoxia-ischemia in near-term fetal sheep

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Hypoxia-ischemia around the time of birth is a major cause of neonatal death and neurodevelopmental disability among survivors. The risk of death and disability is significantly reduced by treatment with therapeutic hypothermia within 6h of hypoxia-ischemia, but we unfortunately lack sensitive biomarkers to select high risk babies in a timely manner. The vasoactive hormones angiotensin II and vasopressin are known to be released during hypoxia-ischemia to promote vasoconstriction. It is unknown whether plasma levels of these hormones may be appropriate biomarkers for evolving brain injury after hypoxia-ischemia.

Near-term fetal sheep (0.85 gestation) were surgically instrumented and underwent sham HI (n=5) or HI (complete umbilical cord occlusion until mean arterial pressure fell below 8mmHg, n=15). Fetuses were studied for a further 3d and were retrospectively grouped based on the severity of histological neural injury (moderate, n=8; severe, n=7).

8/15 fetuses developed discrete seizures during recovery and a pattern of moderate injury (severe hippocampal injury but mild basal ganglia injury). 7/15 fetuses developed status epilepticus and a pattern of severe injury (severe hippocampal and severe basal ganglia injury). Vasopressin and angiotensin II levels were significantly elevated during occlusion in both groups ($p<0.05$). Angiotensin II levels returned to sham levels by 1h after occlusion in both groups. Vasopressin levels remained elevated in both groups compared to shams until 24h after occlusion ($p<0.05$). The severe group showed a further marked increase in vasopressin levels after occlusion and showed significantly higher levels than the moderate group from 1-3h after occlusion.

We conclude that vasopressin is released after hypoxia-ischemia in association with the severity of brain injury, and remain elevated for at least 6-24h. Plasma levels of vasopressin may therefore provide an early biomarker and help guide the timely implementation of cerebral hypothermia within the therapeutic window.

M43. Fetal heart rate variability: a biomarker for evolving fetal hypoxic-ischaemic brain injury

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Many cases of cerebral palsy after preterm birth are associated with perinatal hypoxia-ischaemia (HI), but it is often unclearly when injury occurred. We assessed the utility of the gold standard obstetric measures of fetal heart rate (FHR) and FHR variability (FHRV) to predict evolving neural injury *in utero*.

Preterm fetal sheep (0.7 gestation) were surgically instrumented and 4-5 days post-surgery underwent sham HI (n=8) or HI (25 min of umbilical cord occlusion) (n=8), with 21 days post-HI monitoring of FHR, FHRV and electroencephalographic (EEG) activity. Very low frequency (VLF), low frequency (LF) and high frequency (HF) FHRV power were calculated. Sample entropy (SampEn)[1] was calculated as a measure of disorder within FHRV. The laboratory light phase was 06.00-18.00hrs.

HI was associated with transient suppression of VLF (0-8h), LF (0-3h) and HF (0-1h) power, followed by recovery near-sham values, but a striking secondary reduction from ~18-72h. Reductions were associated with EEG suppression; at least for VLF, the secondary increase corresponded with secondary fetal seizures. In contrast, SampEN was markedly increased from 0–48h post-HI ($P<0.05$), and then partially resolved to near-sham values. EEG power progressively recovered from 24 to 72 h, but remained significantly lower than sham values ($P<0.05$). Diurnal rhythms were initially lost, but returned from 72 h onwards. Intriguingly, the rhythms in VLF, LF and HF became markedly exaggerated after HI, with a significantly greater diurnal fall between 06.00-12.00 h ($P<0.05$). SampEn gradually increased in sham controls with age, but not in HI fetuses, whereas HI was associated with a phase shift of the diurnal rhythm, from a primarily daytime increase to a nocturnal increase and late diurnal fall.

FHRV indices reflect evolving preterm neural injury. Their diurnal patterns may be particularly useful for assessing long-term recovery after HI, both ante- and postnatally.

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M44. Unseen to be seen: A hidden microenvironment regulates ovarian cancer cell behaviour.

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An extracellular tumour-promoting microenvironment is widely accepted to be crucial in tumour initiation and disease progression. Tumour-associated stroma, tumorigenic signalling molecules and regulated transmembrane receptor-ligand interactions are considered to be predominant factors of the microenvironment involved in tumour development. Initially signalling cues sensed by transmembrane receptors like integrin are transmitted mechanically to intracellular partners including the focal adhesion system and the Rho/ROCK pathway. However, the biophysical environment that initiates biological and chemical signalling cues provided by extracellular proteins have been minimally investigated. Consequently, it is still unclear whether the biophysical environment has potential to modulate signalling cues that could modulate cell sensing machinery at the early stages of tumour development and growth.

Here we investigated cell responses to various biophysical microenvironments for ovarian cancer cell lines, using bioimprinted substrate and disrupted collagen culture models. The bioimprint model consists of a polystyrene substrate with either concave or convex cell-like features, providing physical cues distinct from biological cues. In the disrupted collagen culture model, we distort the collagen architecture using collagenase MMP1, providing disoriented spatial collagen architecture for cell culture. We found in the bioimprint model that ovarian cancer cells exhibited transformed morphology compared to cells on flat substrates and increased growth along with the observed upregulated MAP kinase Erk. The bioimprinted biophysical environment was also shown to regulate expression and activation of signalling proteins integrin β1, FAK, Rho and Erk, which is suggestive of focal adhesion and the Rho/ROCK pathway being associated with cellular responses to biomechanical cues as distinct from the chemical and biological factors. We found that also disruption of collagen architecture of the cell environment influenced cell growth. Moreover, it was demonstrated that modulation of collagen architecture affected signalling pathways and modified chemoresponses. In the modified biophysical environments ovarian cancer cells exhibited less sensitivity to Paclitaxel and Carboplatin. We showed inhibition of Src but not of FAK and Rho inhibited the cell growth that was stimulated by bioimprinted substrate and patent collagen architecture.

We revealed that biophysical microenvironment could alter cancer cell behaviour and chemoresponse through mechano-sensing machinery. The study provides an expanded understanding of underlying mechanisms and suggests possible approaches for improved disease management.

M45. Lens channels regulate intracellular hydrostatic pressure

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The transport of water through mouse lens gap junction channels generates a hydrostatic pressure gradient, which varies from 0 mmHg in the surface to ~340 mmHg in the centre. This pressure gradient has been found to be regualted by a feedback control system, which relies on the modulation of mechano-sensivtive TRPV channels to sense either negative or postive pressure changes. The lens is connected to the ciliary muscle by its surrounding fibrous zonules. We hypothsize that contraction or relaxation of the ciliary muscles will through the zonules alter the mechanical tension applied to the lens and via activation of TRPV channels modulate the lens hydrostatic pressure gradient. To determine if altering the mechanical tension applied to the lens via the ciliary muscle regulates the lens intracellular hydrostatic pressures, wild-type C57 mouse lenses were exposed to either 0.1% tropicamide (a muscarinic anatagonist) or 0.2% pilocarpine (a cholinergic agonist) for 2 hours. Changes in lens intracellular hydrostatic pressures were continuously examined using a microelectrode/pico-injector based system. We found that tropicamide induced a decrease in lens surface pressure that was dependent on intact zonules and could be blocked by the TRPV4 inhibitor HC-067047. Pilocarpine caused an increase in lens surface pressure that was dependent on intact zonules and could be blocked by either inhibition of TRPV1 by A889435, or genetic deletion of the p110 catalytic subunit of PI3K. These results show that TRPV1 and 4 mediated a dual feedback system that maintains lens hydrostatic pressure which is also regulated by the mechanical tension applied to the lens via the ciliary muscle. Modulations of the pressure inside the lens could influence its water to protein ratio, gradient to refractive index, and therefore the overall visual acuity.

M46. Role of CaMKII and Alpha-Adrenergic Stimulation in Diabetic Heart Function

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Calcium/calmodulin-dependent kinase II (CaMKII) is a major calcium-handling protein modulator in the heart. Understanding its role in its entirety can help elucidate the pathophysiology behind several cardiovascular diseases, such as diabetic cardiomyopathy. My project focuses on diabetic cardiomyopathy and the role of alpha-adrenergic stimulation and CaMKII in cardiovascular dysfunction. Isolated rat hearts (diabetic or non-diabetic) were stimulated with an alpha-adrenergic agonist in the presence and absence of CaMKII inhibitors followed by measurement of heart rate and contractility. The diabetic rat hearts had lower heart rate and impaired contractility compared to the non-diabetic rat hearts at baseline. When stimulated with the alpha-adrenergic agonist, the diabetic rat hearts had a significant reduction in diastolic duration, a response that was lost when CaMKII was inhibited. The diabetic rat hearts also showed an increased contractility when stimulated with the alpha-adrenergic agonist, a response that was amplified when CaMKII was inhibited. My results suggest that the alpha-adrenergic response may be altered in the diabetic heart, as seen in their heart rate and contractile parameters, and that CaMKII may be playing a role in this altered response.

M47. Toward a cell-based model of metastatic prostate cancer: isolation and characterisation of metastatic prostate cancer cells derived from an orthotopically implanted SCID mouse model

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Introduction. Prostate cancer is a significant health concern, causing about 600 deaths per year in New Zealand. The majority of prostate cancer deaths are caused by highly aggressive castrate-resistant metastatic cancer cells. Metastatic cancer cells are phenotypically distinct from primary tumour cells and have been known to respond differentially to drug therapies. With very limited treatment options, there is considerable need to understand the differences between primary tumour cells and those that have metastasised to further the development of more effective drug therapies.

Aim. We aimed to develop metastatic prostate cancer cell lines from spontaneously metastatic cells arising from an orthotopic mouse model.

Methods. Luminescent PC3 (PC3-Luc) human androgen receptor negative (AR-) prostate cancer cells were injected into the prostate of SCID mice and allowed to incubate *in vivo* for 9 weeks. Primary tumours and lymph nodes were collected and cultured *in vitro*. Distal organs were collected for immunohistochemical metastatic analysis including the lungs, liver, heart, spleen and kidneys. Cultured cells were then assayed *in vitro*, using migration and cell viability assays, and results for primary tumour cells compared with metastatic tumour cells.

Results. Both primary and metastatic prostate cancer cells developed *in vivo* and were successfully isolated and re-cultured for *in vitro* analysis. Cultured metastatic cells isolated from sites distal to the primary tumour had distinct properties compared to primary tumour cells, including altered invasive properties.

Discussion. These results show that it is possible to re-culture AR- human prostate cancer cells developed in mice, both from primary and distal (metastatic) sites. The distinct properties of the metastatic cells are consistent with our aim to produce a novel model of metastatic prostate cancer. In addition, further studies comparing primary and metastatic cells *in vitro* could facilitate greater understanding of the mechanisms of prostate cancer metastasis.

MEDSCI PLENARY:
Challenges in interactive teaching and learning of physiology: how we must all adapt

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Creating interactive teaching and learning opportunities has become a mantra in contemporary curriculum design, and the classic didactic lecture has fallen into disfavour, despite the fact that there are situations in which lecture is the most appropriate medium for delivering content. Students and instructors alike may have difficulty adapting to the new paradigm, especially students who have become accustomed to taking notes in a lecture, then studying on their own. Student struggles with self-directed learning were first noticed during the introduction of problem-based learning at McMaster University but they still occur today. Some instructors also struggle with the move away from a teacher-centred classroom. This talk will examine some of the challenges of adapting to interactive classrooms and discuss findings from the educational psychology literature that can be applied to ease the process for instructors and students.

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1A.1 The impact of glucose and fructose exposure on cardiomyocyte glycogen accumulation and cell viability

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Diabetic cardiomyopathy is characterised by changes in metabolic processes, linked with impaired glucose signalling and increased glycogen storage. Emerging evidence suggests that cardiac fructose levels are elevated in diabetes but whether cardiac fructose exposure has direct effects on cardiac metabolism and cell viability is unknown. The aim of this study was to investigate the effects of high glucose and fructose exposure on cardiomyocyte growth, glycogen, and cell viability using pathophysiologically-relevant concentrations.

Neonatal rat ventricular myocytes (NRVMs) were isolated from 1-2 day old Sprague Dawley rats and maintained in growth media for 2 days. NRVMs were treated with high glucose (30mM) or fructose (1µM to 1mM range) for 24 hours prior to cell lysis. In addition, human pluripotent stem cell derived cardiomyocytes (iPSC-CMs) were treated with high glucose (25mM, 24 hours). Cardiomyocyte glycogen (amyloglucosidase enzymatic assay), protein concentration (Lowry assay) and cell viability (blinded analysis, phase microscopy) were measured.

High glucose increased cardiomyocyte glycogen in human iPSC-CMs (4.8 fold increase, $p<0.05$, n=3) and NRVMs (22% increase, $p<0.05$, n=21-22) following 24 hours exposure. Preliminary evidence of fructose-induced decreased cardiomyocyte glycogen was observed (13.8% decrease, $p<0.05$, n=6). Fructose had no effect on cardiomyocyte growth (ctrl: 1.131 +/- 0.01865 ug/ul vs. 1mM fructose: 1.156 +/- 0.02156 ug/ul protein) or viability (ctrl: 61.16 +/- 3.236% vs 1mM fructose: 60.34 +/- 1.034%).

This study for the first time demonstrates that high glucose and high fructose have opposing effects on cardiomyocyte glycogen content. These findings identify that fructose has direct actions on cardiomyocyte fuel storage *in vitro* and further investigation into the effects of fructose in the diabetic heart is now warranted.

1A.2 The evolution of neuroinflammation and myelination after hypoxia-ischaemia in preterm fetal sheep

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Many cases of cerebral palsy and impaired neurodevelopment are associated with hypoxia-ischaemia (HI) well before birth, during preterm development. HI leads to long-term impairment of myelination and neuroinflammation, both of which likely contribute to poor neural connectivity. We aimed to determine the evolution of neuroinflammation and myelination after an acute HI insult in preterm fetal sheep.
Chronically instrumented fetal sheep (0.7 gestation) underwent sham HI or HI induced by 25 min of umbilical cord occlusion. Fetal brains were processed for histology 3 days (n=9, sham n=12), 7 days (n=8, sham n=8) and 21 days (n=9, sham n=10) post-HI.
HI was associated with a significant increase in Iba-1 positive microglia at 3, and 21 days post-HI ($P<0.05$), but not 7 days. Increased iba-1 area fraction was observed at all post-HI time points ($p<0.05$). A reduction in brain weight (39.5 ±0.89 vs. 32.5 ±1.7 g, day 21), CNPase area fraction ($p<0.05$), and MBP area fraction ($p<0.05$) was observed post-HI vs. shams at all post-HI time points.
Severe HI was associated with reduced brain growth and myelination. A novel finding was the biphasic induction of microglia (resident neural macrophages). The first phase (day 3) is known to be pro-inflammatory, as part of cellular debris clearance. This resolved by day 7, with microglia returning to the ramified morphology. However, a secondary induction of microglia was seen on day 21. Microglia can have dual roles post-HI, depending on their phenotypic expression in response to environmental cues. Secondary M1 microglial activation might reflect chronic inflammation in response to impaired brain development and ongoing injury. This is consistent with reports of prolonged expression of pro-inflammatory cytokines in the blood of preterm newborns. Alternatively, an M2 phenotype expression is associated with repair, through release of protective anti-inflammatory and trophic factors. Phenotypic expression will be examined with further histology.

1A.3 Elucidating the Effect of Shear Stress on the Expression of ENaC in Endothelial Cells.

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The epithelial sodium channel (ENaC) is important for blood pressure regulation through it's function within the kidney and arteries[1]. ENaC has been reported in endothelial cells (ECs) as a prime candidate, facilitating arterial function[2]. ENaC is sensitive to mechanical forces such as laminar shear stress (LSS) and this may be important for endothelial-mediated-vascular function. In cardiovascular pathologies, LSS can become oscillatory (OSS), altering endothelial-mediated-vascular function, which may be associated with changes in ENaC[3].

This project aimed to elucidate the expression of ENaC in response to LSS and OSS within ECs. It was hypothesized that increases in LSS and/or a shift to OSS would lead to upregulation of ENaC expression.

