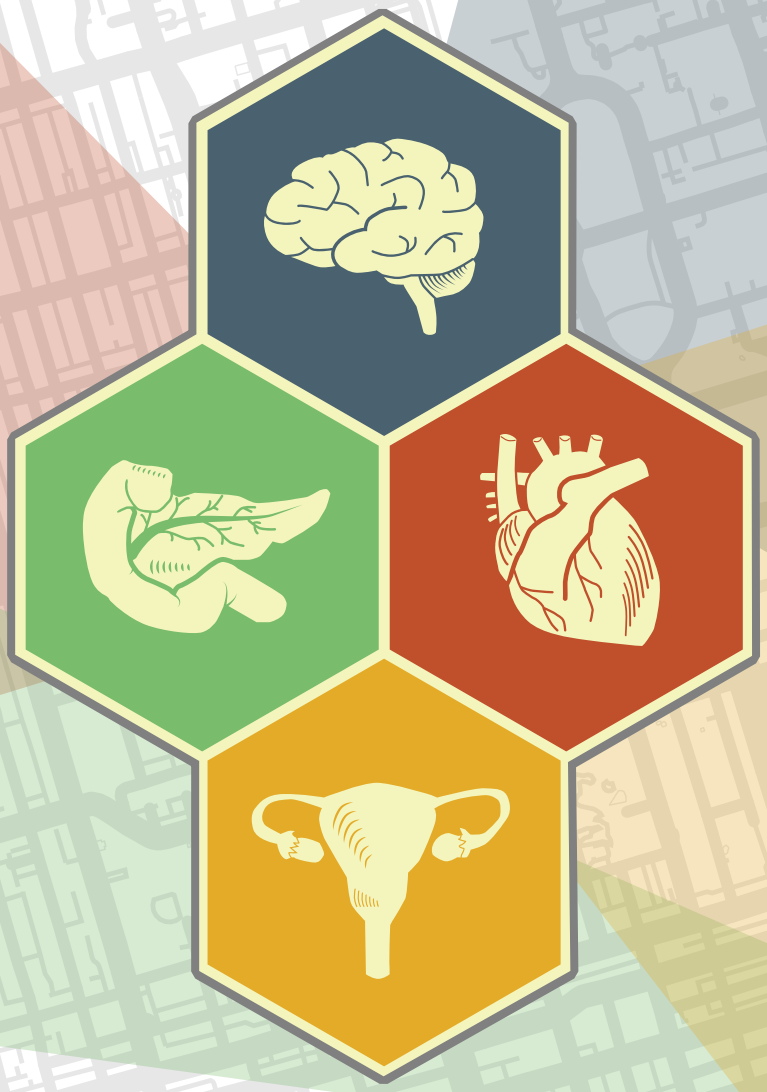

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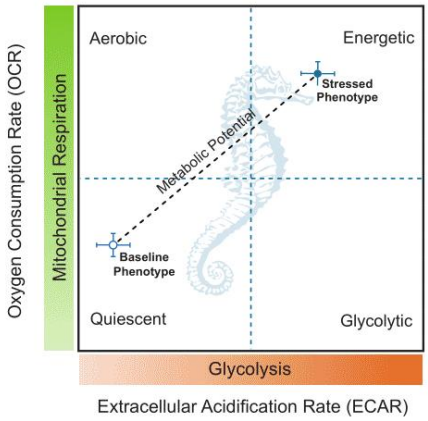

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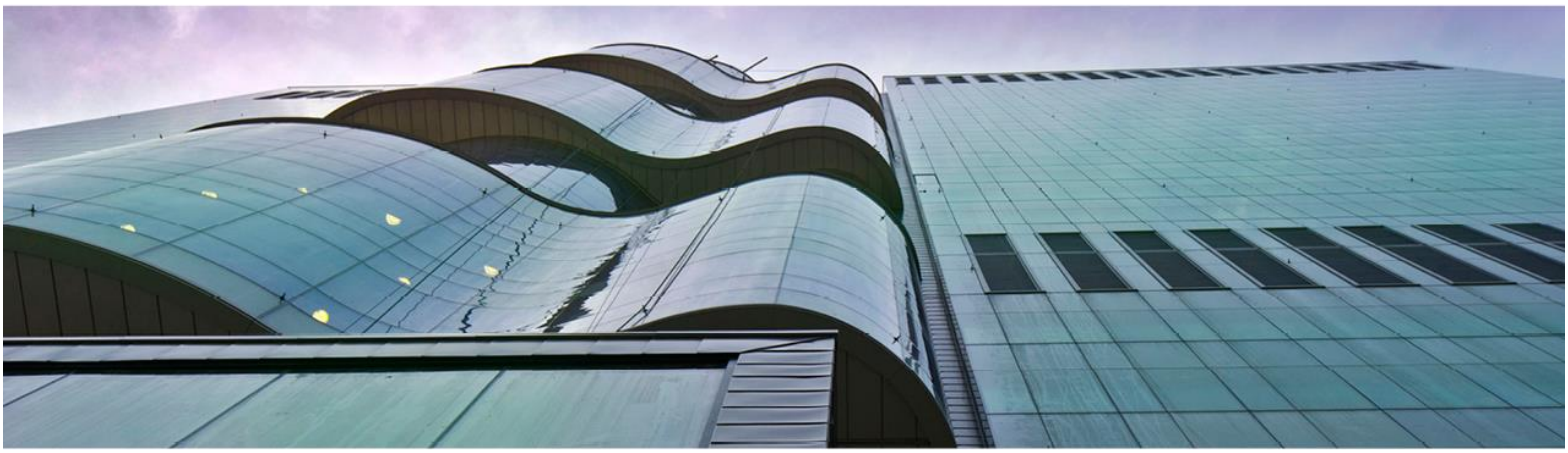

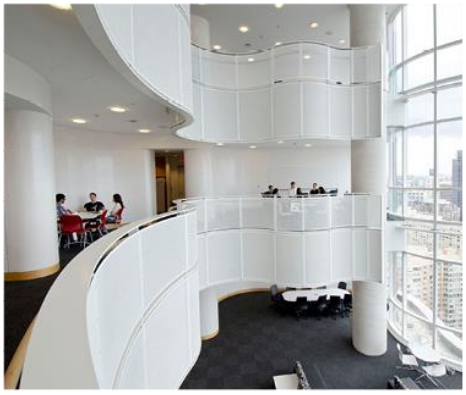

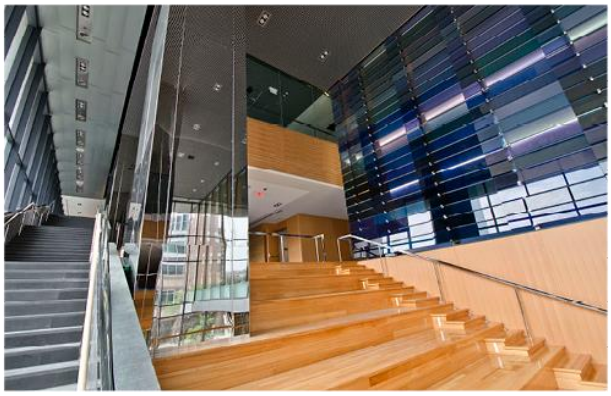

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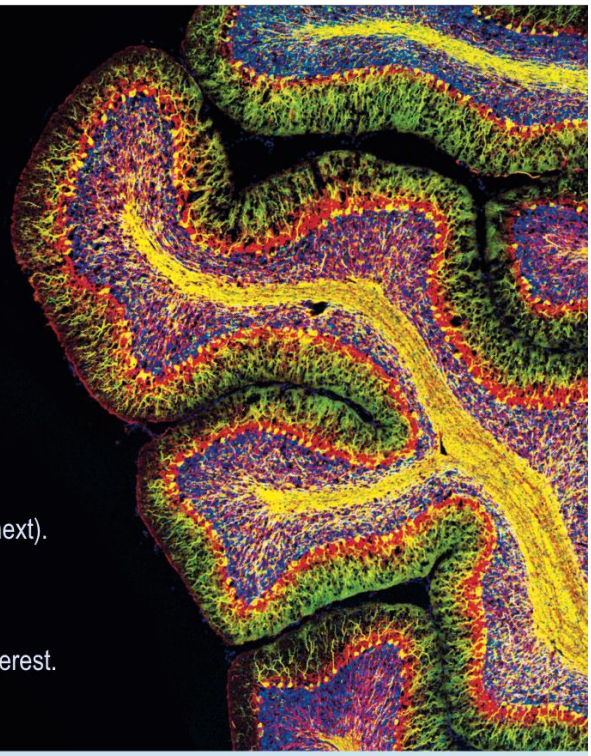

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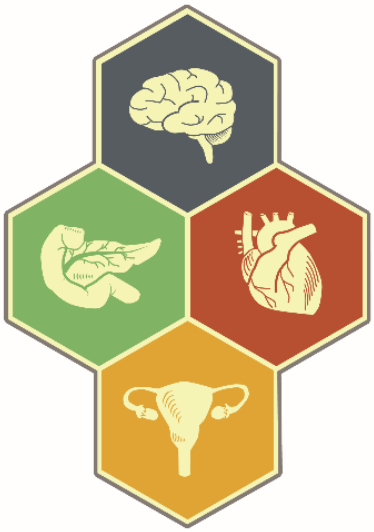

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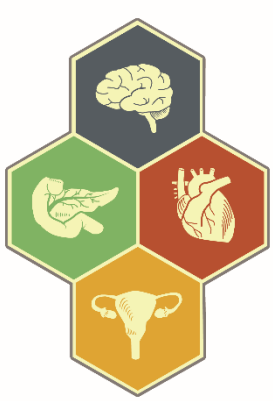

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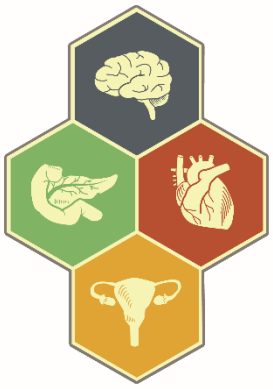


Wednesday, May 11th, 2016

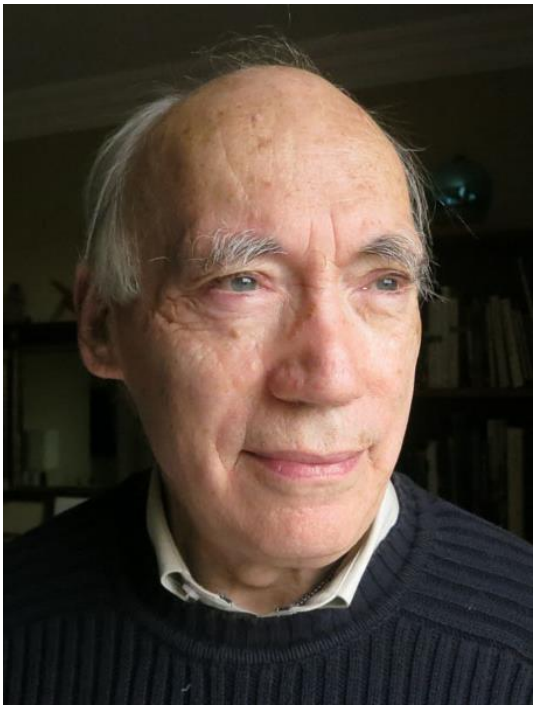
8:00 – 9:00	Registration & Light Breakfast	MSB Student Commons
9:00 – 9:15	Opening Remarks	JJR Macleod Auditorium
9:15 – 10:15	Oral Presentation Session 1	JJR Macleod Auditorium
10:15 – 10:30	Break	-
10:30 – 11:30	Oral Presentation Session 2	JJR Macleod Auditorium
11:30 – 12:15	Lunch	Lobby - JJR Macleod Auditorium
12:20 *sharp*	**Departmental Photo**	MSB Front Steps
12:30 - 1:30	Poster Viewing & Judging	MSB Student Commons
1:30 – 1:45	Break	-
1:45 - 2:45	Oral Presentation Session 3	JJR Macleod Auditorium
2:45 - 3:00	Break	-
3:00 - 4:00	**Macallum Lecture: "Synaptic plasticity and the neural basis of memory", Professor Tim Bliss, FRS**	JJR Macleod Auditorium
4:00-4:30	Macallum & Alumni Reception	Lobby - JJR Macleod Auditorium
4:30-5:00	FIP Awards Ceremony	MSB Student Commons
5:00-6:00	FIP Reception	MSB Student Commons

Thursday, May 12th, 2016

11:00 - 12:00	**FIP Keynote Lecture: "A pre-post-erous debate: expression mechanisms in LTP", Professor Tim Bliss, FRS**	JJR Macleod Auditorium

Prof. Tim Bliss, FRS

Keynote Speaker



National Institute for Medical Research, London, UK

Prof. Tim Bliss' search for the neural basis of learning and memory began in 1964 when he joined the laboratory of Dr. Ben Delisle Burns in the Physiology Department at McGill University in Montreal for his PhD. At the time, although it was widely believed, as it is today, that memory formation relies on long-term changes in the strength of connections between neurons in the brain, researchers had been unable to show that such a phenomenon could indeed occur in the mammalian brain.

After obtaining his PhD in 1967, Prof. Bliss joined the MRC National Institute for Medical Research in Mill Hill, London, where he would later head the Division of Neurophysiology from 1988 to 2006. In 1968, while visiting the laboratory of Dr. Per Andersen in Oslo, that he made his most famous discovery, which remains to this day one of the most important findings in the field of learning and memory. Indeed, in collaboration with Dr. Terje Lømo, Prof. Bliss showed that high frequency stimulation of neuronal fibers could induce long-term changes in the strength of their connections. This phenomenon, now called long-term potentiation (LTP), provided one of the first demonstrations of how memories can form in the brain.

Following this discovery, Prof. Bliss continued to investigate the mechanisms underlying LTP, contributing significantly to our understanding of the learning and memory in health and disease. A fellow of the Royal Society since 1994, he is also a founding fellow of the Academy of Medical Sciences. For his important contributions, he has received many prestigious prizes, including the Bristol Myers Squibb Award for Neuroscience with Dr. Eric Kandel (1991), the Feldberg Prize (1994) and the Croonian Prize Lecture of the Royal Society (2012) which he gave on the topic of "The Mechanics of Memory". More recently, in 2016, he received one of the most coveted awards in science, the Grete Lundbeck European Brain Research Prize, which recognizes researchers who have made highly original and influential contributions in neuroscience. He shares this prize with Profs. Richard Morris and Graham Collingridge, our Departmental Chair. Prof. Bliss' career is an inspiration to young researchers today and we are honoured to welcome him as the keynote lecturer for FIP 2016.

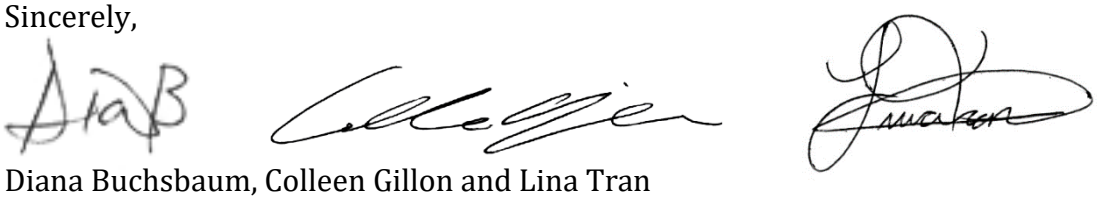
Message from the FIP Co-Chairs

On behalf of the Graduate Association of Students in Physiology (GASP), we are thrilled to welcome you to the 36th annual Frontiers in Physiology (FIP) research symposium! This symposium showcases the cutting-edge research currently being conducted by graduate students, as well as undergraduate students and postdoctoral fellows, in the Department of Physiology at the University of Toronto. Through this exciting event, we hope to facilitate the exchange of scientific ideas amongst students and faculty members, promote intradepartmental collaboration and unite the research powerhouse that is the University of Toronto and its affiliated teaching hospitals and research institutions.

Each year, the Department of Physiology invites a world leader in physiology research to present the FIP Keynote Lecture. This year, we are privileged to welcome **Professor Tim Bliss, FRS**, a researcher at the National Institute for Medical Research, UK, and pioneer in the study of neuroplasticity in the brain. Building upon the brilliant insights of researchers like the Spanish neuroscientist Santiago Ramón y Cajal, and the Canadian psychologist, Donald Hebb, Prof. Bliss and his colleague Dr. Terje Lømo made a ground-breaking discovery in 1968 when they showed that strong electrical stimulation can persistently strengthen the connections between neurons. His research into this process, called LTP, has been instrumental to advancing our understanding of how memories are formed in the brain, and has had important implications for research on Alzheimer's Disease and other illnesses involving dementia. In addition to delivering this year's Macallum Lecture on May 11th on "Synaptic plasticity and the neural basis of memory", Prof. Bliss will present the **FIP Keynote Lecture**, "A pre-post-erous debate: expression mechanisms in LTP", on May 12th. We look forward to learning more about his research and ideas through these talks, which will certainly be both inspiring and informative.

Every year, FIP's success depends greatly on the generous contributions and work of several individuals and groups. Thus, we would like to thank the members of the **FIP Planning Committee** and the **GASP council** under the leadership of **Frances Wong** for their extensive help in planning the logistics and details of this symposium. We are particularly grateful for the continued support of the Department of Physiology, particularly our Chair, **Prof. Graham Collingridge**, Associate Chairs, **Profs. Denise Belsham** and **Scott Heximer**, and Graduate Coordinators **Profs. Zhong-Ping Feng** and **Douglas Tweed**, as well as the members of the department's administrative staff, **Eva Eng, Jenny Katsoulakos, Victor Lee, Rosalie Pang, Colleen Shea, Paula Smellie, and their colleagues**, whose help at every step has been instrumental to the success of this event. We would also like to thank all the trainees who are sharing their innovative research today, as well as the faculty members and postdoctoral fellows who have kindly volunteered their time to act as judges for both oral and poster presentations. Finally, we would like to express our most sincere gratitude to our **institutional and commercial sponsors** for their financial support. Without their generous support of research and training, today's event would not have been possible. We are delighted to have you all with us today and we hope you will enjoy this stimulating and inspirational day.

Sincerely,



Diana Buchsbaum, Colleen Gillon and Lina Tran
FIP Co-Chairs

Message from the Chair of the Department of Physiology

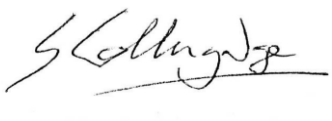
On behalf of the Department of Physiology, it gives me great pleasure to welcome everyone to the annual **"Frontiers in Physiology Research Day".** This Symposium, which celebrates the research achievements of our trainees, has been organized by our graduate students for the past 36 years. Today will showcase the exciting and innovative work conducted by our trainees who represent the future of Physiology in Canada and around the world. We are extremely proud of their accomplishments and dedication to science, and look forward to an outstanding day of presentations.