Human ECs were cultured under LSS (n=6) and OSS (n=3) shear rates of 0, 5 & 10 dyns/cm^2, using the cell culture perfusion system. ENaC subunits (α, β, γ & δ) at mRNA and protein level were quantified using RT-qPCR, immunoblotting. Also the morphology of endothelial cells was assessed using ImageJ.

Results indicate αENaC mRNA was not altered under LSS/OSS; however, βENaC was significantly increased under 5 and 10dyn/cm^2 OSS (p=0.0001). Similarly, γENaC was significantly increased with 10dyn/cm^2 LSS (p=0.0020) while δENaC was decreased under 5dyns/cm^2 LSS (p=0.01). Immunoblotting confirmed α, β, γENaC protein expression in ECs. Endothelial long/short axis was increased with LSS (p=0.006 vs 0dyn/cm^2) and reduced with OSS (p=0.0004 vs LSS & 0dyn/cm^2). Taken together, the results indicate that LSS & OSS effect EC morphology and ENaC regulation. This demonstrates the significance of LSS for vascular function and may help to understand the onset of cardiovascular diseases.

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1A.4 Chronic prolactin administration increases kisspeptin expression in virgin mice

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Oxytocin is secreted from the posterior pituitary gland by hypothalamic neurones in the supraoptic and paraventricular nuclei (SON and PVN) and is required for normal parturition. Kisspeptin fibre density surrounding oxytocin neurones increases during pregnancy and we have previously demonstrated that kisspeptin excites oxytocin neurones only in late pregnancy. Kisspeptin and oxytocin neurons express prolactin receptors and placental lactogen, which acts on prolactin receptors, is elevated in late pregnancy. Thus, we hypothesised that prolactin receptor activation might increase kisspeptin fibre expression to excite oxytocin neurones in late pregnancy. Here, we determined the effect of prolonged prolactin infusion on kisspeptin and oxytocin neurones in virgin mice.

Following subcutaneous infusion of ovine prolactin (1500 µg/day at 1µl/hr for seven days) or vehicle (0.01M $NaHCO_3$), double-label immunohistochemistry (IHC) for kisspeptin and oxytocin was carried out. There was no significant difference in the mean number of kisspeptin-labelled cells in the hypothalamic periventricular nucleus (58.6 ± 23.7 vs 49.1 ± 11.7, P = 0.20) or in oxytocin-labelled cells in the PVN (139.9 ± 22.8 vs 138.1 ± 19.6, P = 0.47) or SON (48.8 ± 8.9 vs 52.1 ± 2.5, P=0.21). Prolactin administration increased kisspeptin fibre density in the SON and perinuclear zone (PNZ) surrounding the SON upon (both $P<0.0001$, Student's t-test), however the fibre density was not different between the two groups in the PVN.

Hence, chronic prolactin receptor activation increases kisspeptin fibre expression in the mouse hypothalamus.

1A.5 Cardiovascular and neural effects of delayed recombinant human erythropoietin treatment after asphyxia in preterm fetal sheep

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Background: Recombinant human erythropoietin (rEpo) has been shown to have neuroprotective potential in preclinical studies. Two large ongoing trials in preterm infants are now testing whether 1000 IU/Kg rEpo given every 48 hours is neuroprotective. However, the cardiovascular effects of repeated exposure to high dose rEpo in conjunction with perinatal insults such as asphyxia are not known. This study examined the cardiovascular and neural effects of a clinically relevant protocol of delayed administration of rEpo after asphyxia in preterm fetal sheep.
Methods: Chronically instrumented preterm (0.7 gestation) fetal sheep received sham asphyxia (n = 8) or asphyxia induced by complete umbilical cord occlusion for 25 minutes (n=14). Fetuses subjected to asphyxia received intravenous bolus injections of saline (n = 8) or 5000 IU rEpo (n = 8) at 6 hours after asphyxia, then every 48 hours for 5 days. Physiological recovery was monitored for seven days.
Results: rEpo treatment was associated with an increase in mean arterial pressure compared to asphyxia-saline between 24-48 hours ($P < 0.05$) after asphyxia. rEpo treated fetuses had higher carotid vascular resistance ($P < 0.05$) and lower carotid blood flow ($P < 0.05$) than the asphyxia-saline group from 9-72 hours after asphyxia. Electroencephalogram (EEG) power in both asphyxia groups was lower than sham-asphyxia group throughout recovery ($P < 0.05$). EEG power in asphyxia-rEpo group was lower than asphyxia-saline group from 48-168 hours after asphyxia ($P < 0.05$).
Conclusions: Delayed treatment with high dose rEpo boluses after asphyxia was associated with a prolonged reduction in cerebral perfusion and altered recovery of EEG power after asphyxia. Histological analysis is now needed to show whether these physiological changes altered the pattern of neural injury. These data support the need for further preclinical examination of different doses and treatment regimens of rEpo for the treatment of hypoxic-ischaemic encephalopathy.

1A.6 Optimisation of an Androgen Bioassay for Measurement of Clinically Relevant Samples – Breast Cancer as a Clinical Focus.

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Menopause is a high-risk factor for estrogen receptor positive (ER+) breast cancer. Adrenal-derived androgens may underpin this risk however little is known about underlying mechanisms. While analytical chemistry techniques measure the concentration of known androgens, net androgenicity of breast cancer patient serum may provide a more informative assessment of breast cancer risk since known and unknown androgens can be measured. Before such studies can be conducted, androgen receptor (AR) bioassays need to be validated as a tool to measure serum bioactivity. The aim of this study was to evaluate a yeast cell- and human embryonic kidney (HEK293) cell AR bioassay for the reliable measurement of serum AR bioactivity. AR bioassays comprise a cell stably co-transfected with an AR expression plasmid and an androgen sensitive reporter plasmid. Serum proteins such as albumin and steroid hormone binding globulin bind 98% of androgens and upon heat denaturation of the proteins, bound androgens are released. In the HEK293-AR bioassay, heating testosterone-spiked serum consistently increased AR bioactivity by up to 137%, whereas the yeast-AR bioassay produced inconsistent results. Expected sex differences in AR bioactivity of male and female serum were observed (average of >73,000 units in male serum versus <1000 units in female serum, $p<0.001$). Heating male and female serum augmented AR bioactivity up to 145% and 145,000%, respectively. Taken together, these results show that the HEK293-AR bioassay reliably outperforms the yeast-AR bioassay for measuring serum AR bioactivity and assay performance is further improved by heat treating serum. This study provides a platform for future endeavours to measure AR bioactivity in the serum of patients at risk of ER+ breast cancer relapse.

1A.7 The effect of ischemia and hypothermia on axonal and myelin integrity

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Hypoxic-ischemic encephalopathy is associated with a high risk of disability. Hypothermia treatment is partially effective, with many infants still suffering disabilities. Cerebral ischemia affects the integrity of myelin in the intragyral white matter in fetal sheep, but is restored with hypothermia. It is not known whether this disruption in myelination results from oligodendrocyte loss, or underlying axonal pathology. We aimed to assess the effect of global cerebral ischemia and hypothermia on myelin and axonal integrity in near-term fetal sheep.

Near-term fetal sheep were randomised to sham control (n=9), ischemia-normothermia (n=8), or ischemia-hypothermia (n=8). Ischemia groups underwent 30 minutes of carotid artery occlusion. Hypothermia was initiated at 3 hours after ischemia, continuing for 72 hours. Axons were immuno-labelled with SMI312 and myelin with MBP, in the intragyral white matter of the first parasagittal gyrus. SMI312 was co-labelled with GFAP for astrocytes and Iba1 for microglia. Axonal and myelin linearity was analysed with ImageJ plugin OrientationJ, and axonal morphology was assessed qualitatively.

Ischemia was associated with a trend towards a 7.1% reduction of myelin area fraction ($p=0.06$), a 13% reduction of axonal area fraction ($p<0.05$), and a 12% loss of myelin and axonal linearity compared to sham control ($p<0.05$). Myelin and axonal area fraction and linearity was restored to sham control levels with hypothermia ($p<0.05$). In sham controls, axons were dense and linear. After ischemia, they were finer, sparser, and had a spheroid morphology. Hypothermia improved this, but some abnormal axonal morphologies persisted. The loss of SMI312 staining was colocalised with increased GFAP and Iba1 staining.

Ischemia was associated with axonal and myelin structural abnormalities. Hypothermia restored axonal area fraction and linearity, but the abnormal axonal morphology was only partially attenuated. Axonal pathology may underlie abnormal myelination, which may contribute to motor deficits seen in infants, even among those treated with hypothermia.

1A.8 Endosomal SNX proteins are required for recycling of the epithelial sodium channel

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The epithelial sodium channel, (ENaC), absorbs Na^+ at the apical membrane of polarised epithelia, and is required for regulation of salt and water homeostasis. ENaC number at the cell surface is strictly controlled, and loss of this control can lead to disorders such as Liddle's Syndrome, a form of hypertension. Retromer is a conserved coat protein complex that localises to endosomes where it mediates protein recycling back to the plasma membrane. In this study we investigated whether retromer is needed for the recycling of ENaC, focusing specifically on the SNX (sorting nexin) heterodimer retromer sub-complex.

Fischer rat thyroid (FRT) and mouse cortical collecting duct (mCCD) epithelia were transiently transfected with siRNA targeting retromer-associated SNX mRNAs, and Western blot analysis showed that significant protein knockdown was obtained for SNX1, SNX2, SNX3 and SNX17. 'Ussing' chamber experiments were then conducted to measure changes in ENaC's amiloride-sensitive short circuit current (I_{sc}-Amil). A significant reduction in I_{sc}-Amil of ENaC was observed in both FRT and mCCD epithelia with a SNX1, SNX2, SNX1/SNX2, SNX3 or SNX17 knockdown, suggesting SNX proteins and therefore retromer are required for ENaC recycling.

The SNX1/SNX2 double knockdown (inhibiting SNX heterodimer formation) decreased ENaC I_{sc}-Amil to a similar value as the individual SNX1 and SNX2 knockdowns, highlighting potential involvement of other SNX proteins. SNX3 and SNX17 are able to form retromer independently of the SNX1/SNX2 heterodimer, and knockdown of these proteins also decreased ENaC I_{sc}-Amil. These results suggest that multiple SNX proteins can facilitate ENaC recycling and compensate for one another. Further investigation into ENaC interaction with SNX proteins and other retromer components is needed to further understand this pathway.

1B.1. Lactoferrin, an endogenous growth factor, immunomodulator and antimicrobial, is anabolic to bone

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Lactoferrin is an endogenous growth factor with multifunctional beneficial properties and therapeutic potential - an 80 kDa bilobal glycoprotein which belongs to the transferrin family. It is an abundant protein in milk and is also present in several other secreted bodily fluids, as well as in the secondary granules of neutrophils. The strong iron-binding properties of lactoferrin can locally create iron deficiency, so creating an important factor in host defence preventing microbes from forming biofilms. Because of the extensive health-promoting immunomodulating effects of lactoferrin, there is considerable interest in the use of bovine or human lactoferrin as a "protein nutraceutical" or as an orally active therapeutic protein. Lactoferrin receptors have been identified on the surface of various cells.

Our studies have centred on the protein's potent anabolic effect in bone and profound bone regenerative activity capacity in the field of tissue engineering. We have assessed the efficacy of local application of lactoferrin on bone regeneration using a preclinical critical-sized calvarial defect models in the rat where lactoferrin released from a collagen scaffold significantly increased bone regeneration.

The effect of lactoferrin in innovative bone regeneration studies has therapeutic potential to improve the poor clinical outcomes associated with bony non-union. Non-united bone fractures occur at an alarming rate due to increases in osteoporosis in the aging population. Furthermore, lactoferrin's anti-microbial and anti-inflammation properties, so modulating the immune response, are very positive properties for bone healing.

1B.2. Central prolactin action is required for maintaining lactational diestrus in mice

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In mammals, lactation is associated with a period of infertility, characterised by reduced pulsatile luteinizing hormone (LH) secretion, cessation of ovulation and loss of kisspeptin input to GnRH neurons. However, the mechanism underlying lactational infertility is currently unclear. The anterior pituitary hormone, prolactin, named for its role in milk production, is chronically elevated during lactation and in non-lactating females, hyperprolactinaemia has been shown to lead to infertility. We aim to test the hypothesis that elevated prolactin is required for the suppression of fertility during lactation. Specifically, we aimed to investigate whether prolactin action in the brain plays a key role in suppressing kisspeptin expression and pulsatile LH secretion during lactation. Mice with a conditional deletion of the prolactin receptor in forebrain neurons (*Prlr*$^{lox/lox}$/CKC-cre), were used to test if prolactin receptor-mediated signaling in neurons is required for lactational fertility. Our preliminary data indicated that all *Prlr*$^{lox/lox}$/CKC-cre mice resume estrus cyclicality early in lactation (lactation day 5-10) compared to control *Prlr*$^{lox/lox}$ mice (does not resume until after lactation day 21). LH pulsatility was examined in virgin and lactating mice by collecting frequent blood samples from the tail tip vein over 3 hours, and LH concentrations measured by ELISA. In the lactating mice, blood sampling was conducted in the *Prlr*$^{lox/lox}$/CKC-cre mice after the resumption of estrus cyclity, with blood samples collected from control *Prlr*$^{lox/lox}$ mice on the equivalent day of lactation (during lactation diestrus). Following collection of blood samples, mice were perfused for immunohistochemistry analysis of kisspeptin cell number in the hypothalamus. We predict that resumption of LH pulsatility will be seen in the *Prlr*$^{lox/lox}$/CKC-cre, and that the lactation-induced suppression of kisspeptin will be absent allowing for the early estrus cyclicity. These results would indicate that high levels of prolactin action in the brain during lactation is crucial for maintaining lactational anovulation.

1B.3. The role of RFRP neurons in puberty onset and depression

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RF-amide related-peptide (RFRP) neurons are thought to modulate reproductive function and stress responses1. Using transgenic mice which have stimulatory and inhibitory designer-receptors exclusively activated by designer-drugs (DREADDs) selectively expressed in RFRP neurons via a Cre-loxP system, we aim to explore the reproductive and behavioural effects of RFRP neurons non-invasively in vivo.
The role of RFRP neurons in puberty onset was investigated by chronically stimulating and inhibiting RFRP neurons through administration of CNO from post-natal days 26-31 (approximately 5mg/day p.o). Stimulation of RFRP neurons in male mice lead to delayed preputial separation (stimulatory mice: 31.7±0.8 days old vs control mice: 29.3±0.3 days old). In female mice, there was no difference in puberty onset. The role of RFRP neurons in anxiety-like and depression-like behaviours was investigated in 8-week-old male mice following acute administration of CNO (1 mg/kg s.c.). There were no changes in anxiety-like behaviours. There was, however, an increase in depression-like behaviour following stimulation of RFRP neurons. Stimulatory mice spent more time immobilised (66.5±4.1%) than control mice (38.4±6.9%) in the last 2 minutes of the forced-swim test. This finding indicates there may be a novel role for RFRP neurons in the control of depression-like behaviour in mice, and more behavioural testing will be conducted to further elucidate this role.
Elucidating the functions of murine RFRP neurons is an important step towards understanding their role and therapeutic potential in human infertility and mental illness.

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1B.4. Preoptic neurons are sufficient to mediate at least some of leptin's effects on fertility

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Leptin communicates information about metabolic status to the gonadotropin-releasing hormone (GnRH) neurons indirectly via afferent neuronal populations in the hypothalamus; a required relay for normal reproductive function[1]. Recent analysis of neurons located in the preoptic area of the hypothalamus suggests these may play a vital role in the rapid communication of leptin with the reproductive axis[2]. We aim to elucidate whether neurons located in the preoptic area are sufficient to mediate leptin signalling; permitting normal reproductive function in the absence of leptin signals from any other region.