We warmly welcome internationally renowned neuroscientist **Professor Tim Bliss FRS**, one of three recipients of the recently awarded **Brain Prize, as this year's** FIP Keynote Speaker. Professor Bliss' pioneering studies on the synaptic basis of learning and memory is legendary, and we look very much forward to his lectures and visit to the Department.

Special thanks and acknowledgement is due today to **Ms. Colleen Gillon, Ms. Lina Tran and Ms. Diana Buchsbaum,** FIP co-Chairs and Vice-Presidents of the Graduate Association of Students in Physiology (GASP). Colleen, Lina, Diana and their team have done a brilliant job in spearheading the process to ensure the success of this day. A special thank you also to **Ms. Frances Wong,** President of GASP, and to everyone who helped make this year's Research Day possible.

We hope you enjoy the day. Thank you for your support and enthusiasm!

Best regards,



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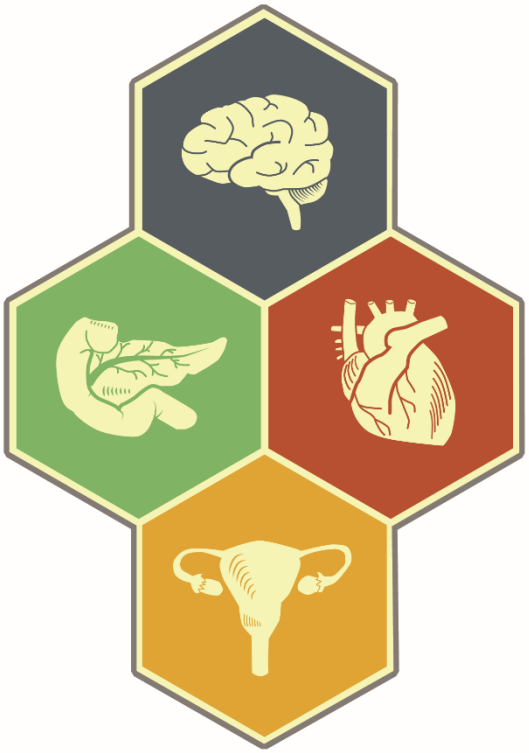

Oral Presentations

Oral Presentation Session 1

1.1. OVEREXPRESSION OF TROPHOBLAST STEM CELL-ENRICHED MICRORNAS PROMOTE TROPHOBLAST FATE IN EMBRYONIC STEM CELLS

U. Nosi[1], F. Lanner[2], B. Cox[1]

Department of Physiology[1]*, University of Toronto, ON, Canada; Department of Clinical Science, Intervention and Technology*[2]*, Karolinska Institute, Sweden*

In the current study, we have investigated microRNA-mediated post-transcriptional control of cell fate, and provide evidence of their involvement in determination of trophoblast versus the simultaneously specified embryonic lineage by using *in vitro* stem cell models representative of these early lineages (TSCs and ESCs, respectively). High throughput analysis identified 3 TSC-enriched miRNAs targeting pluripotency network-associated mRNAs. Transgenic, overexpressing ESCs with inducible expression of the miRNAs were derived, and induction was performed in the presence of an HDAC2 inhibitor. Ectopic expression of candidate miRNAs in ESCs caused a loss of pluripotency as evidenced by the down-regulation of pluripotency-associated genes at both protein and mRNA levels in a time-dependent manner. This loss of ESC identity was associated with upregulation of TSC marker genes Cdx2 and Elf5. Global transcriptional analysis of cells following 6 days of miRNA induction revealed that upregulated genes were enriched in gene ontology terms associated with placenta/trophoblast development. These miRNA-induced ESCs not only adopt a TSC morphology, but also more closely resemble the global transcriptional profile of wild-type TSCs than ESCs, as shown by principle component analysis. Moreover, we note that multiple pathways required for maintaining ESC and TSC fate; including signaling and epigenetics, were significantly affected, indicating that miRNA overexpression has a widespread effect on gene expression. Lastly, when transplanted *in vivo*, these cells contribute exclusively to the trophectoderm of blastocysts, and extra-embryonic tissues of the post-implantation embryo. Using this stem cell conversion model, we show that trophoblast fate-specifying microRNA overexpression in ESCs is sufficient to drive trans-differentiation to TS-like state. The mechanism of miRNA-mediated cell fate conversion is being explored. Moreover, we are currently investigating *in vivo* localization of our candidate miRNAs in pre-implantation-stage embryos. The fundamental knowledge elucidated from this study is crucial in identifying pathways underlying lineage segregation and establishment of the trophectoderm, thus being of great importance for understanding of implantation and development.

1.2. SEROTONIN AND CORTICAL DISINHIBITION: A NOVEL SYNERGY BETWEEN 5-HT1A AND 5-HT2A RECEPTORS IN PREFRONTAL CORTEX

M. Tian[1], E. Lambe[1, 2, 3]
Departments of Physiology[1], Obstetrics and Gynaecology[2], Psychiatry[3], University of Toronto, ON, Canada

In medial prefrontal cortex, layer 6 pyramidal neurons play a critical role in attention and express receptors for serotonin (5-HT), a neuromodulator implicated in many psychiatric disorders with attention deficits. Yet, little is known about the direct electrophysiological consequences of 5-HT for layer 6 neurons themselves and their regulation of local cortical activity. We performed whole cell recording and pharmacological manipulations in acute brain slices of medial prefrontal cortex from transgenic mice expressing either eGFP or a fusion protein of eGFP-channelrhodopsin2 in layer 6 pyramidal neurons. Optogenetic excitation was used to test the effects of 5-HT on the inter-laminar excitatory circuit between layer 6 pyramidal neurons and layer 5 interneurons. Prefrontal layer 6 pyramidal neurons are strongly inhibited by 5-HT through activation of 5-HT_{1A} and, surprisingly, 5-HT_{2A} receptors. This serotonergic suppression of layer 6 neuronal excitability is complex, with the effect of 5-HT_{1A} receptors prominent across a range of membrane potentials and that of 5-HT_{2A} receptors strongest during spiking. Optogenetic investigation of the circuit between layer 6 pyramidal neurons to layer 5 interneurons shows that this connection is suppressed by 5-HT through 5-HT_{1A} and 5-HT_{2A} receptors. These findings reveal a novel modulatory role of 5-HT on cortical excitability through its strong inhibitory effects on layer 6 and its feedforward projections. Dysregulation of normal serotonergic signaling in prefrontal layer 6 would thus alter normal inhibitory cortical tone and change the signal-to-noise ratio within circuits important to attention.

1.3. DEVELOPMENT OF A NOVEL ZEBRAFISH VASCULAR LEAKAGE MODEL FOR HIGH-THROUGHPUT DRUG SCREENS

A. Philip[1,2,3], Y. Wang[1,3], C. dosSantos [1,2] and XY. Wen[1,2,3]
Keenan Research Centre for Biomedical Science and Li Ka Shing Knowledge Institute of St. Michael's Hospital[1], ON, Canada; Institute of Medical Sciences[2], University of Toronto, ON, Canada; Zebrafish Centre for Advanced Drug Discovery[3], St. Michael's Hospital, ON, Canada.

Vascular permeability accompanying normal physiological functions like immune surveillance, macromolecular transport, and wound healing is reversible and tightly regulated. In contrast, dysregulated vascular leakage is a hallmark of several human pathologies like sepsis, influenza and Ebola. Currently there is no disease-modifying therapy to effectively control the vascular leakage associated with these diseases. Therefore, advanced and economical animal models of vascular leakage, and newer strategies for drug discovery are urgently needed. As an alternative to traditional animal models, zebrafish have recently emerged as a powerful vertebrate paradigm to study human pathologies, and also as a key model organism for high-throughput *in vivo* drug screening. Here we show that vascular leakage can be effectively modeled in the zebrafish, and that this model is suitable for high throughput drug screens. Transgenic fluorescent zebrafish lines having flurescently labelled blood vessels were exposed to lipopolysaccharide (LPS) by water delivery. LPS at a concentration of 100µg/ml induces vascular leak in these larvae, accompanied by tail fin edema and swelling. Through the microinjection of microangiography contrast agents (quantum dots and FITC-dextran), we measured the degree and patterning of increased vascular leakage induced by LPS. Time-lapse imaging allowed the realtime-visualization of vascular leakage *in vivo*. These changes in endothelial permeability were also accompanied by a downregulation in the expression of cellular junction proteins namely occludins, claudins and ZO-1. Using a robotic system, hundreds of "leaky" fish in microplates can be treated with drug compounds that are tested for their ability to limit, reverse or/and treat vascular leakage. We further validated the suitability of this model for phenotype-based drug screening using fasudil, a drug known to rescue LPS induced vascular permeability in rodent models. Hence, this zebrafish model is expected to help accelerate the development of novel therapeutics targeting sepsis, as well as other diseases associated with vascular leakage.

1.4. REGULATION OF GLUCAGON-LIKE PEPTIDE-1 EXOCYTOSIS BY THE SNARE PROTEIN SYNTAXIN1A

S. Wheeler[1], H. Stacey[1], H. Gaisano[2], P. Brubaker [1,2]
Department of Physiology[1] and Medicine[2], University of Toronto

Glucagon-like peptide-1 (GLP-1) is an intestinal L cell hormone that potentiates insulin secretion in a glucose-dependent manner. As such, GLP-1-based mimetics are now widely used to treat hyperglycemia in patients with type 2 diabetes. In attempts to develop novel therapeutic approaches for the treatment of type 2 diabetes, the capacity to enhance endogenous GLP-1 secretion from the L cell in a regulated fashion is currently being explored. Although multiple secretagogues able to stimulate GLP-1 secretion have been identified, the mechanism of exocytosis remains unknown. We hypothesized that the core SNARE protein, Syntaxin1a, is required for GLP-1 exocytosis from the primary adult murine intestinal L cell. We thus generated an intestinal epithelial-specific inducible Syntaxin1a knockout mouse model (Syn1aKO), by crossing Syntaxin1a$^{flox/flox}$ mice with villin-creER^{T2} mice. Syn1aKO mice, in addition to age- and sex-matched Syntaxin1a$^{flox/flox}$, villin-creER^{T2}, and Syntaxin1a$^{flox/-}$; villin-creER^{T2} controls were all treated with and without tamoxifen. Syn1aKO and control mice were then challenged in vivo with an oral glucose tolerance test, followed by determination of circulating levels of total

plasma GLP-1. Syn1aKO mice demonstrated elevated blood glucose levels 60 mins post glucose administration in comparison to controls, 24.0 ± 1.9 vs 16.0 ± 1.0 mM ($P < 0.001$), respectively. Accordingly, the area under the 2-hour blood glucose curve for Syn1aKO mice, 2162 ± 126 mM-min, was significantly elevated compared to controls, 1783 ± 80 mM-min ($P < 0.01$). The glucose-intolerant state of Syn1aKO mice was associated with a 17.2% reduction in basal ($P < 0.05$) GLP-1 levels. At t = 10 mins post-glucose, the increment in GLP-1 was even further reduced (by 23.3%, $P < 0.05$), indicating impaired GLP-1 secretion in response to glucose in Syn1aKO mice. In conclusion, therefore, Syntaxin1a plays a vital role in mediating the exocytosis of GLP-1.

Oral Presentation Session 2

2.1. INVESTIGATING SPIKING RESONANCE IN COMPUTATIONAL MODELS OF ORIENS-LACUNOSUM/MOLECULARE (O-LM) HIPPOCAMPAL INTERNEURONS WITH DENDRITIC SYNAPTIC INPUTS

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The theta rhythm (4-12Hz) is correlated with spatial navigation and mnemonic processing in the hippocampus. Inhibitory interneurons of the hippocampus fire action potentials at specific phases of the theta rhythm, pointing to distinct functional roles in shaping this rhythmic activity. Oriens/lacunosum moleculare (O-LM) interneurons regulate the activity of local pyramidal cells in CA1. They express the hyperpolarization-activated, mixed-cation current (I_h) and show spontaneous firing at theta that is impaired upon I_h block *in vitro*. A dynamic clamp study (Kispersky et al. 2012) showed that injecting theta frequency-modulated artificial synaptic inputs into the soma of O-LM cells resulted in theta spiking resonance that did not depend on the presence of I_h. Here, we used multi-compartment models of O-LM cells to examine the effect of dendritically-located synaptic inputs on spiking resonance. We selected models with dendritic I_h from our previous model database work and inserted Poisson-based excitatory and inhibitory synaptic inputs onto the dendritic tree. The input was modulated at various frequencies, including theta. We found that models expressed enhanced resonant firing at theta frequencies given dendritic synaptic inputs compared to somatic-only inputs. Thus, theta-timed inputs, such as from the medial septum, may preferentially target O-LM cell dendrites to maximally recruit firing at theta frequencies. Investigating these network interactions are of critical importance for understanding O-LM cell contributions to theta rhythm activity in the CA1 microcircuit.

2.2 TRANSGENERATIONAL PROGRAMMING OF TRANSCRIPTION IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS (PVN) BY GLUCOCORTICOIDS

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Prenatal exposure to excess glucocorticoids programs hypothalamic-pituitary-adrenal (HPA) function, increasing risk for psychiatric disease. These effects are strongest in female offspring and are heritable over multiple generations. The hypothalamic PVN is the key integrating hub for HPA regulation and is centrally implicated in programming stress responsivity. We hypothesized that prenatal synthetic glucocorticoid (sGC) exposure programs transcription in gene networks in the PVN across three generations following paternal transmission. Pregnant guinea pigs received 3 courses of betamethasone (Beta; 1mg/kg) or saline in late gestation. F1 and F2 male offspring were mated with naïve females to generate F2 and F3 offspring. The PVN was micro-punched from F1 (C; n=5, Beta; n=5) and F3 (C; n=6, Beta; n=4) 40 day-old female offspring. RNA-seq analysis was undertaken and results analyzed using standardized bioinformatic approaches. In F1, 773 genes were significantly ($P<0.001$, $FDR<0.05$) differentially expressed following prenatal Beta. Of these, 540 genes were significantly up-regulated, and 233 genes down-regulated. In F3 offspring, 54 genes were significantly ($P<0.001$, $FDR<0.05$) differentially expressed between Veh and Beta, with 21 genes up-regulated, and 33 genes down-regulated. 10 genes, including genes related to steriodogenesis and food intake, were differentially expressed in both F1 and F3 Beta offspring. Network analysis of F1 Beta animals indicated significant ($p<0.001$, $FDR<0.25$) up-regulation of the Type II Diabetes pathway, and down-regulation of cellular methionine metabolizing machinery. Programmed changes in gene transcription are consistent with the phenotypes associated with prenatal glucocorticoid exposure. Paternal transgenerational transmission strongly implicates the involvement of epigenetic mechanisms. The programmed down-regulation of methionine metabolism pathways would be consistent with demethylation and this may contribute to increased gene expression in F1 offspring. These results show, for the first time, that prenatal exposure to sGC leads to transgenerational programming of the PVN, and provides insight into the mechanisms by which these effects are mediated.

2.3. NOD1 DELETION FROM IMMUNE CELLS PROTECTS AGAINST METABOLIC INFLAMMATION AND INSULIN RESISTANCE

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Type 2 diabetes (T2D) is a systemic disease affecting >3x10[8] people worldwide. Preceding T2D is a prediabetic state characterized by obesity, insulin resistance, and as seen over the last decade, chronic, low-grade inflammation. With obesity or high fat diets (HFD), metabolic tissues such as adipose, liver and skeletal muscle become infiltrated by immune cells such as macrophages and neutrophils. Concomitantly, the intestinal barrier compromises, releasing gut-derived bacterial compounds into circulation that, along with saturated fats, activate inflammatory pathways in immune cells, contributing to insulin resistance. We recently showed that whole-body knockout of NOD1, the receptor for bacteria cell wall peptidoglycan, attenuates HFD-induced insulin insensitivity. However, the tissues where NOD1 acts during HFD and the underlying mechanisms remain unknown. We hypothesize that NOD1 deletion specifically in immune cells will protect against metabolic inflammation and insulin resistance. To test this hypothesis, we generated chimeric mice depleted of NOD1 in hematopoietic cells (KO→WT) or wild-type controls (WT→WT) using bone marrow transplantation, then fed these mice HFD or low fat diet for 18 weeks, assessing metabolic and inflammatory parameters throughout the study. We find that when challenged with a HFD, KO→WT mice show improved glucose and insulin tolerance without differences in body weight or adiposity compared to WT→WT controls. By flow cytometry and qPCR, HFD-fed WT→WT mice displayed elevated neutrophil infiltration and pro-inflammatory macrophage polarization in adipose tissue, but strikingly, these effects were ameliorated in KO→WT mice. Further, this phenotype was attributed to reductions in neutrophil-attracting chemokines (CXCL1, CXCL2, CXCL12) produced by adipose tissue macrophages as determined by immunohistochemistry, and not defects in neutrophil migration. These results demonstrate that hematopoietic NOD1 in part mediates the generation of a pro-inflammatory tissue environment associated with metabolic dysfunction. Our findings reveal potential cell-, tissue-, and gut-specific targets to alleviate diet-induced inflammation before the onset of T2D.

2.4 CAVEOLIN1 MEDIATES THE MIDKINE-INDUCED EPITHELIAL-MESENCHYMAL TRANSITION IN THE LUNG

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We have previously shown that the plasma concentration of midkine (MK), a novel cytokine, increased in patients with acute respiratory distress syndrome (ARDS), and that MK induced epithelial-mesenchymal transition (EMT) by upregulation of angiotensin-converting enzyme (ACE) expression in *in vitro* and *in vivo* models of ARDS and ventilator-induced lung injury. We have also established that a transmembrane protein, Notch2, serves as the cell surface receptor for MK. In the present study, we hypothesized Caveolin1 (Cav1) as a key downstream modulator in the Notch2-mediated ACE activation. Human lung epithelial cells were challenged *in vitro* with HCl or vehicle control followed by 20% elongation of mechanical stretch for 24 and 48h. LDH assay was used to measure cell injury. Protein expressions of MK, Notch2, Cav1, and ACE were assessed by Western blot. To determine the direct interaction between Notch2 and Cav1, the whole cell lysates were extracted for co-immunoprecipitation (co-IP) assays. Additional co-IPs were performed for Cav1 with Notch1, 3, and 4. Knockdown (KD) of Cav1 expression by using Cav1 siRNA was followed by stimulation with recombinant human MK (rhMK) for 48h to determine the direct effects of MK on Notch2-Cav1-ACE pathway on EMT. Exposure to mechanical stretch for 24 and 48h in human lung epithelial cells showed increased expression of MK, ACE, Notch2, and Cav1 expression at 24h which diminished at 48h. Co-IP assays demonstrated a direct interaction between Cav1 and Notch2 but not with Notch1, 3, or 4. Cav1 KD induced EMT as shown by up- and downregulation of Vimentin and E-cadherin respectively. Importantly, Cav1 KD inhibited the rhMK-induced upregulation of ACE. Activation of the MK-Notch2- ACE signaling pathway contributes to EMT in human lung epithelial cells. Cav1 plays a key role in the MK-induced ACE expression, suggesting that Cav1 may be a potential therapeutic target in attenuating ARDS-associated lung fibrosis.

Orals Presentation Session 3

3.1 SEX DIFFERENCES IN MICROGLIA AND P2X4 RECEPTOR MEDIATION OF NEUROPATHIC PAIN IN RATS

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Microglia are fundamental in mediating peripheral nerve injury (PNI)-induced pain hypersensitivity in rodents. PNI produces upregulation of P2X4 receptors (P2XRs) on spinal microglia. P2X4R activation induces release of brain-derived neurotrophic factor (BDNF), which activates neuronal TrkB and induces downregulation of the potassium chloride co-transporter KCC2 in spinal dorsal horn neurons. Recently, we discovered that microglia and P2X4Rs mediate pain hypersensitivity in male but not female mice. Here, we determine whether sexual dimorphism in pain signalling exists in rats. Neuropathic pain was induced using the sciatic cuff model (PNI) and mechanical hypersensitivity measured using von Frey fibers. PNI produces robust microgliosis and upregulation of genes associated with microglial reactivity in the dorsal horn of the spinal cord in both sexes. However, intrathecal application of minocycline (300 ug), a non-specific microglia inhibitor, reverses pain hypersensitivity in male but not female rats. Additionally, intrathecal injection of TNP-ATP (30 nmol), a $P2X_{1-4}$ receptor inhibitor, alleviates hypersensitivity in males only. Furthermore, upregulation of *P2rx4* occurs after PNI in males but not females. Thus, we demonstrate that the sex difference in microglia-neuron signalling in mice is generalizable across rodent species. PNI does produce microglia reactivity in female rats; however, microglia and P2X4Rs do not mediate pain hypersensitivity in females. This sex difference demonstrates the necessity of including female rodents in preclinical pain research.

3.2. UNDERSTANDING THE ROLE OF EPIGENETIC MEMORY ON THE DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS TO RENAL PROGENITORS

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In 2006, Takahashi and Yamanaka showed that adult somatic cells can be reprogrammed back to an embryonic stem cell (ESC)-like state, and they termed these cells induced pluripotent stem cells (iPSC). Like ESC, iPSC have the potential to differentiate into cells from all three germ layers. iPSC can be patient-specific which circumvents the issue of immune rejection in the transplantation setting. With these features, iPSC have great potential for cell therapy and personalized medicine. iPSC can be made from virtually any somatic cell type, and given that studies have shown that iPSC retain epigenetic memory of their cell of origin, it is important to investigate the influence of parental cell type and epigenetic memory on iPSC differentiation propensity and functionality of differentiated cells. Our goal is to use renal differentiation as a model system to better understand the influence of epigenetic memory on the differentiation of kidney-, blood- and fibroblast-iPSC to renal progenitors. We are deriving secondary iPSC lines from the transgenic mouse SLC34a1-GCE; tdTomato; ROSArtTA (neo-in); OKMS-250. Once iPSC lines are established, microarray will be used to determine whether iPSC retain expression of genes indicative of their parental cell type, and ChiP-seq will be used to determine whether key embryonic and mature kidney genes are silenced (H3K27me3), active (H3K4me3) or poised for activation (H3K27me3/H3K4me3) in the different iPSC lines. Our lab has developed a protocol to direct the differentiation of pluripotent stem cells (PSC) to renal progenitors. Our protocol consists of three defined media to sequentially differentiate PSC to T+ mesoderm, Pax2+ intermediate mesoderm and finally, Six2+ renal progenitors. This protocol will be applied to the different iPSC lines to determine whether parental cell type influences differentiation propensity. Results from our study will help better understand the role of epigenetic memory on the differentiation of iPSC to renal progenitors.

3.3 REEP5 IS A CRITICAL MODULATOR OF ENDOPLASMIC RETICULUM MORPHOLOGY AND FUNCTION

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The endoplasmic reticulum (ER) provides many critical functions in eukaryotic and plant cells, but the underlying mechanism for ER organization remains largely unknown. Here, we investigate a mammalian Receptor Expression-

Enhancing Protein 5 (REEP5) as an evolutionarily conserved protein from S. cerevisiae to H. sapiens. Confocal imaging and biochemical assays demonstrates that REEP5 is involved in both tubular ER organization in proliferative cells (human embryonic kidney-293T and murine C2C12 myoblasts) and in the sarcoplasmic reticulum patterning in striated muscles (terminally differentiated C2C12 myotubes, cultured mouse neonatal cardiomyocytes [CMNCs], and adult mouse cardiomyocytes). Immunoblots and immunofluorescence showed that REEP5 is important for ER localization and tubular ER organization in C2C12 myoblasts, myotubes, and CMNCs. shRNA-mediated REEP5 knockdown results in loss of tubular ER morphology in CMNCs and C2C12 myoblasts. Knockdown of REEP5 also results in slower and larger calcium release from CMNCs. In addition, morpholino-mediated REEP5 reduction is associated with defective and delayed cardiac looping and reduced heart rates in zebrafish embryos compared to wild-type littermates. REEP5 homodimerizes via its C-terminal intracellular domain and transient overexpression of a dominant negative REEP5 mutant results in the loss of tubular ER and increases ER vacuoles in HEK-293T, C2C12 myoblasts, and CMNCs. Taken together, these findings demonstrate an important role of REEP5 in balancing tubular and sheet ER levels in eukaryotic cells and play a crucial role during cardiac embryogenesis.

3.4 OAT BLOCKADE TO IMPROVE BETA CELL FUNCTION IN DIABETES

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The furan fatty acid metabolite 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF) is elevated in humans with type 2 diabetes (T2D) and prediabetes, and rapid elevations in CMPF accelerate T2D progression. We previously showed CMPF enters the beta cell through Organic Anion Transporter 3 (OAT3), causing beta cell dysfunction. Here, we deliver intraperitoneal CMPF for 7 days—elevating plasma CMPF to T2D concentrations—prior to 6 weeks high fat diet (HFD)-feeding (CMPF-HFD vs. CON-HFD) to investigate long-term effects of CMPF on islet function. Remarkably, CMPF-HFD mice display persistent islet dysfunction with impaired glucose-stimulated insulin secretion (GSIS) and glucose tolerance up to 6 weeks following cessation of CMPF delivery. Mechanisms behind this persistent CMPF action on the beta cell were uncovered using DNA from human islets treated with CMPF *in vitro* and the Illumina 450K Array which reveals whole-genome epigenetic alterations. To discover novel therapeutic targets to prevent these persistent effects of CMPF in prediabetes/T2D, we investigate whether OAT blockade *in vivo* inhibits CMPF action on the beta cell. To achieve this we block CMPF transport using (i) pharmacological OAT inhibition—by co-treatment with the competitive OAT inhibitor, probenecid—and (ii) genetic elimination of Oat3 (Oat3KO). As above, wildtype mice (co-treated with probenecid) or Oat3KO mice received CMPF prior to 6 weeks HFD-feeding. As anticipated, *in vivo* CMPF treatment alone decreased islet GSIS, increased islet reactive oxygen species, and impaired glucose tolerance. Importantly, Oat3KO mice or mice co-treated with probenecid were protected against CMPF islet phenotypes, rescuing glucose tolerance and islet function. These novel studies demonstrate a persistent effect of CMPF on islet function and a requirement of OAT transport to do so, highlighting the potential for OAT inhibition to prevent CMPF-induced diabetes: a metabolite highly elevated *prior* to diabetes onset which has a persistent effect on islet function, and accelerates disease progression.

Cardiovascular and Respiratory Posters

C1. IDENTIFYING THE FUNCTIONAL ROLE OF TMEM65 IN MAMMLIAN VENTRICULAR MYOCYTES

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The cardiomyocyte (CM) intercalated disc (ICD) is fundamental for signal propagation, cell adhesion, and transfer of molecules and thus essential for CM physiology. Here we investigate ventricular protein Transmembrane protein 65 (Tmem65), identified at the CM ICD in both mouse and human cardiac tissue. Preliminary *in vitro* work in cultured mouse neonatal CM, Ca^{2+} imaging, confocal imaging, qRT-PCR, and cellular biochemistry showed altered expression of gap junction functionality and Ca^{2+} dynamics following Tmem65 knockdown. We also determined a decrease in activation time and velocity of electrical propagation using microelectrode array plates. Our working hypothesis is that decreased expression of Tmem65 orchestrates arrhythmogenic conditions by destabilizing gap junction proteins. To verify this and further identify the molecular mechanisms of Tmem65 *in vivo*, AAV9 containing Tmem65 shRNA (knockdown) or Tmem65 human cDNA (overexpression) will be administered through the tail vein, reflecting whole animal physiology. Overexpression of Tmem65 fused with BirA will also be used in culture myocytes and by AAV9, to obtain a more comprehensive interactome of Tmem65, crucial for analysis of molecular functions. In addition, cultured neonatal CMs are being used to identify altered protein expression following Tmem65 knockdown. These results will help identify Tmem65intracellular mechanisms, and ultimately its functional role in cardiomyocytes, cardio-pathobiology, ICD protein interactions, and potentially provide insight into underlying mechanisms of arrhythmogenic diseases.

C2. INTERROGATING THE ROLE OF PROTON TRANSPORT FOR PH REGULATION OF HUMAN AIRWAY CELLS

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Cystic Fibrosis (CF) is the most common life-limiting genetic disease in the North American population affecting 1≈3000 newborns. The absence of functional CFTR-mediated ion and fluid flux at the apical membrane of epithelia cells causes significant multi-organ disease. The airway surface liquid (ASL) is a thin layer of liquid at the air-facing epithelial surface in the airways and functions as the first line of defense against inhaled pathogens. Next to defective chloride transport and fluid regulation CFTR also conducts bicarbonate. In the absence of functional CFTR, H^+ secretory and exchange mechanisms may mainly determine the acidic pH in CF. Genome wide association studies have linked Na^+/H^+ exchanger type 3 (SLC9A3) to the severity of CF lung disease providing rationale to target these proton transport mechanisms to restore normal ASL pH. We hypothesized that The ASL pH in CF human primary bronchial epithelial cells is more acidic compared to healthy human bronchial epithelial cells, and may depend on the underlying genotype of SLC9A3. Using lung explants from CF and non-CF patients we have optimized a methodology to measure ASL pH by using two fluorophores mixed into a Ringers solution and added to the ASL with or without the presence of specific proton channels and/or transport inhibitors. The fluorescence intensity is measured with a microplate reader and converted to an ASL pH following the establishment of a standard curve. We established a protocol measuring ASL pH over a period of 48 hours, and measuring changes to the ASL pH in the presence of basolateral bicarbonate replacement and apical addition of an ionophore. This proof of principle study will further our understanding of the pathophysiological mechanisms in CF and will pave the way to identify a possible new drug target to treat the basic defect in CF.

C3. MAKAP IS A NOVEL MEDIATOR OF PLN PHOSPHORYLATION IN MOUSE CARDIOMYOCYTES

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Congestive heart failure is the leading cause of death in Canada, and a common character is the dysregulated Calcium ion (Ca^{2+}) cycling. In cardiomyocytes, Sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) is responsible for Ca^{2+} uptake into the sarcoplasmic reticulum (SR). SERCA is reversibly inhibited by binding of Phospholamban (PLN). Normally, PLN inhibition is reversible through phosphorylation of Ser16 by protein kinase A (PKA) while associated with an A- kinase anchoring protein (AKAP). One AKAP, AKAP7, was suggested to be responsible for this reversible inhibition, however AKAP7 null mice were shown to have completely normal cardiac function, indicating that AKAP7 is not the AKAP involved in PLN phosphorylation-dependent regulation. Here, we investigated RNA sequencing data and Human Protein Atlas datasets to identify muscle AKAP (mAKAP) as a potentially novel, cardiac muscle, sarcoplasmic reticulum enriched, regulator of PLN. We hypothesize that mAKAP is responsible for coordinating PLN phosphorylation at Ser16 by PKA in cardiomyocytes. Immunofluorescence of co-transfected HEK cells and cultured neonatal cardiomyocytes show mAKAP is highly expressed in the cardiac SR. Additionally, immunoprecipitation (IP) of PLN and mAKAP in c-transfected HEK cells show co-immunoprecipitation of mAKAP when immunoblotted for PLN, suggesting potential interaction. Further studies will include exploring PLN and mAKAP interactions in the presence and absence of adrenergic stimulation, as well as examining other putative AKAPs using IP-based Mass spectrometry. In conclusion, these results determine if mAKAP is an important candidate for regulation of PLN phosphorylation through PKA in cardiomyocytes, and thus modulator of heart failure.

C4. FUNCTIONAL ANALYSIS USING ANGIOGENIC POTENCY ASSAYS REVEALS SUPERIOR ANGIOGENIC POTENTIAL OF FIRST TRIMESTER UMBILICAL CORD PERIVASCULAR CELLS (FTM HUCPVCS) COMPARED TO OTHER MSC SOURCES TESTED

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Objective: FTM HUCPVCs are a young source of mesenchymal stromal cells (MSCs) that support blood vessels in the umbilical cord. We aimed to determine the angiogenic potential of FTM HUCPVCs and compare their angiogenic properties to older sources of MSCs (term umbilical cord perivascular cells (term HUCPVCs) and bone marrow stromal cells (BMSCs)) using both *in vitro* and *in vivo* angiogenesis assays.

Methods: For aortic ring assay, aortas were sectioned and embedded into Matrigel™ basal membrane extract. Fluorophore-labeled MSCs for testing were added to developing endothelial networks (Day0). MSC integration and network development were monitored by microscopy. Quantification of endothelial networks was performed using ImageJ™ software (Day4) n=3. A Matrigel™ plug assay was setup to confirm *in vitro* results. 5.0 x10^5 MSCs were suspended with equal volumes of Matrigel™ and injected subcutaneously in 11-week-old nude mice and isolated after two weeks. Plug-associated microvasculature was imaged and quantified (N=3).

Results: In the aortic ring assay, FTM HUCPVCs homed to, and integrated with, the developing tubular network with greater efficiency, and also demonstrated greater endothelial cell coverage, when compared to term HUCPVCs and BMSCs. FTM HUCPVCs showed significantly greater network growth when compared to term HUCPVCs ($p \leq 0.001$), BMSCs ($p \leq 0.001$) and untreated endothelial networks ($p \leq 0.001$). FTM HUCPVC contributed to a significantly greater number of closed loops when compared to term HUCPVCs ($p \leq 0.01$), BMSC ($p \leq 0.001$) and untreated networks ($p \leq 0.05$). At two weeks following injection of Matrigel plugs, FTM HUCPVC resulted in significantly greater blood vessel recruitment when compared to term HUCPVCs ($p \leq 0.05$), BMSCs ($p \leq 0.01$) and control media plugs ($p \leq 0.01$). Small tortuous blood vessels were found in significantly higher quantity in FTM HUCPVC injected plugs when compared to term HUCPVCs ($p \leq 0.05$), BMSCs ($p \leq 0.01$) and media plugs ($p \leq 0.001$).

Conclusions: Our results suggest a superior supportive function by FTM HUCPVCs to stabilize endothelial cells and to promote the development of endothelial networks, when compared to term HUCPVCs and BMSCs.

C5. VENTILATION INHOMOGENEITY IN SEVERE ACUTE WHEEZING PRESCHOOL CHILDREN AS MEASURED BY NITROGEN BASED MULTIPLE BREATH WASHOUT

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Multiple breath nitrogen washout (N2MBW) and its primary outcome, Lung Clearance Index (LCI), is a sensitive measure of ventilation inhomogeneity (VI) and has potential utility in younger populations where spirometry is not as feasible. Furthermore, LCI has been used as a monitoring tool for evaluating preschool asthma control. Understanding how LCI changes during acute episodes is essential to furthering the use of MBW in asthma diagnostics and management. N2MBW was attempted in preschool subjects recruited from the emergency department at SickKids Hospital who were discharged with physician diagnosed asthma, and had received salbutamol during their visit. Baseline visits occurred within 5 days of discharge; follow-up visits occurred within 10-14 weeks of the baseline visit. N2MBW was performed using an Exhalyzer D System (EcoMedics AG, Switzerland). MBW was performed per ATS/ERS recommendations, though only 2 trials were used for a successful test occasion. 14 subjects in this ongoing study had acceptable baseline results, and 8 of these had acceptable follow-up results. Of these subjects, 8/14 (57%) had abnormal baseline LCI values (>95th percentile (LCI = 8.43) of local control cohort of similar age – Canadian Healthy Infant Longitudinal Development Study (CHILD)), and 1/8 (12.5%) had abnormal LCI values at follow-up. In those with both measurements, mean (standard deviation) LCI was 8.59 (0.97) at baseline, and 7.73 (0.56) at follow-up. This indicates a significant mean decrease in LCI of 0.87(0.86, p=0.024, 95%CI: 0.149, 1.589). This study demonstrates that VI is present as measured by LCI in the majority of preschool children with acute episodes of wheezing. The VI improves, but does not normalize in all subjects. However, there was difficulty obtaining at least 2 trials for many patients due to their severe wheezing symptoms, indicating that MBW may not be the ideal test in such a severely sick patient group.

C6. TISSUE HYPOXIA AS A UNIFYING MECHANISM OF ORGAN INJURY IN ANEMIA

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Mild to moderate anemia is a prevalent global health issue associated with an increased risk of morbidity and mortality in patients undergoing surgery. We hypothesize that anemia-induced tissue hypoxia disrupts O_2 homeostasis and depletes cellular energetics as a unifying mechanism of anemia-induced organ injury and mortality. To test this hypothesis, we characterized an antibody-mediated model of anemia to assess whether anemia caused by profoundly different mechanisms (i.e. immune vs.hemodilution vs.genetically mediated) will produce comparable cardiovascular and hypoxic cellular adaptations. Anemia was induced with a red blood cell (RBC)-specific antibody (TER119) which binds to glycophorin-A, resulting in intravascular hemolysis and RBC sequestration. A non-RBC binding antibody was administered to control mice. Transgenic (HIF)-ODD luciferase mice, possessing a bioluminescent hypoxia inducible factor (HIF)-1α signal, were utilized to assess the real-time HIF-response *in vivo*. Hemoglobin concentration (Hb,Co-Oximetry), peripheral O_2 saturation (SpO_2,pulse-oximetry) and tissue PO_2 (phosphorescence-quenching of O_2) were measured. The RBC-specific antibody reduced Hb from baseline values of 146±7g/L to a nadir of 89±12g/L after 4-days (n=16,p<0.001). Hb did not decrease in control mice. During anemia, peripheral SpO_2 was increased (n=6,p<0.018) vs.baseline and control values. Despite improved peripheral SpO_2, we observed a decrease in renal tissue PO_2 in anemic vs.control mice (13.0±4 vs.20.8±4 mmHg,p<0.001);while brain tissue PO_2 was preserved. Overall body HIF-Luciferase radiance was not increased in anemic mice; however, a 19±17%increase in HIF-luciferase radiance observed in the dorsal right liver+kidney region (n=12,p=0.039 vs.baseline). Our model of antibody-mediated anemia resulted in characteristic cardiovascular and hypoxic renal responses, which have been previously demonstrated in hemodilutional and genetic models. Kidney tissue hypoxia and appropriate cellular HIF-response were observed, suggesting that anemia-induced tissue hypoxia is sensed at the molecular level. These data support our hypothesis and suggest that this model may enable us to further assess the mechanisms of anemia-induced morbidity and mortality in surgical patients.