Cre-loxP methodology was used to generate mice in which expression of leptin receptor (Lepr) was Cre-dependant. A proportion of these then underwent selective activation ('rescue') of Lepr only in the preoptic area. This occurred by administration of Cre DNA via an adeno-associated virus (AAV) in adulthood, enabling comparison of puberty onset and adult fertility between Lepr-rescue, Lepr-null and wild type (WT) mice (n=5-8 per group). The AAV treatment had no effect on body weight compared to obese Lepr-null mice ($p>0.05$). As expected, a significant difference was observed in the occurrence of puberty onset between WT and Lepr-null mice as only 14% of male and 0 female Lepr-null mice underwent puberty prior to 2 months of age ($P=0.002$). However, 2-4 weeks following AAV treatment, 60% of female and 100% of male Lepr-rescue had puberty compared to only 16% of female and 45% of male Lepr-null mice ($P=0.01$ and 0.19 for males and females respectively). Reproductive cycles were evident in 60% of Lepr-rescue and 0% of Lepr-null mice ($P=0.08$). Fecundity tests currently in progress.

These preliminary results suggest that preoptic neurons, at least in males, are sufficient mediators for much of the regulation of GnRH neurons by leptin. Understanding of the metabolic regulation of reproductive function will enable prevention and treatment of metabolic-associated infertility.

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1B.5. Investigating the projections of suprachiasmatic nucleus vasopressin neurons to preoptic kisspeptin neurons

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The activity of kisspeptin neurons in the rostral periventricular region of the third ventricle (RP3V) drives the surge of gonadotropin-releasing hormone that triggers ovulation. In female rodents, this preovulatory hormonal surge is dependent on circadian inputs from the suprachiasmatic nucleus (SCN). One potentially important input is that from SCN vasopressin (AVP)-expressing neurons to RP3V kisspeptin neurons. We used viral-mediated expression of a cre-dependent red fluorescent reporter to reveal the axonal projections of SCN AVP neurons to the RP3V, in mice expressing cre-recombinase in AVP-expressing neurons (AVP-IRES2-cre). Immunohistochemistry reveals a strong correlation between the level of reporter expression in the SCN and that of reporter-expressing fibres innervating the RP3V (n = 6, p = 0.007). In contrast, other AVP-expressing regions of the brain, the paraventricular and supraoptic nuclei do not appear to project to the RP3V. Quantification of the SCN AVP projections to the RP3V indicates that reporter-expressing fibres make close appositions with 54.1 ± 8.0% of kisspeptin neurons, suggesting putative synaptic inputs.

Functionally, this system was investigated by combining optogenetics and whole-cell patch-clamp electrophysiology in brain slices. AVP-IRES2-cre mice were crossed onto a mouse line expressing the green fluorescent protein in kisspeptin neurons. These were then injected with a cre-dependent blue-light sensitive protein, channelrhodopsin (ChR2). Activation of ChR2 with blue light faithfully drives action potential generation in SCN AVP neurons over 1 – 50 Hz (n = 7). As the SCN is predominantly a GABAergic nucleus, we examined whether blue-light activation of SCN AVP neuron projections would result in GABA release onto RP3V kisspeptin neurons. In the great majority of kisspeptin neurons (13 out of 14), however, no post-synaptic currents were recorded in response to blue-light stimulation. Thus, despite projecting to the RP3V, it appears that SCN AVP population may not communicate with kisspeptin neurons via GABA release.

1C.1 Estimating reference values of aortic pulse wave velocity for the New Zealand population

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Pulse wave velocity (PWV) is a gold-standard measure of arterial stiffness (AS) and has been acknowledged as an independent diagnostic marker of stroke and cardiovascular (CV) risk. New Zealand (NZ) has a high prevalence of people with cardiovascular disease (CVD) with ethnic disparities. However, the use of PWV for routine clinical assessment of CVD risk is not practiced due to a lack of reference values and official recommendations for the NZ population. In this research, aortic carotid-femoral PWV values were estimated (N=92/120) using Doppler ultrasound for a 'reference value population' (RVP, n=26) with CVD risk factors but free from diabetes, high cholesterol, any known heart disease or on medications for these conditions. Whereas, participants with normal BP without any CVD risk factors constituted the 'normal value population' (NPV, n=66). The screened participants were grouped by age (18-30, 30-60, > 60 years) and blood pressure (BP) (normal, elevated, stage 1, stage 2) categories. Peripheral and central systolic/diastolic BP, pulse rate, and augmentation index (AI) were measured by USCOM BP+ monitor.

The data collected so far shows that PWV shares a positive correlation with age (R^2=0.4) and blood pressure (R^2=0.2). The mean aortic PWV were significantly lower in the NVP (5.1 ± 0.92 m/s) compared to RVP (6.3 ± 1.26 m/s) ($p<0.001$). The mean PWV values for the three age categories were 4.8, 5.6, and 6.7 m/s respectively with higher values in the RVP. Effect of age, sex, body mass index (BMI), AI, mean BP, smoking, alcohol consumption, diabetes, dyslipidaemia, and hypertension on PWV was assessed. Multiple regression analysis showed a significant contribution to the prediction of the Mean PWV with age (β=0.5, p=<.001), and BMI (β=0.1, p=.03).

The preliminary results show PWV could have value in a proactive approach to CVD risk assessment that would help in attaining long-term community health goals of the NZ government. The final outcome will assist in establishing the normal and reference values of PWV in the NZ population.

1C.2. Biodegradable polymer blends/composites, with high performance characteristics, for packaging application

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There is an increasing awareness about the environmental pollution cause by the traditional polymers such as polyethylene, polypropylene, polystyrene, PET, PVC etc Together with the growing concern about limited petroleum reserves and government regulations have resulted in increased research activity on environment-friendly, bio-based, biodegradable polymers, for packaging application. Many biodegradable polymers, bio-based as well as petroleum-based, have been developed for packaging applications. However, their wide spread application is limited mainly because their inferior performance characteristics and due to high cost. Most of the commercially available biodegradable polymers does not have the desirable properties. Research on many polymer blends and composites have been reported but considerable knowledge-gap exists especially on barrier properties and biodegradability characteristics of many polymer blends under home composting (ambient temperature) and in seawater environments. Hence additional research work is needed to fill the knowledge- gaps, to improve the packaging material characteristics. The present project aims at development of flexible polymer films, with high performance characteristics, suitable for packaging application. This involves blending various commercially available biodegradable polymers, modification of polymer blends with the addition of biodegradable additives, followed by hot pressing to fabricate flexible polymer films and detailed performance characteristics. The present research output is expected to assist in expanding the usage of biodegradable polymers, reduction of environmental pollution and accelerate the progress towards a sustainable future.

1C.3. Effect of Positive Pressure Oscillations on Cultured Human Epithelial Cells

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Lung supportive devices (LSD) are widely used for respiratory ventilation and therapy to help providing breathing support for patients with various lung diseases including Obstructive Sleep Apnea. These devices deliver continuous air to the patient through a nasal or a facial mask. However, the use of these devices normally results in dryness in the upper airways. Various methods have been developed to improve the continuous positive pressure features using pressure oscillations (PO), which can reduce the patient requirements such as reduction in the titration pressure and humidification requirements.
Thus, the objective of this research is to investigate the differences between continuous positive pressure and positive pressure oscillation on the upper airways humidification. Human nasal (RPMI2650) and airway (Calu-3) epithelial cells grown in air-liquid interface (ALI) on permeable supports are used as a respiratory model. Trans-epithelial electrical resistance (TEER), apparent permeability (P_{app}) and mucus secretion rate are measured before and after each experiment to evaluate the cell layer integrity, permeability and functionality. Significant differences are found between the continuous positive pressure and positive pressure oscillation experiments.

1C.4. Vulnerability of follicular stem cells to sun exposure

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Previous studies have provided evidence which supports the idea that childhood is the most critical period of sun exposure for future melanoma development; more specifically, individuals who receive excessive sun exposure during their childhood tend to have a higher risk of developing melanoma in later life. The melanocyte stem cells in hair follicles (capable of developing into melanoma) are shallower in the skin before puberty, which is hypothesized as the possible cause of this phenomenon by receiving more ultraviolet energy. To investigate this, the Monte Carlo method of photon transport is used to determine the ultraviolet power delivered from the sun to the different depths of the follicular stem cells. The simulated result indicates that the melanocyte stem cells in the bulge regions of vellus hair follicles (major type of hair in children) receive significantly higher UV power than those in the terminal hair follicles (major type of hair in adults). Our results will help to create a better understanding of the vulnerability of children to sun exposure.

1C.5. Two-Wheeled Wheelchair Stability Control Using a Movable Mechanism

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Most conventional Robotic wheelchairs are four-wheeled which are statically stable. However, they suffer from low mobility. To tackle this problem, a two-wheeled wheelchair was designed. In this wheelchair, casters of four-wheeled wheelchair are removed. Without the support of casters, it becomes an inherently unstable system which requires a control system to stabilize it. Most stability controllers used for two-wheeled wheelchair rely on the torques applied to the wheels which cause linear displacement of the wheelchair. Also, when the two-wheeled wheelchair moves on uneven surfaces or with high acceleration, it is likely that the torque and power required from the motors becomes huge and exceeds the capacity of motors. Furthermore, undesirable linear displacement caused by the stability controller decreases safety and comfort of the rider. To solve this problem, we propose a novel method to keep the stability of a two-wheeled wheelchair. In this approach, a movable mechanism is added to the wheelchair to compensate the deviation of the wheelchair from its equilibrium position. The simulation results showed that the proposed method requires less torque to keep the system stable and causes much smaller displacement of the wheelchair than the conventional method.

1C.6. An investigation into the effects of pressure oscillations on airway smooth muscle in chronic asthma.

Roos K.L.T.

The hyperconstriction of airway smooth muscle (ASM) is the main driving mechanism during an asthmatic attack. The airway lumen is reduced, resistance to airflow increases, and normal breathing becomes more difficult. The tissue contraction can be temporarily relieved by using bronchodilator drugs which induce relaxation of the constricted airways. With one of the highest prevalence rates in the world, New Zealand's costs for asthma treatments total an estimated NZD$825 million per year.

While widely used in asthma therapies, pharmacological treatments vary in their effectiveness from one subject to another, as do the side effects of long-term usage. Studies have shown that application of mechanical oscillations which are equivalent to the physiological patterns of normal breathing and deep inspirations in healthy airways can induce airway relaxation. This type of relaxation response is not observed in asthmatics.

Utilizing length oscillations (arising from positive pressure) in association with breathing patterns provides non-pharmacological options for augmenting treatment of the ASM hyperconstriction which is present in many respiratory diseases such as asthma. There is currently little known about the effects of applying superimposed pressure oscillations in combination with breathing patterns to healthy and asthmatic airways during an asthmatic attack.

Results from *in vivo* studies of a chronic murine asthmatic model indicate that the use of superimposed pressure oscillations (SIPO) over normal breathing patterns facilitates relaxation during an induced asthmatic attack in healthy and asthmatic subjects. Oscillation patterns, physiological pressure equivalents, and their effects on key respiratory parameters are presented. Comparisons of healthy and asthmatic lung resistance (R_L) and dynamic compliance (C_{dyn}) values are used as assessments of the changes in airway responses to applied mechanical pressure oscillations. Additionally, a standard respiratory constant is used to normalize acute and chronic asthmatic models' data. Use of the constant assists in modeling the effects of SIPO by transforming R_L and C_{dyn} data into Work and Power equivalents for use in interpreting ASM mechanics.

1C.7. A Review of Different Methods for Skin Stretch Sensing

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Motion artefacts (MA) can lead to incorrect diagnosis of cardiovascular diseases: such noise either leads to false alarms in the automatic diagnosis systems or it leads to hide important information about the subject's health status (Fuad A, 2017). One of the causes of motion artefact in ECG signal is skin stretch during patients' daily activity, which is considered as the most common source of MA (S Ödman, 1982). A proper method to accurately measure the strain of skin is essential to significantly reduce MA. There have been various types of approaches to measure strain of skin in single or multiple directions. Based on their sensing stability, accuracy, sensitivity, hysteresis, comfortability, cost and feasibility, the comparative study further refines the project scope and provide a result of best suitable methods among different sensing mechanisms such as piezo-electric, piezo-resistive, capacitive, disconnection, cracking, quantum tunnelling, optical waveguide with Bragg reflection and RFID method.

Cracking mechanism is considered as one of the best sensing mechanism for skin strain for its high gauge factor, ease of fabrication and suitability for this project. Cracking mechanism along with capacitive and piezo-resistive mechanisms may be combined to design a sensor that simultaneously measures strain field for human skin.

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1C.8. Modelling Thermoregulation of The Human Body Upper Limb

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For the human body to function properly, the temperature of its vital organs must be maintained at approximately 37^{o}C at all times. A model for simulating thermoregulation of the upper limb in human body is presented. The aim of the study is to determine the temperature distribution and heat fluxes in the upper limb. This model includes upper limb muscles, the humerus, radius and ulna. Comparative models for human thermoregulation have been developed which included active and passive systems. These models assume a simple geometry e.g. a cylinder for the arm and a sphere for the head[1]. Thermal manikins have also been developed to obtain skin temperature although these models too have limitations. We use a FEM model for solving Bio-heat equation in an anatomically based geometry. To set the thermophysical properties correctly, we require an anatomically correct geometry as body shape and different tissues determine conduction, radiation and convection heat transfer.

Real geometry of the upper limb is used for our model and created using tetrahedral elements. The heterogeneity of the model is accounted for by assigning different thermophysical properties for different types of tissues (e.g. muscles and bones). Metabolic heat in muscles is modelled as a heat source and assumed to be at the constant basal value. The temperature distribution is then obtained by solving the governing equation using the Galerkin FEM. The results are presented and the model is verified against an analytical solution for the 1D heat equation. Further improvements include the coupling blood flow and the heat equation and implementing a variety of different boundary conditions.

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PLENARY: Effects of early programmed insulin resistance on adult cardiac function in rats

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Genetic predisposition to dyslipidaemia and hyperglycaemia lead to microangiopathy induced autoimmune disease in early life, which might predispose individuals to heart failure with preserved ejection fraction later in life. It is well established that diastolic dysfunction is one of the first diagnostic indicators of heart failure in advanced diabetes, when coronary and renal dysfunction play important roles in the pathophysiological changes in the myocardium. Interstitial fibrosis has also been considered the main factor contributing to the stiffening of the heart and diastolic dysfunction. However, it has been less clear what the impact of peripheral insulin resistance and moderately elevated glucose levels are on cardiac function in prediabetes. We utilised synchrotron microangiography and X-ray diffraction to investigate coronary microvessel and cardiac myofilament contributions to the early origins of diastolic dysfunction in the young Goto-Kakizaki rat. Further, cardiomyocyte passive tension recordings and phosphoproteomics are enabling us to understand the molecular basis of early posttranslational modifications of cardiac contractile proteins. We will show how the combination of programmed insulin resistance and factors such as salt-sensitivity and obesity can aggravate diastolic dysfunction and heart failure.

2A.1 Cardiac mechanoenergetics in right-ventricular failure induced by pulmonary arterial hypertension

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Pulmonary arterial hypertension (PAH) greatly increases the afterload on the right ventricle (RV), triggering RV hypertrophy, which progressively leads to RV failure. In contrast, the disease reduces the passive filling pressure of the left ventricle (LV), resulting in LV atrophy. We investigated whether these distinct structural and functional consequences to the ventricles affect their respective energy efficiencies. We studied trabeculae isolated from both ventricles of Wistar rats with monocrotaline-induced PAH and their respective Control groups. Trabeculae were mounted in a calorimeter at 37 °C. While contracting at 5 Hz, they were subjected to stress-length work-loops over a wide range of afterloads. They were subsequently required to undergo a series of isometric contractions at various muscle lengths. In both protocols, stress production, length change, and suprabasal heat output were simultaneously measured. We found that RV trabeculae from PAH rats generated higher activation heat, but developed normal active stress. Their peak external work output was lower due to reduced extent and velocity of shortening. Despite lower peak work output, suprabasal enthalpy was unaffected, thereby rendering suprabasal efficiency lower. Crossbridge efficiency, however, was unaffected. In contrast, LV trabeculae from PAH rats maintained normal mechanoenergetic performance. Pulmonary arterial hypertension reduces the suprabasal energy efficiency of hypertrophied right-ventricular tissues, as a consequence of the increased energy cost for Ca^{2+} cycling.