C7. GENOME-WIDE ANALYSIS OF EXERCISE-INDUCED ATRIAL REMODELING

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Atrial Fibrillation (AF) is the most common clinical arrhythmia that is linked to intense endurance exercise. Using animal models of intense exercise, our lab has shown that mice exercised for 6-weeks(swim or treadmill) have higher incidences of AF, fibrosis and inflammation. Microarray studies suggested the involvement of tumour necrosis factor alpha (TNFα) in the pathological remodeling with exercise, which was confirmed using pharmacological blockers of TNFα(etanercept) and mice with TNFα-gene ablation. Interestingly, etanercept treatment starting at 3-weeks of commencement of exercise, did not prevent the pathological atrial remodeling, suggesting that pathological remodeling in the atria with exercise occurs at an early stage of intense exercise training. To evaluate the early TNFα-mediated atrial remodeling with exercise, WT and TNFαKO mice swam for 90minutes/session, 2sessions/day for 2-weeks. We performed deep-RNA-sequencing (RNA-seq) analysis on isolated 2-week exercised atria. RNA-Seq reads were mapped and aligned to the mouse reference genome (mm10) using the Tophat tool. Enriched gene sets were identified using Gene Set Enrichment Analysis (GSEA) with curated(c2cp) and mouse specific gene set database (Mouse_GO_AllPathways). Gene sets were ranked and only those with $p<0.05$ and FDR<0.25 were considered. Similar to 6-week exercise(from microarray studies), 2-week exercised atria were enriched in respiratory cell cycle,metabolism and immune system gene sets. Interestingly, in 2-week exercised mice, the NF-κB pathway, which is downstream of TNFα was found to be enriched in sedentary mice. In addition, other pro-inflammatory pathways such as IL-1,IL-6,and TOLL-like receptor pathways were enriched in sedentary mice. We performed the (Swim-Sedentary)WT-(Swim-Sedentary)KO analysis followed by GSEA, in order to refine a subset of enriched gene sets that are specifically mediated by TNFα in exercise-induced remodeling. As expected, NF-κB and P38MAPK pathways were shown to be mediated by TNFα. Additionally, TNFα was also involved in immune system,TGFβ,Wnt,and mechanotransduction pathways such as focal adhesion and integrin signaling pathways. Genome-wide analysis of 2 week-exercised WT and TNFαKO atria via RNA-seq suggests the involvement of TNFα in the early pathological atrial remodeling and a dose-dependent relationship between pathological atrial remodeling and the amount of exercise, which is consistent to the human data that long-term intense exercise greatly increases the risk of pathological atrial remodeling and the incidence of AF.

C8. MODELING ATRIAL FIBRILLATION IN HESC-DERIVED ATRIAL CARDIOMYOCYTES

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Atrial fibrillation (AF) is the most common supraventricular arrhythmia encountered clinically and is associated with increased morbidity and mortality. AF is characterized by rapid electrical signals leading to irregular, uncoordinated activity. Current treatments for AF are largely ineffective and at times unsafe. As the applicability of animal models to understanding human AF is limited, we set out to develop a model of AF using 2D-monolayers of human embryonic stem cell(i.e.HES3 NKX2-5$^{egfp/w}$)-derived atrial cardiomyocytes (CMs). Atrial CMs were enriched in atrial-specific and lacked ventricular-specific genes. Optical mapping was used to measure electrical activation of monolayers during spontaneous firing ("sinus rhythm"(SR)) and following the induction of re-entrant rotors, which are a critical component of AF. Rotors were characterized by slower conduction velocities (CVs) at the centre compared to the periphery (0.96±0.2vs.4.2±0.8cm/s,n=7,$p<0.05$) and increased rates of firing compared to SR (3.12±0.30vs.1.30±0.23 Hz,n=10,$p<0.05$). The application of dofetilide(1μM), a class III anti-arrhythmic (HERG channel blocker) used for preventing AF, resulted in action potential prolongation(n=8,$p<0.05$) at 90% repolarization(APD_{90}) from 181±46 to 290±75ms without significantly affecting CV or firing rates and was associated with "cardioversion" of rotors back to SR in 3 monolayers(n=10). To determine the cellular mechanisms, patch-clamp studies of single CMs were performed and revealed that dofetilide(1μM) prolonged APD_{90} from 227.20±25.83 to 363.09±46.49ms(n=9,$p<0.05$) as a consequence of blockade of HERG currents(4.17±1.02pA/pF,n=3). We also examined the effects of flecainide(10μM), a class Ic anti-arrhythmic used clinically for AF prevention and cardioversion, whose mechanism of action remains unknown. Flecainide slowed firing rates from 3.29±0.34 to 2.01±0.24ms(n=7,$p<0.05$) and slowed CVs at the periphery of rotors from 4.1±0.8 to 2.2±0.4cm/s(n=7,$p<0.05$), with no discernable effect on conduction velocity near the center. Consistent with conduction slowing, flecainide was associated with single rotors fractionating into multiple small rotors in 3 monolayers(n=10). As expected, single CM studies established that flecainide(10μM) had minimal effects on APD_{90} but slowed AP upstroke velocities from 37.75±12.77 to 15.92±7.08V/s (n=10,$p<0.05$) in association with frequency-dependent blockade of Na^+ currents (50.55±1.83, 75.53±2.29 and 85.83±2.08% blockade with 0.5(n=4),1(n=6)and 3(n=4)Hz stimulation rates, respectively). Our studies demonstrate that atrial monolayers are a robust platform for *in vitro* interrogation of the actions

of anti-arrhythmics and have provided new insight into the mechanisms of action of flecainide in AF.

C9. STATIN-MEDIATED MODULATION OF RHOA/ROCK SIGNALLING IN EXPERIMENTAL CHRONIC NEONATAL PULMONARY HYPERTENSION

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Chronic pulmonary hypertension (PHT) arising in the neonatal period is frequently lethal. Currently, there are no available treatment options for chronic PHT and RV failure in the newborn. The activity of RhoA and Rho-Kinase (ROCK) is critical to the pathogenesis of experimental chronic neonatal PHT. Systemic ROCK-specific inhibitors have been shown to prevent and reverse PHT, but at the cost of severe systemic hypotension. Recent studies with statins have revealed pleiotropic effects through inhibition of isoprenoid intermediates, including geranylgeranyl-pyrophosphate (GGPP). As activity of RhoA requires GGPP as a lipid attachment, we hypothesized that statin treatment would safely limit pulmonary vascular RhoA activity, and prevent and reverse experimental chronic neonatal PHT. Neonatal rats were chronically exposed to air or hypoxia (13% O_2) and treated with simvastatin (2 mg/kg/d) or vehicle (20% DMSO in PBS) by intra-peritoneal injection from birth in prevention (d1-14) or from d14-21 in rescue protocols. Lung RhoA/ROCK activity was quantified by Western blot analysis of GTP-RhoA and phosphorylated MYPT1. Arterial medial wall area (MWA) was measured on elastin-stained lung sections. The degree of muscularization was quantified on α-smooth muscle actin stained lung sections. Pulse-wave Doppler was used to estimate pulmonary vascular resistance (PVR). The weight ratio of dried RV to LV and septum (Fulton Index) was measured as an indicator of RV hypertrophy. With exposure to chronic hypoxia, pups treated with 2 mg/kg/d doses of simvastatin had significantly ($p<0.05$) decreased: PVR, RV hypertrophy, MWA, muscularization of the pulmonary arteries, GTP-RhoA and phosphorylated MYPT1 content. Treatment with statins did not adversely affect: systemic blood pressure, body weight, or biomarkers of liver and muscle toxicity. Simvastatin treatment was able to both prevent and reverse experimental PHT by reducing RhoA/ROCK activity in chronic neonatal PHT. Statins may have potential as a safe alternative for therapeutic inhibition of PHT.

Endocrinology and Diabetes Posters

E1. TARGETING INFLAMMATION IN A MURINE MODEL OF ALPORT SYNDROME

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Alport syndrome (AS) is a rare inherited disorder of the kidney that is due to mutations of genes for type IV collagen. The mutations interfere with normal assembly of the collagens of the glomerular basement membrane and this structural abnormality leads to a progressive rise in urinary protein excretion and eventually a decline in glomerular filtration rate. Preliminary studies in our laboratory involving an experimental murine model of AS suggest that inflammation may play an important early role in the pathogenesis of glomerular injury. It further highlights the association of inflammatory pathways with AS disease progression, such as Toll-like receptors, which may lead to the activation of NFkB in glomerular cells and the development of inflammation and fibrosis.
My hypothesis is that early activation of NFkB plays a key role in the initiation and progression of kidney injury in AS. My specific aim is to determine if the blockade of NFkB with MS417, a compound that interferes with the activation of NFkB and NFkB-mediated gene expression, can attenuate kidney injury and increase the lifespan of mice with experimental AS. In order to test my hypothesis, I will treat mice with MS417 and study the effects of MS417 in kidney function, inflammation, fibrosis, as well as the lifespan of mice with experimental AS. I will utilize kidney morphometry, microarray analysis, real-time PCR, and Western blot analysis to study the development of glomerulosclerosis and tubulointerstitial inflammation and fibrosis. These studies will define novel signal transduction pathway(s) responsible for renal disease progression in experimental AS.

E2. INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-4 INHIBITS INTESTINAL EPITHELIAL PROLIFERATION

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Insulin-like Growth Factor Binding Protein-4 (IGFBP-4) is a binding protein that regulates Insulin-like Growth Factor-1 (IGF-1) bioactivity. We recently established that IGFBP-4 plays an integral role in Glucagon-like Peptide-2(GLP-2)-induced intestinal proliferation, for which IGF-1 is also known to be required. However, the pathway that regulates the expression of intestinal IGFBP-4, as well as its mechanism of action remain to be defined. In this study, mice were given degradation-resistant GLP-2, or PBS subcutaneously, 6- and 3h before sacrifice, a time period established to acutely induce intestinal epithelial proliferation. The mice were also given the PI3K inhibitor, wortmannin, or vehicle by oral gavage 30min before each treatment (N=8 per group). Wortmannin administration completely abrogated jejunal mucosa pAkt expression. However, IGFBP-4 mRNA from jejunal mucosal scrapes did not display significant changes in expression in either wortmannin and/or GLP-2-treated groups ($p>0.05$). Similarly, other treatment periods of GLP-2 (0.5-2hrs) also did not induce changes in mucosal IGFBP-4 expression. To examine the effects of IGFBP-4 in vitro, IEC-6 intestinal epithelial cells were pre-treated with epidermal growth factor (6h) to prevent IGF-1 receptor down-regulation, followed by 18h treatment with IGF-1 (12.5 nM) in the presence of IGFBP-4 (0, 6.25, 12.5, 25 nM). IGFBP-4 dose-dependently decreased the proliferative response to IGF-1 (N=9-12, $p<0.05$), while no changes were observed with IGFBP-4 treatment alone. IEC-6 cells also displayed acute increase in IGFBP-4 expression in response to IGF-1 treatment ($p<0.05$) while the colonic intestinal subepithelial myofibroblast cell line, CCD-18Co, demonstrated a time-dependent increase in basal secretion of IGFBP-4 (2 vs 24h, N=5-6, $p<0.05$). The primary mouse jejunal intestinal subepithelial myofibroblasts also demonstrated basal IGFBP-4 release. Overall, these results indicate that intestinal expression of IGFBP-4 is not dependent on acute changes in either GLP-2 levels or Akt signaling in vivo, and that IGFBP-4, plays an inhibitory role in IGF-1-stimulated intestinal proliferation.

E3. THE CIRCULATING FURAN FATTY ACID CMPF DECREASES FAT ACCUMULATION IN THE LIVER

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The furan fatty acid metabolite, 3-carboxy-4-methyl-5-propyl-2-furanopropanoic acid (CMPF) is elevated in the blood of patients with Type 2 Diabetes (Prentice et. al., *Cell Metabolism*, 2014). In mice, CMPF elevation results in beta cell dysfunction, with impaired insulin secretion and a metabolic switch within the islets to a preference for fatty acid oxidation (Liu et. al., *Cell Reports*, 2016). Preliminary results in vivo indicate that CMPF injection into mice for one week prior to four weeks high fat feeding improves insulin sensitivity and liver function, resulting in decreased triglyceride accumulation and enhanced fatty acid oxidation within the liver. We hypothesize that CMPF acts directly on hepatocytes to increase fatty acid metabolism and decrease triglyceride stores. To test our hypothesis we used an acute animal model, whereby isolated primary hepatocytes were treated for 24-hours with CMPF and tested for functionality and changes in cellular signaling. Isolated primary hepatocytes treated with CMPF showed an increase in fatty acid oxidation, demonstrating a direct effect of CMPF on hepatocyte function. Protein lysates generated from isolated hepatocytes after 24-hour treatment displayed enhanced insulin signaling and an alteration in phosphorylation within the AMPK-ACC pathway, a key regulator of energy metabolism in the liver. CMPF enters the beta cell through the use of an organic anion transporter 3 (OAT3). Screening of OAT expression in liver tissue showed high gene expression of OAT2 and OAT7 but no OAT3, indicating a separate entry method for CMPF within the liver. It is important to define CMPFs role within the liver in order to better understand its effects on energy metabolism within the body. Fully understanding its influence on liver function, combined with its negative effects on the beta cell are a requirement before any conclusions can be made about CMPFs therapeutic potential in decreasing excessive fat build up within the liver.

E4. CONTRACTION-INDUCED AUTOPHAGY IN C2C12 MYOTUBES

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Skeletal muscle is a highly regulated metabolic tissue. This is especially true in situations of high energy demand, such as exercise, and during states of low nutrient availability, such as starvation. Autophagy is upregulated under these conditions. However, the role of contraction-induced autophagy in skeletal muscle has yet to be fully elucidated. Our lab has recently established an *in vitro* model of C2C12 myotubes (multinucleated arrays of skeletal muscle cells) that is susceptible to electrically induced contraction. Electrical pulses of desired voltage, frequency and duration are delivered through an electrical pulse generator. This system produces responses that are reminiscent of those of skeletal muscle during acute exercise *in vivo*. In this cellular system it is possible to transfect siRNA and cDNA to score or manipulate autophagy and examine contraction-induced responses. Following 24h of electrical stimulation of myotube cultures in the presence bafilomycin A1, we find that LC3-II protein content and the LC3-II:LC3-I ratio increase relative to unstimulated controls, suggesting increased autophagic flux, while bafilomycin A1 in the absence of electrical stimulation did not significantly increase these parameters. Future experiments aim to examine autophagic flux in contracting myotubes via live cell imaging following RFP-GFP-LC3 transfection. We intend to examine the role of contraction-induced autophagy in vesicular mobilization of the GLUT4 glucose transporter to the membrane, and consequent changes in glucose uptake and oxidative metabolism. This will generate a mechanistic analysis of the importance of autophagy in skeletal muscle metabolism, as proposed based on studies in *Bcl2* mutant mice displaying impaired stimulus-induced autophagy.

E5. ALPHA-HYDROXYBUTYRATE: A METABOLIC MEDIATOR OF BETA CELL DYSFUNCTION

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Both Type 2 Diabetes (T2D) and Gestational Diabetes Mellitus (GDM) are characterized by dysfunction of the pancreatic β-cell, resulting in impaired insulin secretion and, ultimately, improper control of blood glucose. While elevated blood glucose levels are a hallmark of both diabetes types, T2D/GDM are complex metabolic diseases whereby numerous biologically active molecules mediate β-cell dysfunction. To identify novel mediators of impaired β-cell function, our lab previously utilized global metabolomics profiling to identify 30 circulating metabolites differentially present in the serum of patients with T2D/GDM compared to Normal Glucose Tolerant (NGT) individuals. Here, we investigate the effect one of these 30 metabolites, α-hydroxybutyrate (α-HB)—an organic keto-acid elevated 2-fold in T2D/GDM—on insulin secretion and β-cell function. To determine a relationship between α-HB and β-cell function, both, a MIN6 β-cell line and primary mouse islets were treated with vehicle control (VC), physiological (54μM), or pathological (108μM) concentrations of α-HB, and prior to quantifying glucose-stimulated insulin secretion (GSIS). Pathological α-HB concentrations inhibited GSIS in MIN6 cells compared to physiological α-HB or VC. To investigate if this impairment in GSIS was due to a decrease in total insulin

content, total intracellular insulin was measured in MIN6 cells treated with α-HB for 24 hours, revealing no changes in insulin content. Importantly, α-HB did not significantly increase apoptosis in MIN6 cells (measured by cleaved caspase 3/7 activation) or decrease cell viability (assessed via quantification the fluorescent DNA binding Hoechst dye), indicating this impairment in GSIS was not a result of cellular toxicity. Furthermore, pathological doses of α-HB suppressed Reactive Oxygen Species (ROS) production, which may have decreased GSIS as ROS have been shown to act as GSIS signaling intermediates. This study uncovers a causal relationship between α-HB —a highly elevated factor in T2D/GDM—and β-cell dysfunction, identifying a novel metabolite possibly implicated in diabetes.

E6. ELEVATION OF CMPF REDUCES TRIGLYCERIDE STORAGE IN SUBCUTANEOUS WHITE ADIPOSE TISSUE

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An endogenous furan fatty acid metabolite, 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF), is significantly elevated in the plasma of those with type 2 diabetes (T2D). We previously show that elevating plasma CMPF concentrations in mice to diabetic levels recapitulates many characteristics of T2D, including a "metabolic switch" in the islet—decreased glucose metabolism corresponding to an increase in fatty acid (FA) metabolism. Moreover, preliminary results indicate that CMPF accumulates in the adipose tissue 2-hours post injection, prompting us to investigate the effects of CMPF in fat, the major regulator of FA metabolism. We hypothesize that diabetic levels of CMPF act directly on subcutaneous white adipose tissue (sWAT) to up-regulate the gene and protein expression of factors regulating FA metabolism, exhibiting "metabolic switch" in the adipose tissue. To test this hypothesis, mice received 6 mg/kg intraperitoneal CMPF for 1 week followed by 4 weeks high fat diet-feeding (CMPF-HFD). CMPF-HFD mice gained significantly less weight, had significantly smaller subcutaneous adipocyte size, and up-regulated expression of FA oxidation genes compared to Control-HFD mice. Isolating the direct relationship, *in vitro* analyses in (i) adipocyte-like cell line (3T3-L1) and (ii) primary sWAT culture—both of which are validated by insulin sensitivity, adipogenic-specific markers, and triglyceride accumulation—further explore the direct actions of CMPF on adipocytes in decreasing triglyceride storage. CMPF enters through organic anion transporters (OATs) in various metabolic tissues. To scrutinize the mechanism of CMPF in exerting its effect on adipocytes, we further characterize OATs that transport CMPF in adipocytes. These studies define the direct and indirect actions of a novel metabolite, CMPF, in adipose tissue. As we believe CMPF is a potential therapeutic target of T2D and obesity, combatting the detrimental effects of CMPF on the β cells while retaining the beneficial effects on sWAT via specific blockage of OATs in β cells may prove beneficial.

E7. PALMITATE-MEDIATED NEUROINFLAMMATION IN AN IMMORTALIZED MICROGLIAL CELL LINE BV-2 AND CO-CULTURE WITH HYPOTHALAMIC NEURONS

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As in obesity or with a high fat diet, elevated levels of plasma fatty acids induce a state of neuroinflammation in the hypothalamus, hypothesized to be modulated by microglia. The objective of this study was to examine cellular signal transduction in response to palmitate, the most prevalent saturated fatty acid in the diet, in a microglial-derived cell line, BV-2. The cells were treated with 50 μM palmitate for 4 and 24 hours, and the transcriptional regulation of 84 inflammatory markers was assessed with an RT2 Profiler PCR Array, and changes in expression validated by qRT-PCR. The BV-2 cells were also co-cultured using 1.0 micron PET inserts with an immortalized embryonic hypothalamic cell line, mHypoE-46, to investigate potential intercellular communication between neurons and microglia. We found regulation of specific inflammatory and ER stress markers by palmitate, including the Bax/Bcl2 ratio, CHOP, CCL2, IL-13, IL-17, and TNFα in the BV-2 cells. The mHypoE-46 neurons that were co-cultured with BV-2 cells demonstrated significant repression of TNFα expression. The BV-2 microglial cell line exhibits a unique inflammatory profile suggesting a potential anti-inflammatory role for microglia in the hypothalamus. This prompts further investigation into the nature of the microglia and their interaction with neurons.

E8. OLANZAPINE ABOLISHES THE ABILITY OF CENTRAL INSULIN TO SUPPRESS HEPATIC GLUCOSE PRODUCTION

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Atypical antipsychotics (AAPs), such as olanzapine (OLA), are widely prescribed for serious mental illness but are associated with high rates of type 2 diabetes. Historically, the risk of diabetes was attributed to the weight gain propensity of AAPs; however, recent work shows that AAPs: a) can have immediate effects independent of weight gain, and b) can perturb glucose homeostasis via the brain. We aimed to identify the link between AAPs and brain-mediated glucose dysregulation by examining whether OLA causes insulin resistance by impairing hypothalamic insulin sensing. To evaluate this, euglycemic pancreatic clamps were used to measure hepatic glucose production and peripheral glucose disposal. Rats were administered an acute dose of OLA or vehicle (VEH) and an intracerebroventricular (ICV) infusion of insulin or vehicle (VEH) was administered into the 3rd ventricle. Treatment groups were as follows (ICV-peripheral): VEH-VEH; VEH-OLA; INS-OLA; INS-VEH. As expected, there were no differences in glucose or insulin levels between any groups during the basal or clamp phase. The glucose infusion rate, a measure of whole body insulin sensitivity, as well as glucose uptake, was increased in the INS-VEH and INS-OLA groups relative to VEH-VEH and VEH-OLA groups. The INS-VEH group demonstrated significant suppression of HGP relative to basal phase, as to be expected, but this suppression effect was no longer observed with OLA co-administration. Therefore, we demonstrate that OLA, a high metabolic liability AAP, abolishes the well-established ability of a central insulin infusion to suppress hepatic glucose production, suggesting that OLA induces central insulin resistance. Intriguingly, links between central insulin pathways and AAP therapeutic efficacy have been proposed suggesting that this work may be critical not only to treating AAP side-effects, but to maximizing AAP efficacy and furthering our understanding of treatment dimensions in schizophrenia.

E9. THE EFFECT OF ENDOCRINE DISRUPTING CHEMICALS ON HYPOTHALAMIC-FEEDING RELATED NEURONS

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The increasing prevalence of obesity has been linked to the increased exposure to industrial compounds that can act as endocrine disrupting chemicals (EDCs). Of these, bisphenol A (BPA) and bisphenol S (BPS) are known to disrupt estrogen signaling. In the hypothalamus, 17-β estradiol (E2) decreases feeding and increases energy expenditure by downregulating NPY and AgRP expression. We hypothesized that BPA and BPS either interfere with the actions of E2 or have their own effects on hypothalamic neurons, thereby dysregulating NPY/AgRP transcription. Using immortalized murine hypothalamic cell lines (mHypoE-44, mHypoE-41, mHypoA-2/12 and mHypoA-59), representing populations of male, female, embryonic and adult-derived cells, changes in NPY, AgRP and estrogen receptor (ER-α, ER-β and GPR30) gene expression after BPA or BPS (10 uM to 100 uM) treatment for 4 hours was determined by quantitative real-time PCR (qPCR). In contrast to E2 treatment, exposure to BPA increases AgRP gene expression in all cell lines (50 and 100 uM), and NPY expression in the female-derived cell lines (100 uM). Similarly, exposure to BPS for 4 hours increases AgRP in the mHypoE-44, -41 and mHypoA-59 cell lines, whereas NPY increases in only the female-derived lines. Thus, male-derived and female-derived cell lines differ in their response to increase NPY or AgRP expression depending on the concentration of BPA and BPS. In addition, BPA and BPS alter levels of ER-α, ER-β and GPR30, suggesting these EDCs may be acting through these receptors or potentially altering the response of these cells to E2. In conclusion, whereas E2 decreases NPY expression, BPA and BPS increase NPY and AgRP expression, illustrating a potential mechanism behind EDC-induced dysregulation of energy balance and obesity, a primary risk factor for diabetes and cardiovascular disease.

E10. GLUCAGON-LIKE PEPTIDE-2 AND THE INTESTINAL EPITHELIAL INSULIN-LIKE GROWTH FACTOR-1 RECEPTOR PLAY A ROLE IN MICROVILLUS FORM AND FUNCTION

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Glucagon-like peptide 2 (GLP-2) is an intestinal-specific growth hormone, however, due the localization of its receptor, GLP-2 elicits its intestinotropic effects via downstream mediators. We have previously shown that insulin-like growth factor-1 (IGF-1) is required for GLP-2 induced proliferation and enhanced barrier function, effects that are mediated via the intestinal epithelial IGF-1 receptor (IE-IGF-1R). We have also shown that inducible IE-IGF-1R knockout (KO) and control (fl/fl and villin-cre ± tamoxifen, and fl/fl-cre - tamoxifen) mice treated for 11 days with either long-acting (GLY2)GLP-2 or vehicle, demonstrated an increase in microvillus length with GLP-2 treatment through an IE-IGF-1R-dependant pathway.

Furthermore, the IE-IGF-1R is important for microvillus structure, as evidenced by the development of non-uniform microvilli in KO mice. mRNA and IHC localization has been previously reported for microvillus structural proteins (villin, beta-actin, insulin receptor tyrosine kinase substrate-1 (IRTKS-1)), and a brush border enzyme (sucrase). Villin mRNA and localization was previously found to be unchanged in KO mice, but jejunal mucosal protein quantification now indicates that villin expression is up-regulated with GLP-2 treatment in an IE-IGF-1R-dependant manner, and is markedly down-regulated in the KO. These results were confirmed with immunogold electron microscopy, which demonstrated a profound reduction in villin labelling within the microvilli of KO animals. Beta-actin, previously shown to be unchanged at the mRNA level, was increased at the protein level with treatment, and occurred in an IE-IGF-1R-dependant manner. IRTKS-1 did not show significant changes in protein expression, regardless of previous mRNA data indicating a GLP-2 treatment effect. Finally, sucrase protein levels were significantly increased in KO mice, whereas previous data suggested a decrease in mRNA levels with treatment with no change in localization by IHC. Collectively, these findings indicate that both GLP-2 and the IE-IGF-1R play important roles in the regulation of intestinal epithelial microvillus structure and function.

E11. PALMITATE DISRUPTS CIRCADIAN SYNTHESIS AND SECRETION OF GLUCAGON-LIKE PEPTIDE-1 FROM THE INTESTINAL L CELL

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Glucagon-like peptide-1 (GLP-1) is an incretin hormone released by the intestinal L cell in response to nutrient intake. Recent studies in our laboratory identified a circadian pattern of GLP-1 secretion in response to known secretagogues. However, chronic ingestion of a high-fat high-sucrose diet, a large component of which is the saturated fatty acid, palmitate, was found to disrupt the circadian release of GLP-1 in rats. Furthermore, in mGLUTag cells, an established model of the L cell, palmitate pre-treatment for 12 hr reduces GLP-1 secretion and cell content at 4 hr after synchronization, but does not alter mRNA levels for the GLP-1 precursor, proglucagon – these changes occur in the absence of alterations in cell viability. We thus hypothesized that palmitate induces endoplasmic reticulum (ER) stress in the L cell, resulting in decreased levels of translation. Pre-incubation of mGLUTag cells with palmitate for 12 hr followed by synchronization and additional treatment for 4 and 16 hr did not increase the Bax/Bcl2 ratio, indicating no increase in apoptosis; preliminary data also showed no increase in reactive oxygen species. However, after both 4 and 16 hr, there was an increase in CHOP mRNA levels ($p<0.05$-0.01 resp.) indicating ER stress, and cellular GLP-1 content was reduced at both time points ($p<0.05$-0.01). GLP-1 content in the media was also reduced at 12-24 hr by palmitate treatment ($p<0.05$). Furthermore, total protein content in the mGLUTag cells was reduced at 4-24 hr by palmitate treatment ($p<0.05$). In combination with these data, palmitate treatment for 12-24 hr also resulted in a decreased ability of mGLUTag cells to respond to a known GLP-1 secretagogue, glucose-dependent insulinotropic peptide ($p<0.05$). In conclusion, our data suggest that chronic exposure to palmitate reduces overall protein content within the mGLUTag cells by inhibiting translation through ER stress, leading to a specific decrease in GLP-1 production.

E12. NICOTINAMIDE MONONUCLEOTIDE IN THE CONTEXT OF ELEVATED PLASMA LEVELS OF FREE FATTY ACIDS IMPROVES GLUCOSE TOLERANCE BY DECREASING INSULIN CLEARANCE

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The NAD-dependent protein deacetylase, Sirtuin-1 (SIRT1), has been shown to be beneficial to beta cell function by increasing insulin secretion. Nicotinamide Mononucleotide (NMN) has recently been shown to have positive effects on glucose tolerance in mice fed a high fat diet. Our study is the first to examine the effects of NMN on insulin clearance and FFA-induced beta cell function. NMN was i.v. infused, with or without oleate, in C57BL/6 mice over 48h in order to elevate intracellular NAD levels and consequently increase SIRT1 activity. Using hyperglycemic clamps, we demonstrated that administration of NMN in the context of elevated plasma free fatty acid (FFA) levels results in a significant decrease in insulin clearance as well as partial protection against FFA-induced beta cell dysfunction in vivo. This culminated in a large improvement in glucose tolerance, partially due to hyperinsulinemia. Western blots showed that decreased carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) expression in the liver was associated with the decrease in insulin clearance observed in our experiments. Potential mechanisms behind the effects of NMN on insulin clearance and beta cell function are discussed. In summary, NMN may have a therapeutic potential to improve glucose tolerance in conditions of increased plasma FFA levels, such as obesity.

E13. DIETARY CURCUMIN INTERVENTION ATTENUATES BODY WEIGHT GAIN IN HIGH FAT DIET FED MICE VIA INHIBITING FAT TISSUE INFLAMMATION AND INCREASING BROWN ADIPOCYTE UCP1 EXPRESSION

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Adipose tissue inflammation and excessive energy intake are two major causative factors of obesity. Therapeutic agents that can simultaneously target these two pathological events are desired for obesity treatment. A few previous studies have demonstrated the appreciable body weight lowering effect of the dietary polyphenol curcumin in high fat diet (HFD) fed mouse models, and this function was attributed to its anti-inflammatory and anti-oxidative properties in adipose tissues and elsewhere. We show here for the first time that curcumin intervention in HFD fed mice not only reduced fat tissue macrophage infiltration, but also reduced the ratio of macrophage marker expression in Raw264.7 cells. In rat primary mature adipocytes, curcumin intervention in HFD fed mice increased energy expenditure, associated with increased UCP1 and its upstream regulators (PGC-1α, PPARα and PPARγ) in brown adipose tissue. We have also verified that UCP1 promoter activity can be stimulated by curcumin treatment in two naive cell systems. We hence conclude that curcumin intervention plays a dual modulatory role in preventing obesity by targeting adipose tissues: attenuating white adipocyte inflammation and increasing brown adipocyte activity.

E14. MIR-17 UP-REGULATES 3T3-L1 CELL DIFFERENTIATION VIA REPRESSING THE WNT PATHWAY EFFECTOR TCF7L2

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Obesity is a growing worldwide health problem, associated with increased risk of metabolic diseases, including diabetes. Understanding the molecular mechanism of adipogenesis may lead to the discovery of novel therapeutic targets for the treatment and prevention of obesity and diabetes. Wnt signaling is known to repress adipogenesis, while recent studies have shown that a number of miRNAs are implicated in pre-adipocyte proliferation and differentiation. As a previous study indicated the stimulatory effect of miR-17/92 cluster on adipogenesis, we determined here that the first member of this cluster, miR-17, up-regulates mouse pre-adipocyte 3T3-L1 cell differentiation, as miR-17 precursor over-expression increased while miR-17 inhibitor repressed 3T3-L1 differentiation. We then confirmed the repressive effect of the Wnt signaling pathway effector Tcf7l2 on adipogenesis and revealed that Tcf7l2 is a miR-17 target, utilizing both the gain-of-function and the loss-of-function approaches. The natural plant compound curcumin possesses the body weight lowing effect, and is a known suppressor of 3T3-L1 differentiation. We found that curcumin attenuated miR-17 expression but stimulated Tcf7l2 expression in 3T3-L1 cells. Finally, high fat diet consumption in C57BL/6 mice increased miR-17 expression associated with reduced Tcf7l2 expression level in mouse adipose tissue. Together, our observations suggest that miR-17 is among the central switches of adipogenesis. It activates adipogenesis via repressing the Wnt signaling pathway, and its own expression can be nutritionally regulated in health and disease.

E15. ELUCIDATING THE MECHANISM OF GLP-2 INDUCED IGFBP-4 EXPRESSION

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Glucagon-like peptide-2 (GLP-2) mediated intestinal epithelial proliferation is known to require insulin-like growth factor 1 (IGF-1) and insulin-like growth factor-1 receptor (IGF-1R). The human analog of GLP-2, teduglutide has been approved to treat patients with short bowel syndrome (SBS) to reduce the necessity of supplemental parenteral nutrition. IGF-1 activity is regulated by insulin-like growth factor binding proteins (IGFBP). Previous work has demonstrated that intestinal epithelial proliferation through chronic administration of GLP-2 requires IGFBP-4. Additionally, intestinal mucosa IGFBP-4 mRNA levels are increased with chronic administration of GLP-2. These findings suggest that IGFBP-4 expression is induced by GLP-2 stimulation. We hypothesized that intestinal mucosal Akt and ERK were involved in the activation of IGFBP-4 expression as they have been shown to be activated by GLP-2. To determine the mechanism of IGFBP-4 expression through GLP-2 induction, mice were treated with Gly^2-GLP-2 or vehicle acutely for 0.5 -6h. Phosphorylation of intestinal mucosal Akt and ERK were determined by Western blot. Expression of IGF-1 and IGFBP-4 mRNA were determined by qRT-PCR with Sox9 mRNA as positive control. Intestinal Akt signaling was inhibited by the pre-administration of wortmannin, a PI3K inhibitor, or vehicle by oral gavage. Results demonstrated that GLP-2 treatment significantly increases pAKT

expression at 0.5hr ($p<0.05$) and significantly decreases pERK expression at 0.5h and 2h ($p<0.05$). While Sox9 mRNA levels were significantly increased at 1.5h ($p<0.05$), IGFBP-4 and IGF-1 mRNA expression were not significantly different. At 6 hr, pAKT expression was significantly decreased ($p>0.05$) and pERK expression was unchanged. pAKT expression was completely inhibited with pre-administration of wortmannin ($P<0.05$). IGFBP-4 mRNA levels were not significantly different after 6h in response to GLP-2 and/or wortmannin treatment. Through these results, we concluded that acute GLP-2 treatment and the subsequent activation of mucosal pAkt expression do not increase the gene expression of intestinal mucosal IGFBP-4.

E16. EFFECTS OF THE SATURATED FATTY ACID PALMITATE ON CELLULAR NEUROINFALMMATION AND POMC EXPRESSION IN THE MHYPOA-POMC/GFP-1 HYPOTHALAMIC NEURONAL CELL MODEL

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Circulating nutrients and hormones, act on opposing neuronal subtypes within the arcuate nucleus of the hypothalamus to regulate energy homeostasis. Activation of anorexigenic pro-opiomelanocortin (POMC) neurons decreases feeding. However, it is unknown how these neurons respond to excess nutrients, specifically prevalent free fatty acids (FFA) such as palmitate, as detected in high fat diets. Obesity causes hypothalamic neuroinflammation, but it is unknown if neuroinflammation and cellular stress occur in POMC neurons, and whether this leads to insulin resistance and altered *Pomc* expression. This study examines the mechanisms of action of palmitate in the mHypoA-POMC/GFP-1-4 neuronal cell lines, including the effects on *Pomc* mRNA, neuroinflammatory markers, and insulin signaling. We hypothesize that palmitate will promote neuroinflammation, dysregulation of insulin signaling, and changes in *Pomc* expression, ultimately altering energy homeostasis. mHypoA-POMC/GFP-1-4 neurons were treated with 50 μM palmitate for 8 h and relative levels of *Pomc* mRNA and markers of cellular neuroinflammation and ER stress were measured by qRT-PCR. Palmitate caused a significant increase in *Pomc* mRNA, as well as mRNA levels of the ER stress marker, *Chop*, and the cytokine, *Il-6* in mHypoA-POMC/GFP-1 and -2, but no change in *Pomc* mRNA was seen in mHypoA-POMC/GFP-3 and -4. Palmitate is shown to signal through toll-like receptor (TLR) 4 to induce an inflammatory response. Pre-treatment with 10 μM of the TLR4 inhibitor demonstrated significant attenuation of the palmitate-mediated induction of *Il-6* mRNA, but no change in *Pomc* mRNA. These results suggest that palmitate acts through TLR4 to induce an inflammatory response, but through a separate mechanism to affect *Pomc* transcription, potentially through the cellular metabolism palmitate. Metabolic processes will be further studied using pharmacological inhibitors of specific FFA metabolic pathways. Insulin signaling will be examined with palmitate and Il-6 treatments. Together, these data further our understanding of how FFAs act directly on POMC neurons and potentially alters hypothalamic-mediated energy homeostasis leading to the development of metabolic disorders.