2A.2 Impaired Ca^{2+} handling precedes mitochondrial dysfunction in right ventricular hypertrophy

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Pulmonary hypertension causes right ventricle hypertrophy (RVH) and progresses to right ventricle failure (RVF), with evidence of altered cardiomyocyte Ca^{2+} handling and impaired energetics underlying the contractile dysfunction. However, the early stage of the disease is poorly studied. In addition, β-blocker treatment is widely used to treat left-sided heart failure, yet, there are currently no therapies recommended for pulmonary hypertension that target the heart.

Aims: (1) examine Ca^{2+} handling and mitochondrial energetics in a rat model of RVH, prior to the onset of heart failure. **(2)** treat RVH rats with the β_1-AR selective blocker metoprolol and examine mitochondrial function at the onset of RVF.

Methods: RVH was induced in rats with an I.P injection of monocrotaline (60 mg Kg^{-1}) and controls were given saline. Contractility and $[Ca^{2+}]_i$ were measured in isolated RV trabeculae in response to increased frequency, and β-AR stimulation. Trabeculae were subsequently permeabilised and mitochondrial energetics measured. These trabeculae were also processed for confocal imaging and the transverse-tubule organisation (TT_{power}) was measured. Finally, RVH rats were treated with metoprolol (10 mg kg^{-1} day^{-1}) and mitochondrial function determined using high-resolution respirometry at the onset of RVF.

Results: In comparisons to controls, Ca^{2+}-transients in RVH trabeculae had a slower onset, and a negative force-frequency response. β-AR stimulation altered the time course, but not the amplitude, of Ca^{2+}-transients, and increased spontaneous Ca^{2+} release. No difference was found in maximum Ca^{2+}-activated contraction in trabeculae reliant on mitochondrial ATP production and supply, however, TT_{power} was decreased in RVH trabeculae. While mitochondrial function was impaired in RVF rats, metoprolol neither recovered this nor delayed the onset of RVF.

Conclusions: Ca^{2+} mishandling occurs in RVH before mitochondrial energetic deficits. β-blocker treatment did not improve mitochondrial function nor the progression of the RVH to RVF in the time course studied.

2A.3 The impact of chronic activation of arcuate GABA neurons on fertility

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Gonadotropin-releasing hormone neurons (GnRH-N) are heavily innervated by arcuate nucleus (ARN) GABA neurons (GABA-N). In a mouse model of polycystic ovary syndrome, the most common infertility disorder worldwide, an increase of ARN GABA-N inputs onto GnRH-N is observed. However, it is unclear how selective chronic activation of ARN GABA-N directly impacts GnRH-N activity and fertility. To address this question, we are using chemogenetic tools coupled with a Cre/lox approach in mice. We expressed the designer receptor hM3Dq specifically in ARN GABA-N via stereotaxic injection into the ARN of vesicular GABA transporter (VGAT-Cre) mice. The delivery of the designer drug (CNO) to activate hM3Dq was coupled with serial tail-tip blood sampling to detect luteinizing hormone (LH) secretion as a readout of GnRH secretion. *In vivo*, we have been able to accurately target hM3Dq to ARN GABA-N and observed cFos expression specifically in ARN GABA-N after a single peripheral injection of 1.5mg/kg of CNO. However, LH secretion was not affected in gonadally intact male (n=12) and female (n=9) mice or ovariectomised females (n=4). Interestingly, a single injection of CNO directly onto the rostral preoptic nucleus of bilaterally transfected unconscious male and female increased LH release (n=6 male and n=3 female). To investigate longer term chronic activation of ARN GABA-N, CNO was delivered in the water for 2 weeks. This disrupted estrous cyclicity in transfected intact females (n=17). These results show that the specific modulation of the ARN GABA-N can stimulate GnRH/LH secretion. However, a chronic activation of this circuit can cause dysregulation of estrus cyclicity consequently fertility.

2A.4 Can we further refine therapeutic hypothermia for perinatal hypoxic-ischaemic brain injury?

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Therapeutic hypothermia significantly reduces death or disability after moderate to severe hypoxia-ischaemia before birth, but is only partially effective. We investigated whether refining the duration of treatment and rate of rewarming after treatment might help improve neural outcomes.

Chronically instrumented fetal sheep (0.85 gestation) received 30 minutes of bilateral carotid artery occlusion. To determine optimal duration, hypothermia was induced from three hours after ischaemia and continued for either two, three or five days. To determine the effect of rate of rewarming, we compared continued hypothermia for two or three days with rapid rewarming or two days with slow rewarming over 24 hours.

Three days of hypothermia was associated with improved neuronal survival and recovery of EEG power and reduced inflammation ($p<0.05$). Five days of hypothermia was not associated with any additional neuroprotective effects, and unexpectedly was associated with a small reduction in neuronal survival in the cortex and dentate gyrus ($p<0.05$). When hypothermia was stopped at two days, there was a striking deterioration in EEG power over the next 24 hours compared to hypothermia for three days, with a corresponding reduction in neuronal survival in the cortex and hippocampus and a significant increase in microglial induction. Slow rewarming after two days of hypothermia prevented the deterioration in EEG power but did not improve neuronal survival compared to two days with rapid rewarming. Ischemia was associated with a significant increase in astrocyte number and loss of myelin protein CNPase in the white matter tracts, which were partially attenuated by all protocols, albeit slow rewarming was better in some aspects.

In conclusion, hypothermic neuroprotection was not improved by prolonging the duration of treatment and was impaired by early rewarming. We speculate that optimally hypothermia needs to be continued until the cell environment is able to actively support cell survival.

2B.1 Rewarming after therapeutic hypothermia for neonatal hypoxic-ischemic encephalopathy – is fast or slow rewarming better?

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Therapeutic hypothermia partially reduces death and disability in neonatal hypoxic-ischemic encephalopathy. There is very limited evidence that slower rewarming may help optimize outcomes after treatment. The aim of this study was to contrast neuroprotection after controlled slow rewarming with spontaneous rapid rewarming after 72 hours of hypothermia after global cerebral ischemia in the near-term fetal sheep.

Chronically instrumented fetal sheep at 0.85 gestation were randomised to sham control (n=9), ischemia-normothermia (n=8), ischemia-72 h hypothermia-rapid-rewarming (n=8) and ischemia-72 h hypothermia-slow-rewarming over 10 h (n=9). Hypoxia-ischemia was induced by bilateral carotid artery occlusion for 30 minutes. Hypothermia was started 3 h after the end of hypoxia-ischemia. Animals were killed at 7 days for tissue collection.

Ischemia was associated with rapid suppression of EEG power compared to sham-control (p<0.05). At the start of hypothermia, extradural temperature decreased to 31.5±0.2 ^{o}C and 32.3±0.2 ^{o}C in the fast- and slow-rewarming groups, respectively (N.S.). Both hypothermia groups showed a very similar improvement in EEG power from 24 h onward compared to ischemia-normothermia (p<0.05).

Ischemia was associated with significant neuronal loss in the cortex and hippocampus and reduced expression of CNPase and myelin basic protein in the white matter (p<0.05). Both hypothermia protocols significantly improved neuronal survival in the cortex and CA3 (p<0.05), with a small but significantly greater increase in neuronal survival in the cortex, CA4 and dentate gyrus after rapid rewarming compared with slow rewarming (p<0.05). Slow rewarming was associated with increased CNPase expression, while rapid rewarming better preserved myelin basic protein expression.

Slow rewarming after 72 h of hypothermia did not improve recovery of brain activity or overall neuronal survival, and indeed was associated with slightly less neuronal survival in some regions than rapid rewarming. The effects of rewarming strategy on white matter injury was mixed, and the impact on brain development is unclear.

2B.2 A duet of stress adaptation and negative feedback tunes stress neuron activity dynamics

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An organism's responses and adaptations to stressful challenges are critical for survival. Hypothalamic corticotropin-releasing hormone (CRH) neurons orchestrate the neuroendocrine stress response, resulting in corticosteroid (CORT) release. While acute elevations in CORT can be beneficial, persistently elevated stress hormone levels are detrimental to both physical and mental health. Therefore, understanding how CRH neuron output is regulated is a fundamental question in stress physiology. Previously, it has not been possible to study CRH neuron activity in real time. To overcome this, we have used a novel technique called fibre photometry which uses optical methods to record CRH neuron population activity in vivo. Using this technique, we have been able to reveal the natural activity dynamics of CRH neurons in freely behaving mice both in the basal resting state as well as in response to stress. For over 7 decades, it's been thought that CORT acts in the brain after stress to prevent excessive CRH neuron activation, in a negative feedback loop. We observed that CORT negative feedback has rather minimal effects on stress evoked CRH neuron output, but instead has a slow modulatory effect on the basal resting activity. Interestingly, CRH neurons were incredibly adaptive. Habituation of CRH neuron responses to multiple presentations of a mild stressor were observed, however, this adaptation was dependent on stress familiarity and past experience rather than CORT negative feedback. Overall, while the combined efforts of negative feedback and adaptation are required for stress regulation, we find that the two act independently. Negative feedback sets the basal CRH tone whereas adaptation is critical for tuning stress evoked CRH neuron output. This research demonstrates for the first time, the temporal and adaptive dynamics of CRH neuron activity and challenges long standing theories in the field of stress physiology.

2B.3 Targeting the Brain for the Treatment of Heart Failure

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Heart Failure (HF) is associated with increased cardiac sympathetic nerve activity (CSNA), but the mechanisms causing this are unclear. The area postrema (AP), a brainstem circumventricular organ, plays an important role in controlling SNA. We hypothesized that the elevated CSNA in HF is mediated in part by the AP and that lesioning this region would a) reduce CSNA in sheep with HF and b) improve cardiac function in rats with HF.

In sheep with HF, lesion of the AP or sham lesion (n=6/group) was performed when ejection fraction (EF) had fallen to ~50%. CSNA was recorded when EF was <40%. In rats, AP or sham lesion (n=18/group) was performed before HF was induced and 11 weeks later left ventricular end-diastolic pressure (LVEDP) was measured.

In sheep with HF there was a significant decrease in EF (from 74 ± 2 % to 38 ± 1 %, P <0.001) and lesion of the AP significantly reduced the elevated CSNA burst incidence (45 ± 10 vs. 89 ± 3 bursts/100 heartbeats, P<0.01). In rats with HF, LVEDP was significantly lower in the AP lesion group compared with the sham lesion group (10 ± 1 vs 18 ± 2 mmHg, respectively, P<0.05).

Lesion of the AP significantly reduced the elevated CSNA in sheep with HF and resulted in a lower LVEDP in rats with HF. These findings suggest that the AP plays a critical role in setting the high levels of CSNA in HF and indicate that lesion of the AP reduces the decline in cardiac function post-myocardial infarction.

2B.4 The effect of chronic inflammation on the circadian development of fetal EEG activity in preterm fetal sheep

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Circadian rhythms are important regulators of physiological activity, but little is known about fetal patterns, particularly during preterm life, and whether they can be altered by adverse events such as inflammation exposure. Our objective was to determine the effect of inflammation induced by low-dose lipopolysaccharide (LPS) on circadian rhythms of preterm fetuses.

Preterm fetal sheep (104 days) were given either a saline (n=8) or low-dose LPS (n=8) i.v. infusion (ramping dose doubled/day from 200ng for five days) and studied for a further five days without infusion. Fetal EEG activity were continually recorded. EEG spectral edge frequency (SEF) was calculated as the frequency below which 90% of EEG power was present. Sheep were fed *ad libitum*; light period was between 06.00-18.00hrs.

Maternal actigraphy showed ewes ate primarily during the day. In saline fetuses, EEG power increased only during the day, plateauing at night. This pattern persisted in the post-infusion period, although the diurnal rise in power became attenuated. LPS suppressed this rise ($P<0.05$), and post-infusion the circadian pattern was lost with an increase in EEG day and night. SEF activity increased with age, only during the night, until a plateau at 108d. LPS suppressed SEF on day one of infusion ($P<0.05$) but did not affect circadian patterning. However, LPS prevented the plateau phase, with SEF continuing to increase throughout the experiment ($P<0.001$).

Increased EEG power may reflect brain growth, consistent with the primary period of energy intake by ewes. LPS is a growth-inhibitor, but there may be catch-up growth post-infusion at the expense of circadian rhythmicity. Increased SEF at night may reflect energy utilisation for increasing connectivity of the neural network. The SEF plateau and EEG power attenuation in controls reflects the development of sleep-state cycling, which exposure to LPS delays, consistent with the known effects of inflammation on functional connectivity.

2B.5 The role of carotid chemoreceptors in the maintenance of hypertension

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Hypertension is a major health problem of pandemic proportions that is often inadequately controlled, warranting development of new treatments. There is increasing evidence that hypertension is initiated and maintained by elevated sympathetic nerve activity. There is also strong evidence that aberrant afferent signalling from carotid chemoreceptors promotes sympathoexcitation and hypertension. We hypothesized that hypertension is associated with activation of carotid chemoreceptors and that carotid sinus denervation (CSD) would reduce blood pressure.

Adult ewes either underwent unilateral renal artery clipping or sham surgery. Three weeks later, baroreflex control of heart rate was examined and the contribution of sympathetic mechanisms to hypertension was determined using hexamethonium (125 mg/hr). The mean arterial pressure response to activation of carotid chemoreceptors was also determined in both groups. In a second surgery conducted in hypertensive animals only, the carotid sinus nerve was severed bilaterally (CSD) (n=6) or left intact (sham, n=6) and the effect on blood pressure was examined.

Unilateral renal artery clipping induced hypertension (mean arterial pressure 109±2 vs 91±3 mmHg in shams, $p<0.001$). The pressor response to chemoreceptor activation was augmented in hypertensive animals compared to the normal group (15±1 vs 7±2 mmHg, $p<0.05$). CSD decreased MAP (-17±1 vs +2±1 mmHg in shams, $p<0.0001$) and shifted the heart rate baroreflex curve back to the left. The contribution of the autonomic nerves to hypertension was elevated in hypertensive animals compared to normal (-19±2 vs -11±2 mmHg, $p<0.05$). CSD reduced this contribution. In conclusion, we have shown that hypertension in our large animal model is associated with activation of carotid chemoreceptors and that bilateral CSD lowers arterial pressure. Our data implicates the carotid chemoreceptors in the maintenance of hypertension.

2B.6 A century-old cardiac conundrum regarding the force-length relation

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We present the solution to a century-old conundrum regarding the contraction mode-dependency of the cardiac pressure-volume (force-length) relation. The conundrum arose when studies, commencing during the 1960s, queried the pressure-volume diagram published by Otto Frank in 1898. Frank presented two end-systolic pressure-volume relations: one for isovolumic contractions, which located above that of the one for afterloaded-isotonic contractions. Although his results were confirmed by many subsequent investigators, some studies found only a single relation that was independent of the mode of contraction. Attempts to resolve this discrepancy, have been unsuccessful, largely due to the inability to show explicitly how, or under what conditions, the two force-length relations could be unified. We explored the force-length relations of isolated ventricular trabeculae under wide ranges of afterloads as well as preloads. Our exploration has led us to present the solution to this century-old cardiac conundrum.

2B.7 Cardiac muscle samples working against a real-time model of the vasculature dynamic impedance

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The mechanical impedance imposed on experimental samples of cardiac muscle tissue *in vitro* is commonly over simplified and thus these samples do not replicate the dynamic mechanics of ventricular contraction in vivo. Hence, we aimed to develop a system to impose a model-based, time-varying load on cardiac tissue preparations.

We developed a computational model of systemic afterload, by encoding a Windkessel model of vascular fluid impedance with the Laplace law into a hardware-based control system. We compared its performance with that of a typical isotonic loading system by conducting experiments on isolated cardiac muscle preparations. We then observed the effect of loading healthy ventricular trabeculae with a mechanical impedance defined by 'diseased' model parameters. Windkessel-loaded trabeculae developed contraction profiles similar to those typically observed in vivo. Muscle work per twitch varied with variation of model parameters. The trabeculae achieved greater work output using the new loading system at both room temperature and body temperature.

Our implementation of a real-time model of arterial characteristics provides an improved physiologically derived load for use in studying isolated cardiac muscle tissues. This approach is readily applicable to the study of various disease conditions and may help shed light on mechanical impairments underlying many cardiac diseases.