E17. ROLE OF JNK IN GLUCOSE-INDUCED BETA CELL DYSFUNCTION

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Chronic hyperglycemia can cause harmful effects on β-cells, known as "β-cell glucotoxicity". Oxidative stress is present in the diabetic state and has been shown to play a role in β-cell glucotoxicity. C-jun N-terminal kinase (JNK) can be activated by oxidative stress and can impair insulin gene transcription and secretion by inducing the nucleocytoplasmic translocation of Pancreatic and Duodenal Homeobox-1 (Pdx-1). A reduction in Pdx-1 expression or DNA binding leads to suppressed insulin gene transcription. Recent studies in our lab showed that rats infused with JNK inhibitor and JNK-null mice were both protected from glucose induced β-cell dysfunction. However, whether JNK activation is causal in glucose–induced β-cell dysfunction through a direct or indirect effect on β-cell is unclear. The hypothesis is that the inhibition of JNK directly protects β-cells from glucose-induced dysfunction. We investigated the role of JNK activation in glucotoxicity induced β-cell dysfunction by using an *in vitro* model with a JNK inhibitor, SP600125, in rat islets. Islets were incubated for 48hrs in physiological or high levels of glucose with and without SP600125. β-cell function was then assessed with glucose stimulated insulin secretion assays and JNK activity was determined by measuring phosphorylated c-jun, a marker of JNK activation, using western blot. High glucose treatment impaired β-cell function in isolated islets while the inhibition of JNK partially improved the function of β-cells with high glucose treatment. Phosphorylated c-jun was increased with high glucose treatment in isolated islets, which shows that JNK activity is elevated in β-cell glucotoxicity. This study demonstrates that inhibition of JNK protects islets from glucotoxicity induced β-cell dysfunction. Using the *in vitro* model, mechanistic studies can also be done to further understand and support the hypothesized role of JNK. Additionally, our finding suggests that JNK inhibition can potentially be of interest in maintaining β-cell function in T2D.

Neuroscience Posters

N1. USING OPTOGENETICS TO PROBE NEURONAL EXCITABILITY

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Neurons rely on action potentials, or spikes, to transmit information. A certain amount of depolarization can evoke very different spike trains depending on the local spike generation properties. Those properties can vary across neurons, or even between different regions of a single neuron based on the ion channels expressed in each neuron or region. We seek to understand spike generation in primary afferent neurons (PANs), which are responsible for the initial encoding of somatosensory stimuli. How exactly PANs encode information is still not clear because spike initiation in these neurons occurs at axonal terminals, which are inaccessible to intracellular recording due to their extremely small size. To circumvent this problem, we developed an innovative approach that uses light to focally stimulate the axonal terminals while recording from the cell body. To this end, we expressed channelrhodopsin-2, a light-sensitive channel, in PANs in a mouse model. We first validated the use of light to probe neuronal excitability in vitro in the cell body. Using whole-cell recording to measure the excitability of these neurons in response to current injection and light, we show that photo-evoked spiking in the cell body is highly dependent on intrinsic excitability. The majority of small cells were easily excitable in response to current injection (low rheobase and repetitive spiking) and many responded to light with repetitive spiking. However, large cells were hard to excite by current injection (high rheobase and transient spiking) and light did not usually evoke spikes. After validating the use of light in PAN cell bodies to probe spike generation, we proceeded to test spike generation properties of axon terminals. Using in vivo multiunit recording from PAN cell bodies while stimulating cutaneous axon terminals with light, we show that some axon terminals spike transiently while others spike repetitively during sustained stimulation.

N2. THE GLYCOPROTEIN REELIN IN THE BRAINTEM RESPIRATORY NETWORK EXCITES RHYTHMIC BREATHING *IN VIVO*.

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Reelin is a large extracellular glycoprotein involved in brain development and expressed in the central nervous system. In mature organism, reelin facilitates NMDA neurotransmission by activating very low density lipoprotein and apolipoprotein 2 G-protein-coupled receptors. Interestingly, reelin is uniquely expressed in regions of ventrolateral medulla essential for the generation of rhythmic breathing such as the preBötzinger Complex (preBötC), but its functional role in regulating respiratory activity is unknown. We aimed to identify the functional role of reelin in the preBötC in regulating rhythmic breathing in adult rats. To this aim, we microperfused pharmacological agents into the preBötC of adult anesthetized rats using reverse microdialysis while recording diaphragm and upper airway (genioglossus) muscle activities. Our preliminary data showed that microperfusion of reelin (50nM) to the preBötC did not significantly change respiratory rate (*P=0.057*, n=8) or genioglossus muscle activity (*P=0.843*, n=8). Microperfusion of the anti-reelin monoclonal antibody CR-50 (10uM) to block endogenous reelin did not significantly decrease respiratory rate in 5 anesthetized rats (20% decrease, *P=0.054*). Additional experiments are required to determine the significant effects of CR-50. Following brainstem histology, we showed that the proximity of the probes to the preBötC was associated with the latency for CR-50 to decrease respiratory rate (R=0.883, *P=0.018*, n=5) therefore demonstrating that when perfusion was close to the preBötC, respiratory rate

quickly decreased. Our data showed that reelin did not change breathing, likely due to the fact that the high concentration of endogenous reelin found in the preBötC precludes the action of exogenous reelin on its cognate receptors. Blocking the effects of reelin with CR-50 may however decrease the endogenous role of reelin in stimulating rhythmic breathing. Reelin may have an excitatory role at the level of the preBötC and may modulate excitatory neurotransmission involved in the generation of breathing.

N3. BURST STIMULATION PROMOTES LONG TERM POTENTIATION IN THE SUBSTANTIA NIGRA OF PARKINSON'S DISEASE PATIENTS

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Parkinson's disease is a debilitating movement disorder characterized by bradykinesia, rigidity and tremor caused by the loss of dopaminergic neurons of the substantia nigra. The loss of dopamine precludes the long-term potentiation (LTP) of basal ganglia synapses although dopamine-replacement therapy can restore LTP, measured experimentally by high frequency stimulation (HFS). However, many patients develop disabling dyskinesia with long-term dopamine-replacement therapy. Patterned stimulation, in contrast to continuous HFS, has been shown to successfully induce LTP even in a dopamine-depleted state in the rat model of PD. We hypothesized that theta burst stimulation (TBS: 5 Hz) in the substantia nigra pars reticulata (SNr) and the subthalamic nucleus (STN) will induce LTP in the SNr and compensate for the limited dopamine in OFF state PD patients. Microelectrode recordings of the SNr were obtained during intraoperative mapping procedures in PD patients undergoing deep brain stimulation surgery (DBS). We delivered intermittent theta burst stimulation (iTBS, five 100Hz bursts per second, 100μA, 800 pulses total) and compared the results to patients who received sham stimulation (0μA iTBS) or HFS (continuous, 100 Hz, 100μA, 800 pulses). Our preliminary results show that intermittent theta burst stimulation (iTBS) of the SNr induced LTP in OFF state PD patients whereas sham stimulation or HFS did not induce significant LTP. The results have direct utility to combined levodopa-DBS therapy which can be optimized to target the aberrant synaptic plasticity of parkinsonian basal ganglia.

N4. THE CELLULAR AND MOLECULAR MECHANISMS UNDERLYING THE ROLE OF LIMK1 IN SYNAPTIC PLASTICITY

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My research has focused on identifying the role of LIMK1 in synaptic plasticity. Based on previous studies which have shown that LIMK1 plays role in synapses and dendritic spines structure and with an aim to further investigate the role of LIMK1 in neuronal circuits in vivo, my lab has generated LIMK1 knockout mice and has presented genetic and physiological evidences supporting the hypothesis that LIMK1 is critically involved in spine morphogenesis and synaptic function via regulation of actin cytoskeleton. LIMK1 knockout mice have abnormalities in dendritic spines, synaptic function and learning and memory. Recently, In Dr. Chung's lab, it has been found that LIMK1 interacts and phosphorylates CREB in hippocampal neuroprogenitor cells. Moreover, the same study found that LIMK1 phosphorylates CREB at serine-133, which is the site that is widely believed to activate CREB function. Recently in my lab, it has been shown that LIMK1 regulates long-term memory and synaptic plasticity via the transcriptional factor CREB. In my work, I have focused on providing evidences that changes in neuronal activity and synaptic plasticity associates with activation of LIMK1. Furthermore, I found that nuclear translocation of LIMK1 occurs during synaptic plasticity and learning and memory. However, I have yet to delineate the molecular mechanisms, which underlie the nuclear translocation of LIMK1. Furthermore, the role and significance of LIMK1 nuclear translocation remain unanswered question. Therefore, my future work will focus on characterizing the molecular mechanisms of nuclear translocation LIMK1 during synaptic plasticity and the role of LIMK1 nuclear translocation in spine structure, synaptic function and learning and memory.

N5. MUNC13-4 PROTEIN IS A MAJOR CA^{2+} SENSOR FOR IMMUNE CELL EXOCYTOSIS.

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In contrast to highly established role of synaptotagmin-1 as a Ca^{2+} sensor for presynaptic vesicle exocytosis mediating neurotransmissions, exocytosis of secretory lysosomal granules from immune cells is Ca^{2+} dependent yet the critical Ca^{2+} sensor is unknown. Here, we show that point mutations in C2 domains of Munc13-4 dramatically alter Ca^{2+}-dependency of secretion, whereas absence of Munc13-4 or multiple mutations in C2 domains result in almost complete loss of secretion from mast cells. TIRFM analysis reveals that the point mutants strikingly alter Ca^{2+}-dependency of fusion pore opening in addition to the frequency of fusion events. Our results indicate that Munc13-4 is not only a major priming factor but also the key Ca^{2+} sensor in immune cell exocytosis which determines Ca^{2+}-dependency of fusion pore dynamics at the single vesicle level. Thus, immune cells utilize strikingly different mechanisms for Ca^{2+} dependent exocytosis from those in neurons.

N6. RESCUING NMDA RECEPTOR HYPOFUNCTION IN A MOUSE MODEL OF SCHIZOPHRENIA: NEUROPHYSIOLOGICAL CONSEQUENCES IN PREFRONTAL CORTEX

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The cognitive deficits of schizophrenia include impaired executive function, attention problems and distractibility. These deficits are not well addressed by current treatments and are disabling in terms of reintegration into the work place and society. The transgenic mouse model of schizophrenia with reduced expression of the NR1 subunit of the N-methyl- D-aspartate (NMDA) receptor recapitulates not only the cognitive deficits, but also increased locomotor activity, stereotypy, impaired sensorimotor gating, and deficits in sociability. Adult rescue of NR1 expression restored normal function on tests of executive function. Since medial prefrontal cortex is central to executive function in mice, here we probe prefrontal neurophysiology after adult rescue of NMDA receptor function. Whole cell patch clamp recordings in acute brain slices showed substantial attenuation of NMDA receptor currents in layer V pyramidal neurons in the NR1 knockdown mice compared to wildtype, and the almost complete restoration of these currents in the conditional rescue mice after cre-recombinase activation. Ongoing work is investigating genotype differences in intrinsic properties, synaptic transmission and excitation-inhibition balance. As existing treatments for schizophrenia fail to restore normal executive function, we aim to understand the neurophysiological changes that accompany the restoration of normal cognitive performance after a lifetime of NMDA hypofunction.

N7. TLR4 SIGNALLING CONTRIBUTES TO BEHAVIORAL DEFICITS, INFLAMMATION, AND WHITE MATTER DAMAGE AFTER SUBARACHNOID HEMORRHAGE

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Subarachnoid hemorrhage (SAH) from a ruptured cerebral aneurysm is a particularly severe form of stroke that is associated with death in about 35% of cases and permanent morbidity in the majority of survivors. Inflammation is one of the emerging areas of study in SAH research. Our primary research objective is to determine directly the role of toll-like receptor 4 (TLR4) signaling in an experimental model of SAH, since TLR4 is capable of binding to blood products, and increased TLR4 is associated with measures of brain injury and poor outcome. SAH was induced by prechiasmatic injection of 90uL of blood from a donor mouse. Animals underwent behavioral testing and were sacrificed 48 hours after induction of SAH or sham surgery in wild-type or TLR4 deficient animals (n = 6 per group). We studied large artery vasospasm, and various markers of neuronal cell death, inflammation and white matter injury using histological techniques, fluorescence microscopy and electrophysiology. TLR4 deficiency alleviated behavioral deficits observed acutely after subarachnoid hemorrhage. No difference was found on histological analysis of cerebral vasospasm between SAH and sham operated TLR4 deficient mice, though a significant difference was found between the sham and SAH wild-type controls. Moreover, TLR4 deficiency significantly reduced the amount of neuronal cell death and inflammation. Lastly, wildtype animals undergoing an SAH procedure versus sham surgery showed white matter injury, while TLR4 knockouts did not show evidence of white matter injury. Our data demonstrates that TLR4 plays a role in mediating secondary complications that

result after SAH. We hope to delineate the underlying mechanisms and functional consequences of the TLR4 signaling pathway.

N8. THE ROLE OF NEUROLIGIN 2 AND INHIBITORY TRANSMISSION IN THE FUNCTION OF THALAMIC CIRCUITRY DURING EPILEPSY

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Epilepsy is one of the most common neurological disorders. At the cellular level, epileptic seizures are caused by abnormal, excessive or synchronous neuronal electrical activity in the brain. However, the exact mechanisms of epilepsy remain largely unknown. Neuroligin 2 is a postsynaptic cell adhesion protein, which is exclusively located at inhibitory synapses and serves a role in regulating the balance between brain excitation and inhibition. Importantly, the imbalance between excitation and inhibition tends to cause the disruption of neuronal activities, which may lead to epileptic seizures. By using electroencephalogram and electrophysiological recordings, we found that mice lacking Neuroligin 2 display abnormal seizure-like brain activities and an impaired inhibitory neuronal function in an epilepsy-related thalamic circuitry. Importantly, the abnormal brain activity can be rescued by the administration of a drug directed at enhancing the inhibitory synaptic transmission. These results suggest that Neuroligin 2 regulates normal brain function through modulating inhibition. Our findings provide crucial insight into the mechanisms underlying epilepsy generation and to facilitate the understanding and treatment of related brain disorders.

N9. ELECTRON MICROSCOPY ANALYSIS OF SYNAPTIC VESICLE TETHERING BY CALCIUM CHANNELS AT PRESYNAPTIC ACTIVE ZONES

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Neurotransmitter is released from presynaptic terminals by calcium-gated fusion and discharge of synaptic vesicles (SVs) at active zones (AZ). Based on single-channel gated fusion we predicted that SVs are tethered to N-type (CaV2.2) calcium channels (Stanley 1993). Using a direct, in-vitro binding assay we recently reported that SVs can bind to a 49 amino acid region towards the tip of the CaV2.2 C-terminal (Wong et al. 2013, 2014). Resolving SV tethers in presynaptic terminals have previously required cryo-electron tomography (Siksou et al. 2007). We were able to reveal these tethers using conventional transmission electron microscopy (EM) by imaging synaptosome ghosts in which the cytoplasm that aldehyde-fixation would thicken had been ejected by osmotic rupture (Wong et al. 2014). We observed two classes of links that were related to the distance of the SV from the AZ: multiple-short (<45 nm) or single-long (45-175 nm) tethers. Based on its amino acid backbone we estimated that a CaV2.2 C-terminus could extend as far as ~200 nm into the presynaptic interior and suggested that this corresponds to the single-long tethers. We proposed a model where the SVs are 'grabbed' from peri-AZ cytoplasm by a long G-tether and are then 'locked' (L-tethered) by secondary attachments in preparation for exocytosis. To explore the G-tether hypothesis we used EM immunogold-labelling to localize the C-terminal tips of CaV2.2. Gold clusters were enriched on SVs in the peri-AZ region. These findings provide direct support for the idea that SVs bind to the channel distal C-terminal *in situ*.

N10. PREDICTING CELL-TYPE SPECIFIC ACTIVE PROPERTIES BY DEVELOPING MULTI-COMPARTMENT MODELS USING DATABASES AND ELECTROPHYSIOLOGICAL FEATURE CONSTRAINTS: APPLICATION TO INTERNEURON SPECIFIC 3 (IS3) CELLS IN THE HIPPOCAMPUS

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Determining how intrinsic properties govern and modulate neural input-output processing is a critical endeavour for understanding microcircuit functions in the brain. Here we focus on uncovering the intrinsic properties of interneuron-specific type 3 (IS3) cells in hippocampus, a cell type that makes GABAergic synapses onto specific types of interneurons. To date, IS3 cell morphology and synaptic output aspects have been examined, however, IS3 voltage-gated channel (VGC) types, densities and distributions remain uncharacterized. In this work, data on electrophysiological characteristics of IS3 cells was acquired, and two-photon calcium imaging was used to assess the spread of back-propagating action potentials in IS3 cell dendrites. Using this data as a target reference, we developed a semi-automated approach to generate IS3 cell models. In this approach we generated databases of multi-compartment models, each one possessing unique combinations

of ion channel types and conductance values. The rationale for the choices of ion channel types used were based on electrophysiological features, other hippocampal interneurons and *in situ* hybridization data from the Allen Mouse Brain Atlas. From our model databases we identified those with parameter ranges whose measurements most closely resembled those seen in experimental traces, and then analyzed the effects of different intrinsic properties on IS3 cell spike generation. Given the present correspondence with data, our models predict relative conductance balances of different channel types in IS3 cells as well as the impact of different channel type combinations on spike generation. Moving forward, our models can serve to investigate the functional roles of IS3 cells in the hippocampus, a central structure in memory formation.

N11. GLUTAMATERGIC SUBC CELLS ARE AT THE CORE OF THE REM SLEEP NETWORK

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It remains unclear which neuronal circuit and neurotransmitter mechanism triggers REM sleep. Glutamatergic neurons in the subcoeruleus (SubC) are active during REM sleep and are anatomically well positioned to control the muscle atonia and cortical activity that defines REM sleep, but it is unknown if these neurons actually influence or generate REM sleep. Here, we aimed to determine how optogenetic manipulation of glutamatergic SubC neurons impact REM sleep. To control the neuronal activity of the glutamatergic SubC neurons, we bilaterally infused 200nL of AAVs containing either a light-sensitive excitatory opsin or a light-sensitive inhibitory opsin or an inert control protein into the SubC of 27 Vglut2-cre mice. Animals were instrumented for EEG and EMG recordings. Neurons were light-manipulated either independently of behavioral state or specifically during REM sleep. We found that activation of SubC neurons significantly increased the probability of entrance into REM sleep and prolonged the duration of REM sleep episodes ($p<0.01$). Continuous inhibition throughout all behavioral states led to a decrease in REM sleep amounts ($p<0.01$) by abruptly shortening the duration of REM sleep episodes ($p<0.01$). Excitation of SubC neurons led to a stabilization of REM sleep muscle atonia, whereas inhibition led to a significant increase in motor activity above NREM sleep level ($p<0.01$). These results support the hypothesis that glutamatergic SubC neurons are at the core of the circuit which generate REM sleep and its characteristics.