2B.8 Ocular lens function and aging visualised with mass spectrometry

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To function as an effective optical element, the avascular lens exhibits several specialisations that include an ordered cellular structure, a gradient in refractive index, and a circulating current to deliver nutrients and remove waste products from the lens nucleus. The aging lens undergoes many changes to metabolites and proteins that alter these physiological and optical functions in specific lens regions. Some of these changes are associated with formation of age-related nuclear (ARN) cataract, the most prevalent form of blindness worldwide. A combination of human and laboratory-aged bovine lenses have been analysed by advanced mass spectrometry techniques to spatially map these changes and understand how they contribute to ARN cataract formation at the whole organ level.

A range of human lens ages (29y-82y) or bovine lenses, either laboratory-aged by hyperbaric oxygen treatment or organ cultured in artificial aqueous humour containing stable isotopically-labelled metabolites, were analyzed. Axial cryosections (20μm) of lenses were collected on MALDI targets, and matrix applied by a TM-Sprayer. Positive ion mode MALDI-TOF (for proteins) or negative ion mode MALDI-FT-ICR (for metabolites) imaging mass spectrometry was used to map the distribution of protein and metabolite distributions in each lens at 150μm spatial resolution. SCiLS lab software was used to visualise and quantify age-related changes to mass spectral signals.
Several age-related modifications to proteins were observed in specific regions of the lens. In addition, age-related alterations in antioxidant, lipid, and UV filter levels and distributions were spatially mapped. Finally, initial experiments to assess the role of the lens circulating current in lens metabolite transport were performed and will be discussed.

3A.1 Plasticity in the central and peripheral nervous systems and its role in disorders of the brain and heart

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"Plasticity" is a critical process in the brain: it is defined as the ability of neurons to alter their strength of communication at synapses, and it underpins not only learning and memory, but also sensory and motor functions. Plasticity has largely been studied at excitatory synapses, where we and others have shown the importance of dynamic regulation of glutamate receptors and their associated scaffold proteins. In pathological conditions, including in neurodevelopmental and neurodegenerative disorders such as Autism Spectrum Disorders (ASD) and Huntingtons Disease (HD), plasticity processes are known to go awry and this is thought to underpin clinical symptoms such as memory impairment, sensory deficits, movement disorders, and challenging behaviours. In ASD, mutations occur in the synaptic scaffold protein SHANK, and we have recently shown that zinc-dependent regulation of SHANKs can alter synapse function and reverse ASD-related behaviours in animal models. Similarly, in HD, we have shown that relocation of mis-targeted glutamate receptors with the synaptic scaffold protein SAP97 can rescue normal synapse function. These data show that targeting synapse plasticity through these scaffold proteins holds great promise for treatment strategies. Interestingly, both SHANK and SAP97 are also expressed outside of the brain, including in the neurons on the surface of the heart. These neurons are the final site of neuronal control of heart rhythm, and our recent data reveals that plasticity can also occur in these peripheral neurons. We predict that similar to what occurs in the brain, that altered plasticity mechanisms may provide a mechanism for pathological changes such as increases in susceptibility to cardiac arrhythmias. Understanding the molecular underpinnings of plasticity is therefore critical to not only identify pathological processes, but also to harness these plasticity mechanisms to stall or reverse disorders in both the central and peripheral nervous systems.

3A.2 Targeting RyR2 Ca release channels for preventing cardiac arrhythmia – a bench to bedside story

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Ca2+ leak via ryanodine receptor type 2 (RyR2) can cause potentially fatal arrhythmias in a variety of inherited and acquired heart diseases and has also been implicated in neurodegenerative and seizure disorders, making RyR2 an attractive therapeutic target for drug development. The lecture will review the discovery and clinical development of drugs that target Ca leak in the heart. The lecture will also discuss underlying arrhythmia mechanisms in ventricular and atrial arrhythmias, scientific controversies on the mechanism of drug action targeting Ca release, and the discovery of potent and RyR2 selective inhibitors.

3A.3 Regulation of RyR2 by O-linked glycosylation

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O-GlcNAcylation is the enzymatic addition of a sugar, O-linked β-N-Acetylglucosamine, to the serine and threonine residues of proteins, and it is abundant in diabetic conditions due to hyperglycaemia. Recently, it has been shown that O-GlcNAcylation can indirectly increase pathological Ca^{2+} leak through the cardiac ryanodine receptor (RyR2) due to activation of Ca^{2+}/calmodulin-dependent kinase II (CaMKII). However, as RyR2 is well known to be directly regulated by other forms of serine and threonine modification (phosphorylation) this study aimed to determine whether RyR2 is directly modified by O-GlcNAcylation and if this also alters the function of RyR2. Also, this study aimed to ascertain the site for O-GlcNAcylation on the ryanodine receptor 2.

We found that RyR2 is modified by O-GlcNAcylation in human, animal and HEK293 cell models. Using HEK293 cells we found that high glucose increases the level of Ca^{2+} leak through RyR2, and that this effect was enhanced by thiamet-G (an O-GlcNAc promotor) and blunted by diazo-6-oxornoleucine (an O-GlcNAc inhibitor). Intriguingly, when RyR2 triple A and S2808A mutant cells (cells lacking known RyR2 phosphorylation sites) were used in cytosolic and intra-ER imaging using the same protocols as with wild-type RyR2 cells, it was observed that the thiamet-G effect on RyR2 was absent.

These data suggest that the function of RyR2 can be directly regulated by O-GlcNAcylation, potentially at similar sites where phosphorylation has been shown to occur.

3A.4 Role of cAMP binding protein Epac in excitation-contraction (E-C) coupling in rat cardiac muscle

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Recently exchange proteins activated by cAMP (known as "Epac") have been identified in many different cell types, including cardiac myocytes, and are known to regulate intracellular Ca^{2+} signalling via the cAMP-activated protein kinase A (PKA) pathway. In cardiac muscle, the multiple targets and physiological effects of the classical PKA-activated pathway is well understood in excitation-contraction (E-C) coupling. Several studies have shown Epac activation of isolated cardiac myocytes modulates Ca^{2+} homeostasis, but there are conflicting reports of either increased [1] or decreased [2, 3] Ca^{2+} transient amplitude from mice and rats, respectively. The importance of Epac in cardiac E-C coupling therefore remains unclear.

In this study we investigated Epac activation (10 µM cpTOME) of both rat ventricular myocytes and multicellular trabeculae. In isolated myocytes, cpTOME significantly increased Ca^{2+} spark frequency (Fluo-4/AM) from ~7 to 32/100 $\mu m^3/s$ (n = 10), *P = 0.05*, with a reduction in peak amplitude of Ca^{2+} transients. Simultaneous measurements of intracellular Ca^{2+} (Fura-2) and isometric force in trabeculae (n = 7, 1.5 mM $[Ca^{2+}]_o$) showed no effect of Epac activation on either the amplitude of Ca^{2+} transients (Control 0.7 ± 0.1 *vs* cpTOME 0.7 ± 0.1; 340/380 fura-2 ratio, *P = 0.35*) or on peak stress (Control 24 ± 5 mN/mm^2 *vs* cpTOME 23 ± 5 mN/mm^2, *P* = 0.20).

However, an effect of Epac in trabeculae was unmasked by lowering extracellular $[Ca^{2+}]_o$ from 1.5 mM to 1 mM, which mimicked the depotentiated isolated myocytes [4]. In these trabeculae, activation of the Epac pathway increased myofilament Ca^{2+} sensitivity which was blocked by addition of KN-93, a Ca^{2+}/calmodulin-dependent protein kinase II (CaMK-II) inhibitor. This study suggests that Epac activation may be a useful therapeutic target to increase the force of contraction during low inotropic states.

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3A.5 Heretical Thoughts Regarding Cardiac Muscle

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Cardiac muscle is widely presumed to sense the prevailing load and to respond with appropriate force development in a classical feed-back manner. Hence, much effort has been expended seeking the force-sensor. In contrast, we consider that cardiac muscle is blind to load, seeing only sarcomere length – to which it responds choreographically.

Explicitly, at any given sarcomere length, if a myocyte contracts isometrically, then its peak force is pre-determined by the end-systolic force-length relation. If, however, an afterload that is less than the potential peak force is encountered, then the remainder of the contraction occurs auxotonically until the muscle can no longer sustain the load and relaxes. That is, we consider that each cardiac muscle twitch, at any given sarcomere length, is 'all-or-none'- *à la* both the somatic nerve action potential and the skeletal muscle twitch.

In summary, we propose that, whether cardiac muscle contacts isometrically throughout the entire twitch, or for only a brief period, its force-time profile is dictated by the afterload. At no point is the load on a myocyte or its actin-myosin cross-bridges 'sensed' and effort adjusted accordingly. If our proposition is correct, then pursuit of the elusive 'cardiac muscle force-sensor' is doomed to failure.

3B.1. Can androgen receptor blockade restore progesterone sensitivity in a mouse model of polycystic ovary syndrome (PCOS)?

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Androgen excess is a cardinal feature of polycystic ovary syndrome (PCOS), a poorly understood neuroendocrine disorder and a leading cause of infertility in women. Although the aetiology of PCOS remains unclear, evidence from women and animal models suggests that elevated prenatal androgens can elicit PCOS onset in adulthood, indicating that androgens are likely involved in the ontogeny and pathophysiology of PCOS. Recent research suggests that androgen excess in PCOS may alter brain circuits that mediate steroid hormone feedback control of gonadotropin-releasing hormone (GnRH) neurons, cells in the brain which are critical for fertility.

Women with PCOS have impaired progesterone negative feedback[1]. Research in a prenatally androgenized (PNA) mouse model of PCOS has identified that progesterone receptor (PR) expression is markedly reduced in the arcuate nucleus (ARN) of the hypothalamus[2], an area known to be important in the regulation of GnRH neuron activity. PR expression is specifically reduced in ARN GABAergic neurons, which display increased input to GnRH neurons in PNA mice[2]. Recent research has shown that androgen receptor (AR) blockade in adulthood can restore normal GABAergic wiring to GnRH neurons and estrous cyclicity in PNA mice[3], but it is not yet known whether ARN progesterone sensitivity is restored.

To investigate this, PNA and control mice will be treated with an AR blocker or an oil vehicle for 20 days during adulthood and estrous cyclicity will be monitored. Immunohistochemistry for PR will be performed on brain sections from these mice to quantify the number of PR-expressing cells in the ARN. AR blockade has been shown to restore progesterone sensitivity in women with PCOS[1], therefore we hypothesize that AR blockade will restore normal PR expression in the ARN in PNA mice. This research will help elucidate the role of ARN neurons in PCOS pathology.

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3B.2. Are impairments in prolactin signaling in the brain of obese mice associated with deficits in maternal care?

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New Zealand has a well-documented obesity epidemic with two thirds of women of reproductive age being overweight or obese. During pregnancy, obesity represents a significant health issue, including increased complications during pregnancy and impairments with breastfeeding. In rodent models, it has also been shown that diet-induced obesity (DIO) leads to poor outcomes for offspring, with a high pup mortality rate in obese mice. However, the mechanisms leading to these deficits in obese mothers are poorly understood. Using a DIO mouse model, we aim to firstly characterise the impact of maternal obesity on maternal behaviour and pup survival, and to secondly investigate whether there are deficits in signalling of the pregnancy hormone, prolactin, in the brain of obese pregnant mice. To characterize maternal behaviours in the pre and perinatal period, control and obese mice will be mated, individually housed once pregnant, and parturition will be video recorded. Footage will be analysed for length of parturition, time spend nesting and interacting with pups following birth. We will also assess pup survival at days 0, 1 and 3 postpartum. Recently, we have revealed a critical role for prolactin action in the medial preoptic area (MPOA) in modulating the appropriate display of maternal behaviour following birth. We hypothesise that maternal obesity attenuates prolactin signalling in the MPOA. Brains from day 17 pregnant obese and control mice have been collected and immunohistochemistry for phosphorylated signal transducer and activator of transcription 5 (pSTAT5), a marker of activated prolactin receptors, will be performed. We predict maternal obese mice will have impaired maternal behaviour and pup survival, accompanied by a reduction in pSTAT5 in the MPOA.

3B.3 Dissecting estrogen positive feedback mechanisms using CRISPR-Cas9

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Estrogen feedback mechanisms are arguably the most important component of fertility regulation; however, the hypothalamic circuitry of these mechanisms is currently uncertain. The estrogen receptor alpha (ESR1)-expressing GABAergic neuronal afferents to gonadotropin-releasing hormone (GnRH) neurons are likely to be involved in estrogen positive feedback, but their location in the brain have not yet been determined. The CRISPR-Cas9 system allows for precise and efficient manipulation of genes by interacting with engineered guide-RNAs.

This study aims to use CRISPR-Cas9 to knockdown ESR1 in GABAergic neurons in particular brain-regions of mice, and to examine the reproductive phenotype of each knockdown-mouse. Firstly, CRISPR-Cas9 will be established as a tool for *in vivo* knockdown by using adeno-associated viral vectors (AAV) containing Cas9 and gRNA to target ESR1. Secondly, transgenic mice that express Cas9 in GABAergic neurons will then have gRNA applied by stereotaxic injection to the rostral periventricular region of the third ventricle (RP3V), arcuate nucleus (ARN) or the amygdala to achieve a brain-region and phenotype specific ESR1 knockdown. In both the AAV and the transgenic mice, immunohistochemistry will be performed to label ESR1 and verify the expression of genetically-encoded proteins.

We predict that the CRISPR-Cas9 approach will reduce ESR1 expression following AAV injection in wildtype and VGAT-Cas9 mice. This study should determine the particular brain-regions containing GABAergic-ESR1 neurons that are critical for estrogen positive feedback. Improving the characterization of the circuitry involved in estrogen positive feedback will lead to more targeted treatments of infertility and the development of safer contraceptives.

3B.4. The Role of Prolactin in Reward Aspects of Maternal Behaviour

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Maternal care is crucial for the survival of newborn offspring in mammals. The medial preoptic area (MPOA) of the hypothalamus plays a critical role in the neural circuitry controlling maternal behaviour, and acts as a key site for hormonal modulation of this behaviour. Acute deletion of prolactin receptors in the MPOA disrupts postpartum maternal behaviour, indicating an essential role for prolactin in maternal behaviour[1]. Our preliminary data indicates that a population of MPOA prolactin-sensitive neurons project to the ventral tegmental area (VTA), a region known for its importance in reward behaviour. Therefore, we hypothesise that prolactin action in the MPOA drives maternal behaviour by activating reward circuitry in response to interactions with offspring.

Firstly, we aim to identify populations of prolactin-sensitive neurons that are activated by interactions with pups. Groups of mice expressing the red fluorescent reporter *td tomato* in prolactin receptor (Prlr)-expressing cells were used to identify prolactin-responsive cells. Postpartum mice (lactation day 3) had pups removed, and four hours later pups were returned to one group. Immunohistochemistry will be performed on brain sections for cfos, a marker of cellular activation, to identify prolactin-sensitive neurons that are activated during exposure to pups. Our goal is to investigate reward behaviour in mice with a MPOA-specific deletion of the Prlr. In order to do this, we are investigating behavioural paradigms that test whether female mice find pup-interactions rewarding. These include the conditioned place preference (CPP) test, that tests whether mice prefer a chamber associated with the presence of pups. We are also investigating motivational aspects of maternal behavior using a climbable transparent barrier, that is placed between a mother and her pups and barrier crossing is recorded. In the future we aim to use these tests to investigate prolactin's role in reward aspects of maternal behaviour.

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3B.5. Long term effects of androgen receptor blockade in a mouse model of Polycystic Ovarian Syndrome

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Polycystic ovarian syndrome (PCOS) is a leading cause of infertility in women worldwide. PCOS is characterised by three cardinal features, including hyperandrogenaemia, a polycystic-like appearance of the ovaries, and impaired reproductive cycling. Research in animal models has shown that prenatal androgen exposure can elicit a PCOS-like phenotype in adulthood, including impaired estrous cyclicity and altered ovarian morphology. Although the key features of PCOS lie in the periphery, recent evidence has implied that the brain may be driving this condition. Prenatally androgenised (PNA) mouse models of PCOS display increased GABAergic inputs to gonadotropin releasing hormone (GnRH) neurons in the brain[1]. This altered circuitry may drive the underlying neuroendocrine abnormalities in PCOS. Recent evidence has revealed that long term blockade of androgen signalling in adulthood is able to restore normal GABAergic inputs onto GnRH neurons, estrous cyclicity and improve ovarian morphology[2]. However, it is not yet known whether this restoration persists long-term, following treatment cessation.

To investigate this, estrous cyclicity, GABAergic inputs to GnRH neurons, and ovarian morphology will be examined following long-term flutamide treatment cessation. GnRH-GFP PNA or control mice will be injected daily for 20 days with flutamide or oil from post-natal day 40. Vaginal smears will be collected across 20 days of treatment and an additional 20 days post-treatment to examine changes in estrous cyclicity. Following this, immunohistochemistry of brain slices will be completed to visualise and quantify the numbers of GABAergic appositions to GnRH neurons. Histological staining of ovarian slices will be used to examine changes in ovarian morphology. This research will determine whether key PCOS features are permanently or transiently reversed following long term androgen receptor blockade.

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3B.6. Kisspeptin regulation of oxytocin neuron activity in late pregnancy

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Oxytocin causes uterine contraction during birth and milk ejection during lactation. Oxytocin is secreted from the posterior pituitary gland by oxytocin neurons. We have recently shown that oxytocin neuron firing rate increases in response to intracerebroventricular (ICV) kisspeptin only in late pregnancy. Immuno-histochemistry with retrograde tracing showed increased kisspeptin projections from the periventricular nucleus of the hypothalamus to the perinuclear zone surrounding the supraoptic nucleus in late pregnancy. To determine whether the stimulatory effects of central kisspeptin in late pregnancy result from a local action at the supraoptic nucleus, in vivo extracellular single unit recordings made from supraoptic nucleus neurons in urethane-anaesthetised late pregnant rats during microdialysis application of kisspeptin-10 (100 μM in the dialysate) into the supraoptic nucleus. Preliminary data show that microdialysis of kisspeptin-10 does not significantly change the firing rate of oxytocin neurons (average 4.9 ± 0.9 spikes s^{-1} before and 6.3±0.7 spikes s^{-1} during kisspeptin, n = 4, ($F_{8,17}$ = 0.64, P = 0.7, one-way ANOVA analysis). Microdialysis of kisspeptin also had no effect on the firing rate of vasopressin neurons (n = 4, $F_{8,20}$ = 0.5, P = 0.8 one-way ANOVA analysis) that are also found in the supraoptic nucleus. Therefore, further recordings are being conducted to determine whether the excitatory effect of ICV kisspeptin on oxytocin neurons is mediated by actions within the supraoptic nucleus.

3B.7 Impaired cardiomyocyte diastolic function is linked with glycogen accumulation in the diabetic heart

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Diabetic cardiomyopathy is characterized by early metabolic changes linked with a disturbance in cardiac glucose handling and increased glycogen storage. Recently, a glycogen-specific autophagy pathway, 'glycophagy', has been identified. The aim of this study was to investigate the role of glycophagy in the diabetic heart and determine whether cardiomyocyte glycogen overload is correlated with diastolic dysfunction. Male type 1 diabetic Sprague Dawley rats (streptozotocin (STZ), 55mg/kg i.p., 8 weeks duration) were injected with an inhibitor of autophagosome-lysosome fusion (chloroquine (CQ), 50mg/kg i.p) 4 hours prior to tissue collection. Glycogen was measured using an amyloglucosidase enzymatic assay. Cardiomyocytes isolated from a separate cohort of STZ rats were apportioned to glycogen analysis or loaded with Fura2 Ca^{2+} fluorescent dye for assessment of Ca^{2+} handling (ratiometric signal F360:380nm, IonOptix). A 2-fold increase in glycogen in the diabetic heart was observed ($p<0.05$). CQ-induced lysosomal blockade increased cardiac glycogen by 45% in control rats ($p<0.05$) but not STZ rats, suggesting that glycophagy throughput may be impaired in diabetes. Glycogen accumulation in cardiomyocytes isolated from diabetic rat hearts was significantly correlated with delayed Ca^{2+} uptake into the sarcoplasmic reticulum during myocyte relaxation (Tau constant of Ca^{2+} decay, $r=0.76$ $p<0.05$). This study is the first to show that cardiac glycophagy throughput is impaired in diabetes, and diabetic cardiomyocyte glycogen overload is linked with diastolic dysfunction. These findings provide a novel mechanism for diastolic dysfunction in diabetes and further investigation into the role of glycophagy in the diabetic heart is now warranted.

3B.8 Characterising the role of vascular ENaC for blood pressure regulation

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Epithelial sodium channel (ENaC) in the kidneys plays an important role in maintaining salt/water homeostasis and blood pressure. Recently, ENaC has been identified in blood vessels and is proposed to regulate vascular tone. An increase in vascular tone is observed in diseases such as hypertension, indicating that ENaC in the vasculature may contribute to hypertension. The hypothesis is that, in hypertension, ENaC expression in arteries increases and will contribute to development of vascular dysfunction.

Carotid and mesenteric arteries from hypertensive transgenic rats (Cyp1a1-ren2) were isolated and $\alpha\beta\gamma$ENaC expression was analysed by qRT-PCR, immunostaining and western blotting. Analysis of the influence of ENaC on vascular function was assessed by pressure myography.

Expression analyses show significant upregulation of αENaC in hypertensive carotid (mRNA and protein, $P<0.05$) and mesenteric (mRNA:$P<0.001$, protein:$P<0.01$) arteries. The analyses revealed an artery dependent expression pattern of β and γENaC, where βENaC mRNA was significantly upregulated in carotid ($P<0.05$) but γENaC mRNA was upregulated in mesenteric arteries ($P<0.01$) from hypertensive rats. Investigation of vascular responses revealed a significant attenuation of endothelium-dependent dilator responses in hypertensive carotid ($P<0.05$) and mesenteric ($P<0.001$) arteries, an indication of endothelial dysfunction. This loss of vasodilation was significantly rescued in hypertensive mesenteric arteries ($P<0.05$) by ENaC inhibition. The intraluminal flow was increased in both hypertensive arteries and ENaC inhibition reduced this flow.

In summary, an upregulation of vascular ENaC mRNA in arteries was observed during hypertension and this increased expression of ENaC is potentially associated with vascular dysfunction. This data suggests a link between pathogenesis of hypertension and vascular ENaC expression.

3B.9 High-density Lipoprotein Cholesterol: towards reversing the pathology of Facioscapulohumeral Muscular Dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is genetic myopathy affecting 1 in 8,333. FSHD is characterised by progressive skeletal muscle weakness and wasting, resulting in a severely diminished quality of life. FSHD is the results of a genetic mutation leading to the misexpression of DUX4. DUX4 is known to induce a pathological cascade leading to increased oxidative stress, inflammation, apoptosis, and altered myotube formation. There is currently no cure for FSHD and current treatments options have poor efficacy. High-density lipoproteins (HDLs) are well-characterised for their antioxidant and anti-inflammatory properties. Currently, HDL-based therapies are in clinical trial for several other oxidative stress-related diseases. Therefore, we hypothesise that HDL treatment will abrogate DUX4-mediated cellular damage *in vitro* and *in vivo.* To test this hypothesis we developed two *in vitro* (early and progressive) and one *in vivo* models of FSHD. C2C12 (murine skeletal muscle) cells were cultured for 48 hours and during the final 16 hours were treated with a physiological concentration of HDLs (21µM), after which they were assessed for FSHD pathology. Male and female C57B/l6 mice received an intramuscular injection of DUX4 lentiviral particles. Mice were treated with HDLs or vehicle tri-weekly for 14 days, following which the hind-limb was analysed for DUX4-mediated damage. *In vitro*, HDLs protected against DUX4-mediated oxidative stress, cell death and restore myotube formation in both early and progressive stage disease models. HDL treatment *in vivo* was able to decrease cell death in both sexes. Moreover, HDL treatment decreased oxidative damage in males. Within female mice, HDL treatment was also able to partially preserve skeletal muscle fibre diameter. The results reveal that HDLs offer promising results within FSHD and we propose that the administration of HDL-based therapy is a potential therapeutic strategy to treat FSHD.

3B.10 Annexin II light chain p11 interacts with ENaC to increase functional activity at the membrane

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The epithelial Na^+ channel (ENaC) facilitates Na^+ absorption in various epithelia including the kidney, lung and colon, where ENaC is localised to the apical membrane to enable Na^+ entry into the cell. The degree of Na^+ entry via ENaC largely depends on the number of active channels localised to the cell membrane. While regulation of ENaC endocytosis has been well studied, relatively little is understood of the proteins that govern ENaC exocytosis. We hypothesised that the annexin II light chain, p11, that controls exocytosis of other epithelial ion channels, could participate in the transport of ENaC along the exocytic pathway. Our results demonstrate that all three ENaC channel subunits interact with p11 in an *in vitro* binding assay. Quantitative mass spectrometry of affinity-purified ENaC-p11 complexes recovered several trafficking proteins. We also found that p11 is expressed in cortical collecting duct epithelial cells, however, the expression of p11 in these cells was not influenced by either short- or long-term exposure to aldosterone. To determine whether the p11 interaction affected ENaC function, we measured amiloride-sensitive Na^+ currents in *Xenopus* oocytes or mammalian epithelia coexpressing ENaC and p11, or a siRNA to p11. Results from these experiments showed that p11 significantly augmented amiloride-sensitive current, whereas knockdown of p11 decreased current. Further, knockdown of p11 reduced the ENaC cell surface population suggesting p11 promotes membrane insertion of ENaC. Overall, our findings reveal a novel protein-protein interaction of p11 with ENaC that controls the number of ENaC channels inserted at the plasma membrane via the exocytic pathway.

3B.11 Oxytocin is Sympathetic to a broken heart

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Myocardial infarction (MI) triggers an adverse and sustained increase in sympathetic nerve activity (SNA) to the heart, provoking arrhythmias and is a leading factor for the high mortality rate within the ensuing hours. The mechanism(s) responsible for this adverse increase in SNA following MI remain to be fully elucidated, although both peripheral afferents to the brain, and the central integration of these inputs within the CNS, dictate overall sympathetic output. We have previously reported novel findings that show hypothalamic oxytocin neurons within the paraventricular nucleus appear to be activated immediately following an acute MI and, importantly, intravenous administration of an oxytocin receptor blocker, retosiban, appears to ameliorate the adverse increase in SNA. However, it remains uncertain as to whether the retosiban is acting peripherally on the afferent fibers projecting to the CNS, or centrally within the CNS, to prevent sympathetic activation following acute MI. Accordingly, the primary aim of this study aimed to discriminate between the central vs peripheral effects of oxytocin receptor blockade on SNA.

SNA was continuously recorded from the cardiac sympathetic nerve of urethane-anaesthetized rats before, and three hours after acute MI (ligation of the left anterior descending coronary artery). Rats received an injection of either retosiban (3 mg/kg, i.v.) or atosiban (4.5 µg in 5 µl, i.c.v.) one minute after the infarct. Acute MI induced a maximal 190% increase in SNA, which was completely prevented in those rats that received an oxytocin receptor blocker, regardless of whether it was administered i.v. (retosiban) or i.c.v (atosiban). These novel results show that the therapeutic properties of oxytocin receptor blockade in preventing sympathetic activation following acute MI appear to be centrally mediated and, given that safety profile of retosiban, potentially clinically translatable.

3C. Overcoming ongoing challenges in teaching medical sciences: A structured workshop using Design Thinking to embed 21st century learning skills into your lessons and courses.

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Introduction/background:
21st century learning skills (CLS) are identified by the World Economic Forum as essential for thriving in a complex, fluid and uncertain future environment. Design Thinking is an iterative, human centred, practical approach to creating the best ideas and solutions.

Purpose and outcomes:
Embed one new graduate capability mapped to a 21st CLS in a lesson you teach, using the Design Thinking process.

Outline of workshop activities
Presentation [15min] What are 21st century learning skills, why do we need them? What is the Design Thinking process, and how does this apply to education?

Activity [5min] Map your institution's graduate capabilities to the 21st century learning skills template.

Structured workshop [60 mins]. Working in small groups, participants design a lesson using a Design Thinking canvas. Instructions for steps followed by timed segments for each activity.

1. Empathise with archtype student
2. Define one 21st CLS to embed
3. Ideate with wild ideas, be visual, go for quantity
4. Prototype on a lesson template
5. Test by presenting to next group. Use I like, I wish, what if … feedback framework.

4a Prototype revision

5a Test again on a different group. Feedback.

Discussion [10 mins]
Are there gaps between the 21st CLS and your institution's graduate capabilities?
What other situations could you use this I like, I wish, What if… feedback framework?
What 21st CLS/graduate attributes would you find most difficult to embed, ideas from the group

Prerequisite:
BYO internet connected device such as laptop, tablet or smartphone.

Limits:
Limited to 30 participants.

MedSci Plenary Lecture: Commercialising research; problems, pitfalls and potential

Malpas, S.

Auckland Bioengineering Institute, University of Auckland

Technology commercialisation is the sexy hot-topic in academia as Universities and Governments around the world attempt to show how our research endeavours leads to new jobs, taxes paid and a wealthier economy. This all sounds great but in general completing a PhD, writing papers, supervising students and getting grants often doesn't leave much time for commercialisation. Indeed one can legitimately ask; why bother, am I cut out for this, where do I go for help and finally will I get rich?

Simon has been involved in three medtech companies and is currently Chair of the Board of Directors for Millar Inc, a US based medical device company and Kaha Sciences Ltd a NZ life sciences company. He freely admits that he has made many many mistakes with regard to the commercialisation of research. One of his aims is to help others to avoid the same mistakes. Simon is also a program director for large scale research funding at the Auckland Bioengineering Institute which is focused on the sensing of pressure for medical applications. This presentation therefore will draw on the pitfalls and possibilities of trying to commercialise research whilst keeping one foot in academia.

S1A.1. Preclinical studies to inform the use of cord blood stem cells for preterm brain injury

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Human clinical trials have recently reported safety data and preliminary evidence of efficacy following treatment of children with cerebral palsy using umbilical cord blood (UCB) stem cells. UCB is an appealing neuroprotective treatment – it is already used safely for transplant in blood disorders, and UCB is made up of many different cell types, including mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs), T regulatory cells (Tregs) and monocyte derived suppressor cells (MDSCs), where each may contribute towards reducing neuroinflammation and/or repair of brain injury.

The etiology of damage to the developing brain, that underlies cerebral palsy, is variable, however it is well described that about half of those individuals with cerebral palsy were born preterm (<37 weeks gestation). Currently there are no standard neuroprotective or neuroreparative treatments that are offered to infants born preterm, despite strong knowledge to demonstrate this cohort as high risk for long term neurological deficits. Therefore, we set out to use large animal (sheep) models of preterm brain injury associated with hypoxia-ischaemia or inflammation to examine the neuroprotective benefits of UCB stem cells.

Our results to date have shown that UCB stem cells are neuroprotective for the developing white matter of the preterm sheep brain, mediated via anti-inflammatory, anti-oxidant and anti-apoptotic actions of the UCB cells. The neuroprotective benefits of UCB cells are optimised when cells are administered as early as possible after an insult to the preterm brain. We also show that the combined mix of whole UCB stem and progenitor cells is more efficacious than administration of MSCs alone. Combined, these data support that early administration of UCB cells after preterm birth may protect normal brain development and prevent cerebral palsy.