N12. INVESTIGATING THE ROLE OF M6A MRNA METHYLATION IN MEMORY FORMATION

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Memory relies critically on the synthesis of new proteins, both at the soma and locally in dendrites, as evidenced by the amnesic effects of translation inhibition. How cells are able to precisely and independently coordinate translation at each of their synapses remains an important question. Recently, the most abundant modification of mRNA, N6 methyladenosine (m6A), was found to be involved in translational control. Just as epigenetic modifications of DNA are able to precisely and flexibly coordinate transcription during memory formation, mRNA modifications may underlie fine-tuned regulation of local translation. This project aimed to uncover whether the m6A mRNA methylation pathway is regulated memory formation, and develop viral tools that will enable further probing of its potential role in memory. We found that components of the m6A pathway, in particular the demethylase *Fto* (fat mass and obesity-associated protein), are highly expressed in the mouse brain. Furthermore, contextual fear conditioning, a hippocampus-dependent form of learning, modulates expression of several of these components, including *Fto*, in area CA1 of the hippocampus, while increasing overall levels of m6A in mRNA. To enable future causal investigation of the role of the m6A pathway in memory, we developed and validated herpes simplex virus (HSV) gene delivery vectors, including a CRISPR/Cas9 knockdown vector, to rapidly and selectively overexpress or knockdown FTO in neurons. Our findings suggest a promising role for m6A mRNA methylation in memory formation, and the development of Cas9-based HSV tools in particular will allow precise and flexible interrogations of this pathway.

N13. AROUSAL AND RESPIRATORY NETWORKS INTERSECT IN THE LOCUS COERULEUS WHERE CO2-RESPONSIVE NEURONS CONTROL BOTH THE AROUSAL AND RESPIRATORY RESPONSES TO HYPERCAPNIA

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The control of breathing and that of arousal are linked. Changes in blood gases can elicit a coordinated response from the respiratory and arousal control networks, which may rely on connecting hubs joining these networks that control the responses of both circuits to shared stressors. The CO2-responsive locus coeruleus (LC) — implicated in respiratory and sleep-wake regulation — may be such a connecting hub. We used unilateral microdialysis to pharmacologically inactivate

the LC in freely-behaving rats (n = 13) instrumented to record sleep-wake states and respiratory muscle activities. The GABAA agonist muscimol (50μM) was microperfused into the LC during normocapnia and hypercapnia (7% inspired CO2). Hypercapnia increased time spent awake by 87%. Hypercapnia also shifted the distribution of wake bout lengths from short (<50s) to long durations: wake-sustaining effect. Comparably, hypercapnia shifted the distribution of NREM bout lengths from long to short durations: wake-inducing effect. While LC Inhibition did not affect wake time under normocapnia, it did prevent 48% of the hypercapnia-induced increase in wake time. LC Inhibition reduced the wake-sustaining effect of hypercapnia by 98%; however, the wake-inducing effect of hypercapnia was not significantly attenuated. Hypercapnia reduced EEG power at all frequencies from 1-32Hz with the exception of the θ-band in NREM sleep and the δ-band in wake (mean reduction, 24%). Muscimol blocked hypercapnia-induced EEG activation at all frequencies in wakefulness; however, muscimol only reversed EEG activation in the α-band during NREM-sleep. The hypercapnic ventilatory response was significantly attenuated by LC inhibition in a state-independent manner (in wake and NREM-sleep respectively, hypercapnia-induced 2.1- and 2.6-fold increases in diaphragm minute amplitude were attenuated by 24 and 31% by LC inhibition). These results indicate that the respiratory and arousal control networks intersect in the LC forming a connecting hub that is required for the full response of both control circuits to hypercapnic stress.

N14. CALCIUM RESPONSES TO SINGLE ACTION POTENTIALS IN SPINAL CORD LAMINA I NEURONS

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Lamina I neurons of the spinal cord are a hub of nociception, taking in nociceptive information from the periphery, processing it, and relaying it to the brain. In chronic pain models, lamina I neurons exhibit hyperexcitability and decreased inhibition. Voltage-gated calcium channels (VGCCs) have been implicated in the development of chronic pain, however their function in lamina I neurons is poorly understood. Here, we develop an approach to measure calcium responses evoked by single action potentials (APs) in these neurons. We made current-clamp recordings of lamina I neurons, loaded via the patch pipette with the calcium indicator Oregon Green Bapta-1 (OGB1). APs were induced by current injection. Simultaneous two-photon imaging of OGB1 fluorescence in the somata, dendrites, and dendritic spines of lamina I neurons enabled calcium response analysis. Single APs induced robust ΔG/R increases in the somatic cytosol (peak ΔG/R = 0.1, n= 58 cells), nucleus (peak ΔG/R = 0.04, n= 58 cells), dendrites (peak ΔG/R = 0.2, n=75 dendrites) and dendritic spines (peak ΔG/R = 0.1, n=14 spines). Calcium responses were ablated by tetrodotoxin, and greatly reduced in the presence of nickel and NNC-55-0396, demonstrating a major role of T-Type VGCCs. The role of other VGCCs was also investigated. These findings suggest that single APs induce a calcium rise in the dendritic arbour and nucleus which occurs predominantly through T-type VGCCs. Action potential induced calcium influx through VGCCs could aid integration of inputs and alter gene transcription to upregulate excitability.

N15. ACTIVATION OF A MEDULLARY RESPIRATORY MOTOR CIRCUIT BY REMOTE CONTROL.

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Reductions in tongue muscle tone can precipitate obstructive sleep apnea (OSA). The hypoglossal motor nucleus (HMN) is the source of motor output to the tongue, and pharmacological activation of the HMN may increase tongue activity and reduce OSA. However, there is currently no pharmacological agent able to selectively manipulate a receptor or channel that is restricted in its expression to the cranial motor pools. To identifying the feasibility of pursuing such a "druggable" target at the HMN, we modelled a restricted drug-receptor interaction by introducing "designer" receptors into the HMN and selectively modulated them with a "designer" drug that exclusively interacts with these receptors. Using Cre-dependent viral vectors, hypoglossal motoneurons of ChAT-Cre$^+$ mice (n=7) were transduced with AAV8-hSyn-DIO-hM3Dq-mCherry receptors. After 2 weeks the mice were instrumented for sleep and respiratory muscle recordings. One week later, mice were studied before and after intraperitoneal injection of vehicle and clozapine-N-oxide (CNO; 1mg/kg); CNO activates hM3Dq receptors but is otherwise biologically inert. Systemic administration of CNO, to activate the hM3Dq receptors transduced at the HMN, increased tongue muscle activity across all sleep-wake states (p=0.013). Notably, tongue muscle activity increased in non-REM and REM sleep by 254±28% and 205±26% respectively compared to vehicle injection, and approached activity levels recorded during quiet wakefulness (79.8±14% and 53.3±9.1% respectively). Histology confirmed effective trans-gene expression at the HMN. There were no significant effects of CNO on diaphragm or neck muscle activity, or sleep-wake architecture. Selective activation of a "designer" pharmacological target that is locally expressed in the HMN results in sustained reactivation of tongue muscle tone throughout sleep. This result establishes proof of principle for pursuing a selective and restricted "druggable" target at the HMN - such as Kir2.4 or other channels with similarly restricted expression - as a potential pharmacotherapy for OSA.

N16. NEURONAL REACTIVATION CHANGES DUE TO NEUROGENESIS-MEDIATED FORGETTING

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Memories allow experiences to have some permanence in our minds. Increasing adult neurogenesis in the dentate gyrus of the hippocampus has been shown to reduce this permanency, leading to forgetting. The hippocampus encodes memories through the activation of a selection of neurons and successful memory retrieval is believed to involve reactivation of these neurons. Although successful memory retrieval has been linked to reactivation, we investigate whether decreased reactivation is the mechanism that underlies forgetting. This is achieved by permanently tagging neurons activated during the acquisition of a contextual fear conditioning task and analyzing dentate gyrus neuronal reactivation levels of forgotten memories after 28 days of exercise-induced neurogenesis. The data shows less reactivation in animals that demonstrated neurogenesis-induced forgetting, and a positive correlation between retrieval success and reactivation levels. These results indicate that neurogenesis-mediated forgetting can be explained at the cellular level as a reduction of complete reactivation of encoding neurons.

N17. GSK-3B INHIBITOR, TDZD-8, REDUCES HYPOXIA ISCHEMIA-INDUCED BRAIN INJURY IN NEONATAL MICE

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Glycogen synthase kinase 3β (GSK-3β) is a constitutive multifaceted kinase that plays a critical role in several neurodevelopmental processes including neuronal polarization, neurogenesis and axonal growth. Dysregulation of GSK-3β leads to neuronal cell death following ischemia-reperfusion injury in adults but its effect in neonates is less clear. However, it is known that expression levels of GSK-3β are higher in the neonates than the adults implicating a role of GSK-3β in neonatal hypoxic ischemic brain injury. Neonatal hypoxic-ischemic (HI) brain injury and its related disease hypoxic-ischemic encephalopathy (HIE) is a major cause of childhood mortality and morbidity with an incidence rate of 2-6 affected neonates per 1000 live births. TDZD-8 is a non-ATP competitive inhibitor specific for GSK-3β that has neuroprotective, antioxidative and anti-inflammatory properties. In this report, we investigated the effects of GSK-3β inhibitor TDZD-8 on neuroprotection against hypoxic-ischemic brain injury in neonatal mice. Hypoxic-ischemic injury was induced by unilateral ligation of the right common carotid artery on postnatal day 7 (P7) CD1 mice followed by 60 minutes of hypoxia with 7.5% O_2 and 92.5% N_2. We showed that pre-treatment with TDZD-8 significantly reduced brain infarct volume and improved sensorimotor function following the hypoxic-ischemic brain injury. TDZD-8 reversed the reduction of phosphorylated protein kinase B (Akt) and GSK-3β, and suppressed the activation of caspase-3 induced by the hypoxia-ischemia. Furthermore, we found that TDZD-8 reduced the hypoxic-ischemic induced apoptotic cell death and reactive astrogliosis which could link the Akt/GSK-3β/caspase-3 pathways. We conclude that GSK-3β plays an important role in neonatal hypoxic-ischemic brain injury, and can serve as a potential drug target for neonatal hypoxic-ischemic brain injury.

N18. MOLECULAR PATHWAYS RESPONSIBLE FOR NMDA RECEPTOR-MEDIATED BEHAVIOURAL PLASTICITY

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Impaired glutamate signalling through NMDA receptors is known to cause cognitive deficits in various neurodegenerative disorders, however it is unknown whether this impairment can be overcome in adulthood. Our laboratory has developed a new mouse line (NR1-CreTg) with a 90% reduction in NMDA receptor levels. The mutation can be restored to normal by an inducible Cre recombinase. We used this line to restore NMDA receptor levels and measured fear memory to investigate the reversibility of developmentally impaired glutamate signalling. The three genotypes studied were wildtype controls (WT), homozygous mutant NMDAR deficient (NR1KD), and homozygous mutant mice with a Cre transgene (NR1KD-Rescue). Molecular analysis was performed in the hippocampus, a brain region that is plastic and reliant on NMDAR mediated learning. The molecular events that occur in the process of behavioural plasticity will be explored using gene expression analysis of learning induced genes (ARC, CAMKIIα, FOS, NR4A1, EGR1, DUSP1, HOMER1A and BDNF). Although there were no genotype differences in basally expressed genes, upon further characterization we expect there to be differences in gene expression induced by learning. We expect such results due to partial recovery of associative fear memory in NR1-CreTg mice following rescue of NMDAR expression ($F_{3,23}$=80.58, $p<0.05$). Using the NR1-CreTg mouse line to rescue NMDA receptors, we would like to determine genes and molecular mechanisms mediating plasticity in the hippocampus.

N19. PROBING THE UNDERLYING MECHANISMS OF POSTANESTHETIC MEMORY DEFICITS

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Anesthetics cause postanesthetic memory deficits, which contribute to postoperative cognitive dysfunction (POCD). Understanding the causes of POCD and developing treatments is important because POCD is associated with prolonged hospital stays, increased costs, loss of independence and increased mortality. We previously showed that persistent memory deficits caused by etomidate result from an increase in tonic $GABA_A$ receptor-mediated inhibitory current in the hippocampus (*J Clin Invest*, 2014). The underlying mechanisms are uncertain. The same tonic current is increased by the pro-inflammatory cytokine IL-1β via its downstream factor p38-MAPK (*Cell Rep*, 2012). The two aims of this study were to determine whether persistent increase in tonic GABA current: 1) can be induced by other anesthetics; 2) is mediated by an IL-1β-dependent signaling pathway. Co-cultures of murine hippocampal neurons and cortical astrocytes were treated with drugs for 1h. Drugs were then washed out and whole-cell voltage clamp techniques were used to record tonic current 24h later. Western blotting was performed to measure IL-1β and phosphorylated p38-MAPK levels in hippocampal tissue collected from mice 24h after injection with etomidate (8 mg/kg, i.p.). Isoflurane (250 μM), sevoflurane (266 μM), propofol (3 μM) and midazolam (200 nM), but not ketamine (1 mM), increased the amplitude of the tonic current. The anti-inflammatory drug minocycline (100 μM) prevented etomidate-induced increase in tonic current. Etomidate enhanced IL-1β levels and p38-MAPK phosphorylation. Etomidate-induced increase in tonic current was reversed by the IL-1β receptor antagonist, IL-1Ra (100 ng/mL), and an inhibitor (SB 203,580, 20 μM) of p38-MAPK. Collectively, several inhalational and injectable anesthetics, but not ketamine, triggered a persistent increase in tonic GABA current. Etomidate-induced increase in tonic current is mediated by an inflammatory signaling pathway involving IL-1β and p38-MAPK. Thus, anesthetics may trigger a pro-inflammatory signaling pathway to increase the tonic current, and likely thereby cause persistent memory deficits.

N20. RYANODINE RECEPTOR-MEDIATED ION IMBALANCE PLAYS A ROLE IN NEONATAL HYPOXIC ISCHEMIC BRAIN INJURY

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Ryanodine receptors (RyR), located on the membranes of endoplasmic reticulum (ER), are potent provider of intracellular calcium levels upon activation. Imbalance of Ca^{2+} homeostasis is characteristic of hypoxia-induced brain injury, ultimately causing neurodegeneration. Thus, it has been implicated that RyRs contribute to Ca^{2+} imbalance post-hypoxic ischemia. In this study, we investigate the effects of RyR blocker, dantrolene on hypoxic-ischemic (HI) brain injury in neonatal mice. We found that application of a RyR blocker, dantrolene (i.p.) on 7 day-old mice reduced the infraction volume and morphological changes of the brain induced by HI. Neurobehavioural testing demonstrated dantrolene treatment enhanced neurological functional recovery. The neuroprotective effect of dantrolene was further demonstrated on neuronal cell culture *in vitro*, in which dantrolene significantly reduced oxygen-glucose deprivation (OGD)-induced cells death. Fura-2 calcium imaging analysis confirmed that dantrolene reduced calcium level in culture cortical neurons *in vitro*. Finally, western blotting analysis showed dantrolene treatment reduced caspase-3 and caspase-9 apoptotic proteins, and elevated PKC, survival protein levels. Taken together, our results showed a promising outcome of RyR blocker on neuroprotection compared to vehicle-treated group, suggesting a potential role RyRs might play in mediating ionic imbalance following hypoxic-ischemia, thus placing itself as a potent drug development target for HI.

N21. ROLE OF PAK SIGNALING WITHIN THE ENTORHINAL CORTEX IN THE REGULATION OF SYNAPTIC PLASTICITY AND SOCIAL MEMORY

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Neurodevelopmental disorders including Autism spectrum disorders (ASD) and intellectual disability (ID) are characterized by social impairments that impact adaptive functioning. PAKs (p21-activated kinase) are a family of serine/threonine protein kinases that are central regulators of both the actin cytoskeleton and neuronal morphology. Through genetic screening and post-mortem studies, mutations in the PAK gene are implicated in ASD and ID. However, how PAK coordinates synaptic transmission and plasticity, and the cellular and molecular mechanisms governing the neural circuitry in social memory remain elusive. We have generated a novel transgenic mouse model (CamKII/tTA tetO-PAK3) where the spatiotemporal expression of a dominant negative PAK mutation is modulated in the entorhinal cortex (EC). We find that dTg mice demonstrate specific deficits in social memory but normal sociability and novelty recognition. To analyze the role of PAK without potential developmental perturbations, we employ a tetracycline inducible "tet-off" system, where the

administration of a tetracycline analog, doxycycline (DOX), blocks transgene expression in the EC and restores social memory in dTg mice. We also demonstrate that the EC projections to the hippocampus along the perforant pathway (PP) show reduced basal synaptic transmission and impaired short-term plasticity due to inhibited neurotransmitter release. This suggests that PAK-mediated EC projections to the DG are critical for social recognition memory. To directly manipulate this circuitry more precisely, we utilize optogenetic inhibition in the EC of wild-type mice during memory retrieval and find similar social memory deficits as observed in dTg mice. We further demonstrate that infusion of an allosteric PAK inhibitor, IPA-3, via bilateral guide cannulas to the EC of wild-type mice also impairs social memory. Together these findings provide a novel cellular and molecular mechanism to understand the role of PAK signaling in the neural circuitry of the EC in regulating social memory processes.

N22. OPTOGENETIC MANIPULATION OF LOCUS COERULEUS NEURONS TRIGGERS RESPIRATORY MOTOR PLASTICITY

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The respiratory system is capable of plasticity. For example, repeated airway obstruction – as experienced in sleep apnea – can trigger a form of respiratory motor plasticity that strengthens the ability of hypoglossal motoneurons to trigger contractions of the tongue. This type of respiratory plasticity is known as long-term facilitation (LTF), and has been suggested to be dependent on noradrenaline acting at the hypoglossal motor pool. Despite its obvious functional importance, the source of noradrenaline remains unidentified. This study provides evidence that the locus coeruleus (LC) is one component of the neural circuit mediating LTF. LTF was triggered in anaesthetized, spontaneously breathing rats after exposure to an established repeated obstructive apnea protocol (10x 15s apneas separated by 1 minute). c-Fos, a biomarker for cell activation, was measured identify the source of noradrenaline mediating LTF. Intermittent bilateral optical stimulation of the LC (AAV5-hsyn-ChR2(H134R)-mCherry) was then performed in anaesthetized rats. Animals with histological verification of ChR2/mCherry expression in the targeted region were used for analysis. Apnea-induced LTF triggered increases in c-fos expression in one noradrenergic cell population – the LC (~77%±10% increase in the number of c-fos positive cells compared to controls) (n=5, unpaired t-test, $p<0.001$). This increased expression of c-fos was specific animals that exhibited LTF. To functionally test the role of the LC in mediating LTF, we found that repeated optical stimulation matching the pattern of stimulation induced by repeated apneas triggered LTF (~55%±24% increase at 60 mins after stimulation, n=6). These findings provide a novel insight into the neural circuitry associated with the generation of respiratory motor plasticity. Identifying the role of the LC in mediating LTF contributes to our understanding of this adaptive phenomenon and may help develop novel treatments for obstructive sleep apnea.