S1A.2. The role of microglia in perinatal brain injury

Fleiss, B [1-3]

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Neuroinflammation is a primary driver of damage in almost all injuries and insults to the brain. Microglia are the chief mediators of neuroinflammatory processes and these specialized innate immune cells also play central roles in brain development and homeostasis. Microglia are capable of acquiring numerous complex functional states dependent on the specific nature of an insult or injury, including cytotoxic responses, immune regulation, or injury resolution. Limiting the cytotoxic activation of microglia while promoting injury resolution represents a rational neuroprotective strategy, that requires an in-depth understanding of the molecular mechanisms controlling their phenotypes across forms of injury. My research has involved studying these activation states and their roles in injury in models of encephalopathy in the term infant associated with hypoxia-ischemia, in the preterm infant associated with chorioamnionitis and in paediatric traumatic brain injury. We are continuing work focusing on the role of sex in microglial responsiveness, novel nanoparticles to deliver drugs to microglia in vivo, the molecular regulators of activation states, and the persistence of changes in microglia and how this impacts the brain across the lifespan. This work is uncovering novel avenues for therapeutic design to improve the outcomes for infants who suffer from perinatal brain injury.

S1A.3. Gestational age and associated health and educational outcomes: Life outcomes to adolescence for premature infants in New Zealand

Tim Foster[1,2], Max Berry[1,3], Bridget Robson[1], Oliver Robertson[1], Kate Rowe[3], Nevil Pierse[1]

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Background and objectives

Advances in perinatal medicine over the last 20 years have dramatically improved survival for preterm infants [1]. With increasing survival [2-7], we urgently need more information about the long-term impact of preterm birth

One of the most challenging areas of perinatal medicine is the care of periviable infants (gestation < 25 weeks and/or birth weight < 500 g). Recommendations around intervention and resuscitation at the threshold of viability vary greatly [8]. In this context, there is a need for accurate, contemporary information describing long-term and short-term outcomes [9].

Methods

We performed a retrospective national cohort study of all New Zealand registered births appearing in a minimum of two independent national data sets at a gestational age of 23 weeks or more. We report 2 separate cohorts followed up to 2016: Cohort 1, born 1/1/2005 – 31/12/2015 (n = 613,521) used to study survival and mid-term health and educational outcomes; Cohort 2, born 1/1/1998 – 31/12/2000 and surviving to age 15 (n = 146,169) used to study high-school educational outcomes.

Outcomes described by gestational age include survival, hospitalization rates (with main admission diagnosis), national wellbeing assessment 'Before School Check' outcomes at age 4, rates of special education support needs in Primary School and national High School examination (NCEA) results.

Results

Ten-year survival increased with gestational age from 66% at 23/24 weeks to > 99% at term. All outcomes measured were strongly related to gestational age. However, most extremely preterm children didn't require special educational support and were able to sit for their NCEA examinations.

Conclusion

Within a publically funded health system, high-quality survival is achievable for most infants born at periviable gestations. Outcomes show improvement with gestational ages to term. Outcomes at early-term gestation (37 & 38 weeks) are poorer than for children born at full-term.

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S1A.4. Protecting the injured newborn brain - are cocktails better served with ice?

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Department of Physiology, the University of Auckland

Perinatal brain damage after hypoxia-ischaemia (i.e. oxygen deprivation) around birth occurs in approx. 8-48 and 1-4 babies per 1000 preterm and term births, and is associated with death or major neurodevelopmental disabilities. One seminal insight from research studies was that perinatal brain injury is not a static event, but rather an evolving process that results in neural and glial cell death long after hypoxic-ischaemic insults, thus offering a potential window for treatment. Following from this, therapeutic hypothermia has become standard-care for injured term newborns with hypoxic-ischaemic encephalopathy, but its neuroprotection is partial and it is not available for preterm babies. Hence, many babies still die or survive with debilitating handicaps and current research is now focussed on finding neurotherapies that could improve outcomes in preterm newborns or further improve brain cooling at term. Recombinant human erythropoietin (rEpo) is safe and perhaps one of the most promising neurotherapeutics. Using a translatable model of perinatal brain damage, my research has shown that delayed treatment with rEpo can reduce neural and white matter loss after severe hypoxia-ischaemia in preterm-and term-equivalent brains, although it was not additive to hypothermic neuroprotection. This research highlights the practical limitations with preclinical therapeutics that share protective mechanisms with cooling, and suggests future studies should focus on neurorepair strategies.

S1B.1. Optimizing Ventilation in the Inured Lung Using Multi-Frequency Oscillation

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We have recently demonstrated that oscillation of a heterogeneous lung with multiple simultaneous frequencies improves gas exchange and maintains lung recruitment at lower distending pressures compared to traditional 'single-frequency' oscillatory ventilation (SFOV)[1]. We termed this novel ventilatory modality 'multi-frequency oscillatory ventilation' (MFOV), and hypothesized that such short-term physiological improvements were due to a more even distribution of ventilation to different lung regions, in accordance with local mechanical properties. Various lung regions may be characterized by different 'preferred' frequencies for oscillatory flow, thus MFOV is uniquely capable of enhancing more uniform participation of mechanically heterogeneous lungs in gas exchange[2]. As a result, MFOV may produce more efficient oxygenation and CO_2 elimination compared to traditional SFOV, along with possible reductions in parenchymal strain heterogeneity and potential for ventilator-induced lung injury (VILI)[3]. More importantly, MFOV may be a more efficacious approach to protective ventilation in the heterogeneously injured lung, in comparison to SFOV or conventional mechanical ventilation (CMV) with low tidal volumes. In this presentation, we will discuss the theoretical rationale for the use of MFOV in structurally heterogeneous pathologies such as the acute respiratory distress syndrome (ARDS). We will then demonstrate how the spectral content of MFOV waveforms may be algorithmically designed using anatomically explicit computational models of the mammalian respiratory system. Finally using dynamic xenon-enhanced computed tomography and 4-dimensional image registration, we will elucidate the mechanisms by which MFOV improves regional ventilation distribution, aeration, and parenchymal strain compared to SFOV and CMV in a porcine model of ARDS. We expect that these pre-clinical studies of MFOV will be ultimately translatable and testable in eventual human clinical trials, with potential to reduce morbidity and mortality associated with ARDS and other heterogeneous lung diseases.

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S1B.2. Physiological insights into the pathogenesis and treatment of obstructive disease

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Airflow limitation (reversible or irreversible) is the defining characteristic of obstructive airway disease. Broad pathological features include airway inflammation and structural remodelling of the large and small airways. Treatment is naturally directed at reducing airway inflammation based partly on the assumption that inflammation drives airway remodelling events. However, there is increasing evidence to suggest that inflammation and remodelling develop independently, necessitating other direct approaches to reverse structural abnormalities such as bronchial thermoplasty. The utility of any approach that specifically targets remodelling will likely be improved by new imaging technologies, and in this regard optical coherence tomography offers promise, although has yet to deliver. The other intuitive clinical intervention in the treatment of obstructive disease is the use of airway smooth muscle (ASM) relaxants (e.g., salbutamol) to induce bronchodilation. Notably, in addition to the actions of pharmacological agents, mechanical-induced bronchodilation arises due to the cyclical stresses and strains exerted on the airway wall through breathing movements, particularly deep breaths. Patient response to inhaled bronchodilators may in fact be determined by the interactive effects of pharmacological and mechanical dilation. In view of such findings (driven predominantly through classical physiological experimentation) a new research field has emerged 'mechanopharmacology' which considers the impact of the mechanical environment of the lung on drug discovery. With the above preface, this presentation will now focus on the origin of remodelling, its direct assessment by optical coherence tomography and the emergence of non-inflammatory therapies in obstructive disease.

S1B.3. SleepStrong: A Pilot Sleep Health Wellbeing Initiative for New Zealand Employees at Fisher & Paykel Healthcare.

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Sleep is a salient human requirement, essential for life. However, despite accounting for around one third of our lives, sleep is seldom an active health consideration unless sleep concerns arise[1]. With the advent of modern technologies such as artificial light, through to our 24-hour society pervaded by internet capable "smart" devices, humans are constantly engaged and connected.

Despite this, our biological need for sleep remains unchanged. One in three adults frequently achieves an inadequate sleep duration[2], one in three has at least mild insomnia[3], and around one in five adults is employed in a role requiring shift work. In New Zealand, one quarter of adults self-report chronic sleep concerns[4], with socioeconomic deprivation, unemployment, and Maori ethnicity[5] positively identified as risk factors associated with various inadequate sleep health metrics. These findings demonstrate significant levels of sleep health concern amongst New Zealanders, and have important consequences for the health, safety, and wellbeing of individuals, whanau, and communities.

Fisher & Paykel Healthcare is a world-leading designer and manufacturer of sleep therapy products for people with sleep disordered breathing. Based in Auckland, NZ, Fisher & Paykel Healthcare today employs more than 4000 people worldwide, across a wide variety of roles. SleepStrong is a sleep health wellbeing initiative created by Fisher & Paykel Healthcare specifically for employees, to foster positive sleep health and wellbeing improvements. Developed to incorporate sleep health education, sleep health assessment, and improved access to sleep health care, SleepStrong has been created and piloted over the last 18 months. This session will focus on the motivation behind this initiative, the development processes, initial participant feedback, and ongoing opportunities.

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S1B.4. Ian Sun, Fisher and Paykel Healthcare
New Trends in CPAP development

S2A.1. Modulation of tissue integrity via stabilization of desmosomes in health and disease

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Desmosomes are adhering junctions present in all epithelia but most abundant in cells of tissues subjected to extensive mechanical stress such as in keratinocytes of the epidermis and in cardiomyocytes of the heart muscle. The core of desmosomes consists of desmosomal cadherins which are tethered to intermediate filaments via adaptor proteins including plakoglobin (Pg) and desmoplakin. Desmosomal diseases affect the skin in the autoimmune blistering disease pemphigus via autoantibodies against desmogleins (Dsg) or impair heart function by mutation of desmosomal components in Arrhythmogenic cardiomyopathy (AC). There is evidence that desmosomal cadherins besides their adhesive properties orchestrate multiple signaling pathways and thus desmosomes should be regarded as signaling hubs. To identify specific treatment options for desmosomal diseases it is required to better elucidate the mechanisms regulating desmosome adhesion. We identified a disease-relevant adhesion receptor consisting of Dsg3 and p38MAPK and propose that signaling pattern may define the clinical phenotype of pemphigus. A new experimental approach to treat pemphigus in vivo is to stabilize Dsg binding by peptide-mediated crosslinking. Similarly, a Dsg linking peptide is effective to reduce arrhythmia in a mouse model of AC. Moreover, since in the intercalated discs of cardiomyocytes the β-adrenergic receptor is located also, we investigated whether adrenergic signaling regulates cardiomyocyte cohesion. We identified positive adhesiotropy as a new function of sympathetic signaling in the heart which is caused by protein kinase A (PKA)-mediated phosphorylation of Pg. Taken together, stabilization of desmosomal contacts appears to be relevant under physiologic conditions and may serve as new therapeutic strategy to treat desmosomal diseases.

S2A.2. Vascular epithelial Na^+ channels mediate myogenic arterial responsiveness

Drummond, H.

University of Mississippi, USA

Modulation of tissue integrity via stabilization of desmosomes in health and disease Jens Waschke Institute of Anatomy and Cell Biology, Ludwig-Maximilians-Universität (LMU), Munich, Germany Desmosomes are adhering junctions present in all epithelia but most abundant in cells of tissues subjected to extensive mechanical stress such as in keratinocytes of the epidermis and in cardiomyocytes of the heart muscle. The core of desmosomes consists of desmosomal cadherins which are tethered to intermediate filaments via adaptor proteins including plakoglobin (Pg) and desmoplakin. Desmosomal diseases affect the skin in the autoimmune blistering disease pemphigus via autoantibodies against desmogleins (Dsg) or impair heart function by mutation of desmosomal components in Arrhythmogenic cardiomyopathy (AC). There is evidence that desmosomal cadherins besides their adhesive properties orchestrate multiple signaling pathways and thus desmosomes should be regarded as signaling hubs. To identify specific treatment options for desmosomal diseases it is required to better elucidate the mechanisms regulating desmosome adhesion. We identified a disease-relevant adhesion receptor consisting of Dsg3 and p38MAPK and propose that signaling pattern may define the clinical phenotype of pemphigus. A new experimental approach to treat pemphigus in vivo is to stabilize Dsg binding by peptide-mediated crosslinking. Similarly, a Dsg linking peptide is effective to reduce arrhythmia in a mouse model of AC. Moreover, since in the intercalated discs of cardiomyocytes the β-adrenergic receptor is located also, we investigated whether adrenergic signaling regulates cardiomyocyte cohesion. We identified positive adhesiotropy as a new function of sympathetic signaling in the heart which is caused by protein kinase A (PKA)-mediated phosphorylation of Pg. Taken together, stabilization of desmosomal contacts appears to be relevant under physiologic conditions and may serve as new therapeutic strategy to treat desmosomal diseases.

S2A.3. An Alliance of Biophysics, Biochemistry and Genetics initiates cancers

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Appropriate mechanical forces on cells are vital for healthy cell behaviour and this paper discusses the possibility that the initiation of a tumour depends on the disruption of the normal physical architecture of the extracellular matrix (ECM) around a cell. The alteration that occurs thence promotes oncogene expression. Some questions that are not answered with certainty with current consensus mechanisms of tumorigenesis (e.g. the late onset of cancers even when most mutations occur early in life) are more elegantly explained by executive control of tumours being within the physical and mechanical characteristics of the ECM rather than at the level of gene activity. Clinical relevance of that alternative hypothesis is illustrated in (i) studies that recognise effects of mutations in the ECM (e.g. chondrosarcoma, glioblastoma, dermatofibrosarcoma protuberans, hepatocellular carcinoma, pancreatic cells), (ii) orthotopic transplants (compared to heterotopic tissues), (iii) aging, and (iv) physical trauma and scars (including skin, breast and liver). Further, studies are described where returning extracellular signals to normal (e.g. by using modified culture substrates) or inhibiting aberrant signals (e.g. by using anti-integrin antibodies or affecting *COL* mutation expression) induced cells exhibiting a cancer phenotype to revert to having a normal healthy phenotype. Overall, reported observations suggest that restoring a pristine ECM or targeting the related signal transduction mechanisms may possibly be utilised to modify or control the progression of cancers. Thus this discussion suggests that the ECM may have both executive function in induction of a tumour and also may be a route for controlling cancer. This approach provides a coherent skeleton for discussing the notion, in the context of contemporary knowledge, that tumorigenesis is an alliance of biochemistry, genetics and biophysics in which physical architecture provided by the ECM may be a fundamental component.

S2A.4. Epithelial sodium channel, ENaC, as a potential therapeutic target for breast cancer

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Breast cancer is the most common cancer affecting New Zealand women. Despite a huge global effort pathways of breast cancer progression and metastasis are unknown. Ion channels are emerging as novel regulators of cancer cell proliferation and metastasis. The epithelial sodium channel, ENaC, is well known for its role in Na^+ reabsorption in epithelia. A number of novel roles for ENaC have been described, including potential roles for ENaC in cancer. However, a role for ENaC in breast cancer has yet to be described. Therefore, in this project, we investigate a novel unrecognised role for ENaC in the growth and migration of breast cancer cells. We identified that ENaC mRNA expression is significantly reduced in more aggressive tumours and in metastatic breast cancer cell lines, suggesting ENaC mRNA level correlates with breast cancer prognosis. Breast cancer cell proliferation was determined using both the MTT and EdU assays and showed that ENaC overexpression reduced, and ENaC knockdown increased, breast cancer cell proliferation. These alterations were due to changes in ENaC activity suggesting active ENaC promotes an epithelial phenotype. In breast cancer cell migration (scratch) assays, inhibition or promotion of ENaC activity alters cell migration. ENaC is attached to both the extracellular matrix and the cytoskeleton, allowing it to sense changes in the environment and cause intracellular changes in response. Therefore we are using atomic force microscopy to determine if ENaC has a role in breast cancer cell stiffness and morphology.
In conclusion, changing ENaC levels and activity is able to alter breast cancer cell proliferation and migration highlighting ENaC as a potential target for breast cancer treatment.

S2B.1. Role of the medial preoptic area in parental care and infanticide

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Mammalian neonates are born immature and require intense care for nutrition, protection, and locomotion. Mammalian mothers are equipped with motivation to nurture them. And in the species that live in a family group, fathers and older siblings may also provide extensive care to the young, in a manner similar to maternal care except for nursing. By studying those highly social species, such as laboratory mice, marmosets, and humans, we are trying to elucidate the neural mechanisms of parental care in general.

Neuronal activity mapping and site-specific functional suppression identified the central part of the medial preoptic area (cMPOA) as the hub of parental care network in the mouse brain[1]. In addition, the rhomboid nucleus of bed nuclei of stria terminalis (BSTrh), a part of the extended amygdala, was shown to facilitate male infanticide of non-offspring pups. The circuit between the cMPOA and BSTrh suppressed infanticide and enabled paternal behavior even toward non-offspring after mating in male mice.

The anterior commissural nucleus (ACN) adjacent to the cMPOA is the third-largest nucleus of magnocellular oxytocin neurons. The role of oxytocin in maternal behavior, in particular in stress resilience required for motherhood, will also be discussed based on the recent results[2].

1. Tsuneoka, Y., Tokita, K., Yoshihara, C., Amano, T., Esposito, G., Huang, A.J., Yu, L.M., Odaka, Y., Shinozuka, K., McHugh, T.J., et al. (2015). *Distinct preoptic-BST nuclei dissociate paternal and infanticidal behavior in mice*. EMBO J *34*, 2652-2670.
2. Yoshihara, C., Numan, M., and Kuroda, K.O. (2017). *Oxytocin and Parental Behaviors*. Curr Top Behav Neurosci. https://doi.org/10.1007/7854_2017_11

S2B.2. Rebalancing the addicted brain: prosocial compounds for treating substance use disorders

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One of the most debilitating aspects of substance use disorders is the profound social withdrawal and social isolation that can often occur as a result of chronic substance abuse. Considerable potential benefit might therefore be derived from developing pharmacological interventions for addiction that serve to enhance social motivation. Over the past decade there has been growing interest in exploring the anti-addictive effects of the so-called social neuropeptide, oxytocin. However, despite promising preclinical findings, pharmacokinetic issues will likely prevent oxytocin itself from unlocking the full potential of targeting the social brain to treat substance use disorders. We are thus developing a novel small-molecule, KNX100, with powerful prosocial and anti-addictive effects in preclinical models. KNX100 has excellent oral bio-availability, readily enters the brain and has a long half-life, overcoming the substantial pharmacokinetic challenges presented by oxytocin. Peripherally administered KNX100 increased social preference and social interaction in rats and restored normal social behaviour in a mouse model of autism spectrum disorder. It inhibited self-administration of opioids in rodents, and alcohol and stimulants in both rodents and non-human primates. KNX100 blocked prime-induced reinstatement of methamphetamine-seeking in rats, cue-induced reinstatement of oxycodone seeking in rats, and reduced the severity of nicotine withdrawal symptoms in mice. Importantly, KNX100 itself does not appear to have abuse liability. In initial preclinical safety studies KNX100 was well-tolerated with a wide therapeutic windows. KNX100 is now being developed by a University of Sydney spinout company, Kinoxis Therapeutics, with first-in-human trials due to commence in 18 months.

S2B.3. Prolactin and parental care in the zebra finch

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

S2B.4. The modulation of maternal behaviour by prolactin

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Parental care is critical for the survival of dependent offspring, with maternal care being the predominant form of care in mammals. The interactions between a mother and child are controlled by complex neural circuitry, with the medial preoptic area of the hypothalamus forming a critical nexus that integrates hormonal and sensory inputs into this circuitry. The specific actions of the anterior pituitary hormone, prolactin, on this circuitry and its role in modulating maternal behaviour has been unclear. By deleting prolactin receptors specifically from the MPOA using an adeno-associated virus expressing Cre-recombinase injected into prolactin receptor flox mice, we have recently shown that prolactin signalling is critical for the normal onset of maternal behaviour. Using a conditional transgenic strategy to remove prolactin receptors from specific neuronal populations, we have also found that prolactin acts on glutamatergic neurons to modulate the display of maternal aggression towards an intruder. These data are the first to demonstrate that prolactin action is important for both the onset of maternal behaviour and for modulating specific aspects of maternal behaviour in the postpartum period.

S3A.1. Nanodomains and Electrical Conduction at Cardiac Gap Junctions

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Computational modeling indicates that cardiac conduction may involve ephaptic coupling – intercellular communication involving electrochemical signaling across narrow extracellular clefts between cardiomyocytes. We hypothesized that β1(SCN1B) –mediated adhesion scaffolds *trans*-activating $Na_V1.5$ (SCN5A) channels within narrow (<30 nm) perinexal clefts adjacent to gap junctions (GJs), facilitating ephaptic coupling. Super-resolution imaging indicated preferential β1 localization at the perinexus, where it co-locates with $Na_V1.5$. Smart patch clamp (SPC) indicated greater sodium current density (I_{Na}) at perinexi, relative to non-junctional sites. A novel, rationally designed peptide, βadp1, potently and selectively inhibited β1-mediated adhesion, in electric cell-substrate impedance sensing studies. βadp1 significantly widened perinexi in guinea pig ventricles, and selectively reduced perinexal I_{Na}, but not whole cell I_{Na}, in myocyte monolayers. In optical mapping studies, βadp1 precipitated arrhythmogenic conduction slowing. In summary, β1-mediated adhesion at the perinexus facilitates action potential propagation between cardiomyocytes and may represent a novel target for anti-arrhythmic therapies.

S3A.2. Elucidating Nanoscale Determinants of Synaptic Function

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In the brain, fast excitatory synaptic transmission is mediated by AMPA and NMDA-type glutamate receptors, enabling rapid communication between neurons. The strength of synaptic communication is 'plastic', i.e. it can be altered, and these changes underpin learning and memory at the cellular level. The advent of super-resolution imaging has revealed at nanoscale resolution the ultrastructure of excitatory synapses, in which synaptic proteins display precise distribution patterns that could directly influence how synapses function in the brain. Using d-STORM super resolution imaging we have recently shown that AMPA receptors are localised in discrete pools at both synaptic and extrasynaptic sites, and that the location of these receptor pools is highly regulated by postsynaptic scaffold proteins. Moreover, alternative splicing of these scaffold proteins induce different receptor localisations, suggesting that individual synaptic proteins have divergent functional and structural roles in both physiological and pathophysiological synaptic states. Extrasynaptic receptors are of significant interest as they are known to trigger cell death signalling pathways in neurodegenerative diseases such as Huntington's disease (HD). Using dSTORM super-resolution imaging, we reveal that mutant *HTT* drives the elevation of extrasynaptic NMDA but not AMPA receptor clusters located 100-500 nm from the postsynaptic density. This was accompanied by a decline in synaptic NMDAR-mediated currents. Intriguingly, upregulation of synaptic scaffold proteins α- or βSAP97 increased synaptic and/or perisynaptic NMDAR localisation and prevented the shift of NMDARs to extrasynaptic sites in mutant *HTT* neurons. This was accompanied by the rescue of normal synaptic NMDAR-mediated currents. Taken together, our high resolution data reveals plasticity in surface NMDAR localisation driven by mutant *HTT*, and identifies the similar but independent roles of SAP97 isoforms in rescuing normal synaptic function in pathological states.

S3A.3. Super resolved calcium channel organisation in the heart

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Contraction of the heart relies on a process called excitation-contraction coupling, in which the controlled cycling of calcium (Ca^{2+}) in the cardiomyocytes is crucial. The ryanodine receptor (RyR) is a Ca^{2+} release channel localised to the sarcoplasmic reticulum (SR) within the cardiomyocyte and is responsible for the generation of the Ca^{2+} transient – a cellular event critical for enabling contraction. Impaired RyR function is often associated with the development of heart disease, including over-activity of the channels in atrial fibrillation (AF), the most common form of arrhythmia. RyRs are organised into clusters within the terminal SR, with changes in cluster organisation linked to altered Ca^{2+} handling properties of the RyR channels. However, the nanoscale nature of these clusters leads to the inability to detect subtle changes in their organisation using conventional imaging modalities.

Using super resolution imaging (dSTORM), we have investigated the nanoscale organisation of RyR clusters in both human and animal models of heart disease. In transgenic mice, changes in the expression of junctophilin-2 (JPH2) – a protein associated with RyR clusters, can alter cluster organisation including size and channel density, providing insights to the altered Ca^{2+} release properties observed in these animals. In human AF patients, there is an increased mean cluster size compared to non-AF patients, indicating the potential for more individual RyR channels per cluster. There is also evidence of reduced nearest-neighbour distances between RyR clusters in AF, suggesting enhanced propagation of abnormal Ca^{2+} release throughout the cardiomyocyte, contributing to the generation of arrhythmic events. These findings reveal how subtle changes in the organisation of RyR clusters can be implicated in functional alterations to the cardiomyocyte and cardiac function, which could not have been identified without the use of super resolution imaging.

S3A.4. High content super-resolution microscopy

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Super-resolution microscopy methods are becoming increasingly mainstream. The typical PALM/STORM experiment, however, is still time consuming, labour intensive, and looks only at a very small number of cells. The interpretation of results obtained is often also rather qualitative. I will discuss our realization of a high–throughput platform capable of automatically imaging of 10,000 cells in day, as well as efforts to improve quality, ease of use, and to extract as much information out of data as possible. This combination allows us to image large populations of cells and to compare the results to population based methods such as genomics.

S3A.5. Fibrosis at the Nanoscale in the Failing Human Heart

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Fibrosis is considered an important mechanism of pathology in many diseases, including the development of heart failure. Through the use of super-resolution method of Stochastic Optical Reconstruction Microscopy, commonly referred to as STORM, we have identified aberrant deposition of collagen may directly disrupt the electrical activity that regulates contraction of cardiac myocytes. A key nanostructure of the myocytes is the transverse tubules. These structures are tubular invaginations of the plasma membrane, ~300 nm in diameter, that penetrate the cell in spoke like pattern at regular 1.8 μm intervals along the entire length of the myocyte. Their function is to conduct the cardiac action potential deep into the cell interior where they facilitate a synchronous calcium release and contraction. Pathological remodelling t-tubules is known to contribute to loss of contractility in the failing heart but the mechanism that drives this remodelling remains a mystery. For the first time, we have identified increased accumulation of collagens I, III, and VI within the lumen of enlarge t-tubules of the failing human heart, leading us to propose that fibrosis at the nanoscale is responsible for their remodelling. This work was recently featured on the cover of the journal Cardiovascular Research (doi:10.1093/cvr/cvx055).

S3B.1. Obesity epidemic fuelling the surge of endometrial cancers: How fat cells drive endometrial hyperplasia and cancer development?

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Endometrial cancer is the most common gynecological cancer. Obesity is an independent risk factor for this disease and approximately 50% of cases are associated with high body mass index. Intentional weight loss in women significantly lowers their risk of endometrial cancer. With global rise in obesity, the significant increase in endometrial cancer incidence and mortality is expected to pose a major challenge to healthcare services. At present, how adipocytes drive pathogenesis of endometrial hyperplasia and cancer is unclear. Understanding the molecular mechanisms involved in crosstalk between endometrial cells and adipocyte will help in developing new preventive and therapeutic measures for this disease. Using human patient samples, endometrial cell lines, and preclinical hyperphagic animal models, we have established that adipocyte-derived VEGF-mTOR signalling drives the growth of endometrial cells leading to hyperplasia and cancer. This work provides a mechanistic explanation for the occurrence of endometrial cancer in obese women and reveals that VEGF-mTOR signalling may be an attractive therapeutic target against endometrial cancer.

S3B.2. Beyond GWAS – how do we utilize genetic information to better understand a complex disease like endometriosis?

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Endometriosis is a common, oestrogen-dependent disease in which endometrial-like tissue is found in ectopic locations, causing pelvic pain and infertility. While endometriosis has a heritable component, there is no single causative gene. Instead, multiple gene variants or single nucleotide polymorphisms (SNPs) contribute to disease risk, each with small effect size. To date, 16 genomic regions have been associated with increased risk of endometriosis, of which 14 have been replicated in multiple studies[1]. The task now is to determine how these regions contribute to disease aetiology. Since 2010, we have recruited women undergoing laparoscopy for diagnosis of potential endometriosis (n=792 to date) for a programme of research aimed at understanding the genetic regulation of endometriosis. In addition to blood and endometrial samples, and access to pathology samples, we have collected comprehensive patient data including symptom profiles, menstrual history and surgical and other clinical data. Using a subset of samples, we have identified endometrial cis- expression quantitative trait loci (eQTLs) for 408 genes and trans-eQTL for 82 genes. Two eQTLs were located within known risk regions for endometriosis (*VEZT* and long non-coding RNA *LINC00339)*. The contribution of *VEZT* and *LINC00339* to disease aetiology are unknown and studies investigating their function in endometrial tissues are currently underway. In parallel work, we are employing cutting-edge Fourier-transform infrared spectroscopy (FT-IR) and mass-spectrometry imaging to characterise patterns of metabolites and proteins in endometrium and endometriotic lesions. While still in the very early stages, ultimately, we hope these studies will allow us to investigate metabolite and protein-based QTL as a means of advancing our understanding of the genetic mechanisms contributing to endometriosis. In combination, our extensive bank of patient tissues and data provide an invaluable resource for ongoing studies aimed at enhancing understanding, identifying diagnostics, and improving therapeutic options for this complex gynaecological disease.

1. Yadav, S. et al. (2017) *Meta-analysis identifies five novel loci associated with endometriosis highlighting key genes involved in hormone metabolism*. Nature Communications. 8: 15539.

S3B.3. Treatment of preeclampsia in pregnancy: pitfalls and possibilities

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Preeclampsia (PE) is characterised by new-onset hypertension after 20 weeks of gestation, in association with proteinuria, maternal kidney and liver dysfunction and/or uteroplacental insufficiency. Affecting 2-8% of pregnancies worldwide, it contributes to >60,000 maternal and >500,000 infant deaths annually. For women with severe, early-onset PE, the baby has to be delivered preterm to save the mother. This leads to serious risks and long-term childhood consequences associated with premature birth. Underlying the diverse organ dysfunction in PE is the widespread vascular dysfunction in maternal systemic and placental blood vessels.

For the last 60 years, treatment of PE has focused almost exclusively on controlling the hypertension. Despite our expert clinical use of anti-hypertensives drugs, research over the past decade has revealed that managing the hypertension only temporarily masks the main symptom of PE. But it fails to correct the underlying cause of the clinical symptoms – the systemic vascular dysfunction.

Our research has identified the peptide hormone relaxin as a potential effective short-term treatment for PE. Relaxin is a naturally occurring peptide in pregnant women and has gained considerable attention as a “vasoprotective” drug, largely through its direct beneficial effects on the vasculature. Importantly, there are no identified safety risks associated with relaxin infusion in pregnant women. I will present data from animal and human blood vessel studies to demonstrate why relaxin is a strong candidate drug to treat women at any stage of PE once diagnosed due to its actions on a broad spectrum of vascular pathways, with rapid onset of clinical improvement (hours) after a short duration of treatment.

S3B.4. Potential therapies for fetal growth restriction secondary to placental insufficiency

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Placental insufficiency is the commonest cause of fetal growth restriction (FGR) in developed countries. FGR increases the risk of fetal and neonatal death, preterm birth and postnatal morbidity, including both neurodevelopmental and metabolic impairment. Currently, there is no therapy for FGR and management aims to identify the optimal time for birth to balance the intra-uterine risks with the postnatal risks associated with preterm birth.

We have been investigating potential maternal and fetal therapies for FGR, aimed at improving fetal oxygen and nutrient supply without increasing the risk of fetal compromise. These studies have been conducted in fetal lambs, enabling detailed assessment of fetal health throughout treatment and, more recently, the consequences through to adulthood following intra-uterine therapy.

Maternal sildenafil citrate (Viagra®) has shown promise in short-term animal studies and currently is in clinical trials. Intra-amniotic IGF-1 therapy also has been shown to be effective in several fetal sheep studies and appears to act through effects on the fetal gut, liver and on placental amino acid transport without adverse effects through to adulthood.

Translating fetal therapies from animal studies through to clinical trials is challenging, particularly when assessments of fetal wellbeing are relatively crude. Preliminary data suggest that the aminoterminal product of C-type Natriuretic Peptide may have potential utility as a marker of fetal hypoxia.