N23. A NEURODEGENERATIVE MODEL OF REM SLEEP BEHAVIOUR DISORDER

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During rapid-eye-movement (REM) sleep, wake-like brain activity is accompanied by muscle paralysis (atonia) interspersed with phasic twitches. The motor characteristics of REM sleep are thought to be controlled by the pontine subcoeruleus (SubC) region. REM sleep behavior disorder (RBD) is a disruption of motor control during REM sleep, manifesting as increased muscle activity with complex movements. Understanding the progression of RBD is vital, as >80% of RBD patients develop a neurodegenerative disorder associated with toxic aggregates of the protein alpha-synuclein (αsyn), most often Parkinson's disease. While the SubC region of RBD patients show αsyn aggregates and cell loss, there is no direct evidence of how SubC αsyn-related pathology affects REM sleep. We hypothesize that pathological αsyn in the SubC region will cause a dysfunction in motor control during REM sleep reminiscent of RBD. We drove αsyn expression bilaterally in the SubC region using 200nL of an adeno-associated virus harboring the human αsyn gene (AAV2-CBA-αsyn-GFP) in wild type mice (n=7). AAV2-CBA-GFP was used as control (n=7). Eight weeks later, sleep/wake states were determined via video polysomnography. During REM sleep, we analyzed basal muscle tone as well as the properties of phasic twitches. Immunohistochemistry was used to assess expression of αsyn in post-mortem tissue, and silver-staining was used to identify degenerating cells. Aggregates of αsyn were observed within SubC cells following viral transduction. Also, SubC cells of αsyn mice were positive for silver-staining, indicating αsyn promoted neurodegeneration. These αsyn mice showed muscle twitches with exaggerated duration (*αsyn: 68 ± 2 milliseconds; control: 60 ± 2 milliseconds; t-test, $p<0.05$*) during REM sleep*.* Our study supports the hypothesis that pathological αsyn in the SubC region can cause protein aggregation, neurodegeneration, and dysfunctional control of motor activity during REM sleep. Further studies are required to elucidate how the progression of αsyn pathology in the brain contributes to RBD. This research is vital to further the understanding of the development of RBD, and could lead to future interventions that stop the advancement of more severe αsyn-related neurodegenerative disorders.

N24. ENHANCED THALAMIC SPILLOVER-INHIBITION DURING NON-REM SLEEP TRIGGERS AN ELECTROCORTICAL SIGNATURE OF ANESTHETIC HYPNOSIS

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Modulation of thalamic GABAergic signaling can trigger state-associated changes in electrocortical activity. Such alterations in thalamic signaling are thought to underlie the increase in frontal alpha-beta electrocortical activity that signals anesthetic-induced loss-of-consciousness with $GABA_A$ receptor ($GABA_AR$) targeting general anesthetics. Importantly, the general anesthetic etomidate elicits phasic extrasynaptic $GABA_AR$ activation ("spillover" inhibition) at thalamocortical neurons *in vitro*. We hypothesize that enhanced phasic extrasynaptic $GABA_AR$ activation in the thalamus is sufficient to trigger the same electrocortical signature elicited by the presence of etomidate at the thalamus (*i.e.* increased frontal alpha-beta activity). Delivery of etomidate into the thalamus of a murine model ($n = 9$) elicited an increase in frontal alpha-beta electrocortical activity and sleep spindle-like oscillations *in vivo* (all $p < 0.031$). Importantly, this effect of etomidate was restricted to non-REM sleep, was not altered by blockade of thalamic T-type Ca^{2+} channels, and was *fully* recapitulated by pharmacological enhancement of thalamic spillover inhibition ($n = 18$). These findings suggest that when thalamic activity enters the mode of signaling set-up by transitions into non-REM sleep, then etomidate at the thalamus enhances GABAergic spillover inhibition to elicit the increase in frontal alpha-beta electrocortical activity that signals anesthetic hypnosis. These findings provide a mechanistic distinction between the electrocortical signatures elicited by thalamic activity during natural sleep and with an anesthetic, and are consistent with modeling studies implicating the thalamus as a key site orchestrating the changes in brain activity associated with anesthetic hypnosis.

N25. PARVALBUMIN+ INTERNEURONS CONSTRAIN THE SIZE OF THE LATERAL AMYGDALA ENGRAM

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During emotional memory formation, changes in the strength of connections between active neurons of the amygdala leads to the formation of specific neuronal ensembles, whose reactivation at later time points forms the basis of memory recall. These neuronal ensembles have been shown to include only a small, consistently sized fraction of the total population cells that are capable of responding to any particular stimulus. This sparsity has been proposed to be the result of a competitive allocation process, where only the most excitable cells are selectively recruited to the engram. Modeling studies that have attempted to describe how this process occurs have identified the importance of inhibition in constraining the engram to a sparse population. However, evidence for this form of inhibitory control has yet to be demonstrated in vivo. Here, we show that the lateral amygdala (LA) engram is actively constrained to 10-15% of neurons, and that this proportion expands when the activity of parvalbumin-containing interneurons is impaired during memory encoding. Using Arc expression as a measure of neural activity during retrieval, we show that the engram population in the lateral amygdala may be lower than previously estimated. Furthermore, using genetically targeted inhibitory DREADDS, we provide the first evidence that interneurons may play a role in determining the size of the lateral amygdala memory trace. These results confirm the predictions made in modeling studies regarding the role of inhibition in memory allocation and suggest that the lateral amygdala can be characterized by a high degree of sparsity.

N26. ASTROCYTES PLAY A CRITICAL ROLE IN DEXMEDETOMIDINE PREVENTON OF POSTANESTHETIC MEMORY DEFICITS

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Increasing evidence suggests that general anesthetics contribute to postoperative cognitive deficits (POCD) and delirium. We recently showed that anesthetics trigger a sustained increase in the activity of γ-aminobutyric acid type A receptors ($GABA_ARs$) in neurons and this increase in current causes postanesthetic memory loss (*JCI*, 2014). Further, anesthetics activate $GABA_ARs$ in astrocytes to trigger the release of soluble factors that, in turn, increase the tonic current in neurons. Dexmedetomidine (Dex) is an alpha2-adrenergic receptor agonist that is commonly used as sedative in the ICU. Dex reduces postoperative delirium (Psychosomatics, 2009) and may prevent anesthetic-induced neurotoxicity (*N Engl J Med.*, 2015). The goal of this study was to determine whether Dex prevents postanesthetic memory deficits by targeting alpha2-adrenergic receptors in astrocytes. Whole-cell voltage clamp methods were used to record the tonic GABA current from cultured hippocampal neurons. Cells were treated with etomidate (Etom, 1 μM) ± Dex, 10 μM for 1 h and then washed. Tonic currents were recorded 24 h later. For some studies, neurons were treated with conditioned medium from astrocyte cultures treated with Etom + Dex. The alpha2-adrenergic receptor agonist (clonidine, 100 μM) or antagonist (yohimbine, 5 μM) was used for some studies of astrocyte-neuron co-cultures. All data are expressed as mean ± SEM and were analyzed

by ANOVA ($p < 0.05$). The tonic GABA current was increased to 180% of control in Etom-treated neurons and this effect was prevented by co-treatment with Dex-treated astrocyte conditioned medium. Co-treatment with clonidine mimicked the effects of Dex, whereas the alpha2-adrenergic receptor antagonist yohimbine abolished the Dex effects on tonic current. Dex acts on alpha2-adrenergic receptors in astrocytes to prevent anesthetic-induced increase in tonic current. Studies are currently underway to determine whether Dex can be 'repurposed' as a strategy to reduce postanesthetic memory loss.

N27. THE EFFECT OF INHIBITORY CELL NETWORK INTERACTIONS DURING THETA RHYTHMS ON EXTRACELLULAR FIELD POTENTIALS IN CA1 HIPPOCAMPUS

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Oscillatory local field potentials (LFPs) are extracellularly recorded potentials with frequencies of up to ~500Hz whose biophysical origin is well understood in the framework of volume conductor theory. They reflect physiological functions in health and disease and complement the information obtained by analysis of spikes. A prevalent output from hippocampal networks is a 4-12 Hz "theta" oscillation. This LFP theta rhythm is tightly correlated with spatial navigation, episodic memory and rapid eye movement (REM) sleep. Our goal is to understand mechanisms of cellular contributions to this prominent rhythm. Using "LFPy", a python package that implements the biophysical framework of volume conductor theory, we constructed a pyramidal cell model of the CA1 region in hippocampus which generates extracellular potentials. Our pyramidal cell model receives inhibitory synaptic input from four different types of CA1 interneuron populations which have been suggested to be critically involved in theta. We investigated the contribution of each inhibitory cell type to the extracellular potential by varying the synaptic strengths and connectivities between the different inhibitory cell types – a virtual electrode probe along the vertical axis of the pyramidal cell recorded output in a layer dependent manner. As the balances among the cell types of the inhibitory network changed, important features of the signal such as polarity, amplitude and frequency were affected in distinctive ways. We obtained a mechanistic understanding underlying these effects thus illustrating the dynamic role that different inhibitory cell types could play in theta rhythms. Specifically, we were able to distinguish between regimes where synaptic connection strengths preserved the extracellular potential frequency versus those that led to lag or abolishment of the extracellular rhythm. Finally our simulations helped us assess the rate of spatial attenuation of the LFP thus allowing us to estimate the biological tissue volume contributing to the LFP signal.

N28. DIFFERENT FORMS OF DISINHIBITION HAVE DISTINCT EFFECTS ON DORSAL HORN CIRCUITS

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Pain caused by damage to the nervous system (i.e. neuropathic pain) is a notoriously intractable condition. Increases in spontaneous activity and receptive field sizes of somatosensory neurons have been found in animal models of neuropathic pain, and are thought to contribute to its debilitating nature. Reduced synaptic inhibition in the spinal dorsal horn plays a role in the pathogenesis of neuropathic pain; however the mechanism through which this disinhibition manifests is not clear. We aimed to investigate differences between two potential mechanisms of disinhibition: reduced $GABA_A$/glycine receptor activation and chloride dysregulation. Using Morris-Lecar model neurons, we built a computational neural network that modelled touch sensitive circuits in the dorsal horn. We fit the model using relevant properties of the circuit, determined through multi-channel in vivo recordings in adult male Sprague-Dawley rats. Our model was able to reproduce the effect of co-stimulation in different regions of the network's receptive field, and firing characteristics of excitatory and inhibitory neurons in the dorsal horn. Simulations of each mechanism of disinhibition determined that chloride dysregulation produced a pronounced increase in the spontaneous firing rate of neurons, whereas reduced receptor activation did not. Similar results were found experimentally, by pharmacologically inducing each form of disinhibition. Differences were also found in how each form of disinhibition modulated the receptive field of dorsal horn neurons. These results argue that different forms of disinhibition manifest in touch sensitive circuits in fundamentally different ways, and furthermore that chloride dysregulation is an important contributor to the development of neuropathic pain.

N29. COMPLEX MOLECULAR AND FUNCTIONAL OUTCOMES OF SINGLE VERSUS SEQUENTIAL CYTOKINE STIMULATION OF MICROGLIA

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Microglia are the 'professional' phagocytes of the CNS. While phagocytosis is crucial for normal CNS development and maintenance, it can be both detrimental and beneficial after injury or disease. Microglia can acquire pro-inflammatory (M1) or anti-inflammatory (M2) activation states, which can affect cell functions. Although microglia are exposed to a changing cytokine environment after injury or CNS diseases, little is known about molecular or functional consequences. Therefore, we applied several microglial activation paradigms (with or without phagocytosis of myelin debris) to primary rat microglia: M1 (IFN-gamma+TNF-alpha; 'I+T'), M2a (interleukin-4), M2c (interleukin-10), M1→M2a, M1→M2c, M2a→M1, M2c→M1. We assessed: (i) gene expression, reflecting their activation and inflammatory state, receptors and enzymes related to phagocytosis and reactive oxygen species (ROS) production, and ion channels; (ii) myelin phagocytosis and production of ROS, and (iii) expression and contributions of several ion channels that are considered potential targets for regulating microglial behavior. We found that M1 stimulation increased pro-inflammatory genes, phagocytosis and ROS production, as well as expression of KCa3.1, Kv1.3 and Kir2.1 channels. M2a increased anti-inflammatory genes, ROS production, and KCa3.1 and Kv1.3 channel expression. Myelin phagocytosis enhanced the M1 profile and dampened the M2a profile, and both phagocytosis and ROS production were dependent on NOX enzymes, and on Kir2.1 and CRAC channels. Importantly, microglia showed some capacity for re-polarization between M1 and M2a states, based on gene expression changes, myelin phagocytosis and ROS production. This study illustrates complex changes in gene transcription profiles and behavior in microglia. These changes could influence CNS inflammation and thus, should be considered in future experimental, pre-clinical studies.

N30. EFFECT OF NORMAL AND PARKINSON'S DISEASE-MUTANT ALPHA-SYNUCLEIN ON SYNAPTIC VESICLE RECYCLING IN HUMAN CNS PRESYNAPTIC TERMINALS

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α-Synuclein has been associated with a number of severe and progressive human brain disorders that have been grouped as α-synucleinopathies. Parkinson's disease (PD) is a key member of this group and several cases of the disease have been attributed to α-synuclein point mutations including A30P and A53T. α-Synuclein is known to be concentrated at presynaptic terminals and, while its precise presynaptic role remains elusive, the protein has been associated with synaptic vesicle (SV) recycling. Altered SV recycling was observed with normal and PD mutant α-synuclein overexpression in rodent tissue (Nemani et al. 2010). To explore if these effects also occur in humans, we introduced normal and mutant α-synuclein into isolated live human synaptosome (SSM) nerve terminals. SSMs were obtained from human cortex removed for epilepsy surgery. The test proteins were introduced into the SSMs by cryoloading (Nath et al. 2014) and SV recycling was assessed by depolarization-induced FM-dye uptake. Introduction of wild type or A53T mutant form of the protein had no detectable effect on FM-uptake but we did observe a small, but statistically significant reduction with A30P. Thus, our results did not reproduce in human terminals the significant pathological effects of α-synuclein on SV recycling observed in rodents and suggest that the evaluation of the cellular basis of α-synucleinopathies may require study using human-specific experimental models.

N31. GABA CELLS IN THE CENTRAL NUCLEUS OF THE AMYGDALA CONTROL CATAPLEXY

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Cataplexy is a debilitating symptom of narcolepsy characterized by the sudden loss of muscle tone during wakefulness. The neural mechanism underlying cataplexy is not well understood, but since it is often triggered by strong positive emotions, the amygdala is hypothesized to control cataplexy onset. We used chemogenetic, electrophysiological, and behavioural techniques to identify a GABA circuit in the central nucleus of the amygdala (CeA) that may play a causal role in promoting cataplexy. The CeA of 13 orexin-/-,VGAT-Cre mice was bilaterally injected with 200 nL of an AAV containing an excitatory DREADD expressed in GABA cells (AAV/hSyn-DIO-hM3Dq-mCherry). Neurons expressing this receptor are activated by clozapine-N-oxide (CNO). EEG, EMG, and video data were collected overnight following CNO or saline (control) injections. We found CNO-induced activation of GABA CeA cells increased time mice spent in cataplexy ($p<0.001$), triggering more episodes ($p<0.01$) without changing their duration. Levels of muscle atonia, theta activity, and episode duration were

identical under both saline and CNO conditions. We also found the CNO-induced cataplexy was mainly associated with positive stimuli such as wheel running ($p<0.01$), and the increased attack frequency arose from a reduction in the threshold to elicit cataplexy ($p<0.05$). Our data suggest that emotionally rewarding stimuli may trigger cataplexy by activating GABA CeA cells. Understanding downstream regions through which the CeA produces cataplexy is an important next step in dissecting cataplexy mechanisms.

N32. DISSECTING THE CIRCUITRY UNDERLYING MUSCLE PARALYSIS IN CATAPLEXY

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Cataplexy is generally triggered by strong positive emotions, which suggests that the central nucleus of the amygdala (CeA; a limbic structure that mediates emotional stimuli) is involved. Indeed, we recently showed that targeted chemogenetic activation of the CeA worsens cataplexy in narcoleptic mice. However, it remains unclear how the CeA connects with the circuits that control muscle paralysis. We hypothesize that emotional stimuli trigger cataplexy by activating the CeA, which in turn recruits the pontine circuits that induce REM sleep paralysis (i.e., the subcoeruleus, SubC). Specifically, we hypothesize that GABA cells in the CeA project to the ventrolateral periaqueductal grey (vlPAG) and lateral pontine tegmentum (LPT), which normally function to support waking muscle tone by inhibiting the SubC circuits that generate REM sleep paralysis. The following neuro-anatomical experiments aim to identify how the CeA communicates with the vlPAG and LPT. We hypothesize that GABA cells in the CeA project to GABA cells in both the vlPAG and LPT. In order to determine if GABA cells in the CeA project to GABA cells in the vlPAG and LPT, we injected cell-specific viral vectors expressing either yellow or red fluorophores into the CeA and vlPAG/LPT, (respectively) of VGAT-Cre mice. Using confocal microscopy, we found that GABA neurons in the CeA project to GABA neurons in the vlPAG and LPT, thus confirming our hypothesis. We suggest that emotional stimuli activate GABA cells in the CeA cells, which in turn project to and inhibit GABA cells in both the vlPAG and LPT. CeA-induced inhibition of GABA vlPAG/LPT cells results in cataplexy by disinhibiting SubC cells, which leads to the muscle paralysis/weakness that defines cataplexy. These data bring us one step closer to defining the circuit connections and mechanism that cause cataplexy.

N33. THE VENTRAL MEDULLA CONTROLS REM SLEEP PARALYSIS

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A defining feature of rapid eye movement (REM) sleep is muscle paralysis. Circuits that control this loss of muscle tone are not well understood. The ventral medulla (vM) may represent a site that generates this muscle paralysis, as vM cells are active during REM sleep and vM lesions produce REM sleep without paralysis. We sought to determine the role of the vM region in producing REM sleep paralysis. We bilaterally injected 100nl of AAV8-hSyn-DIO-hM4D(Gi)-mCherry (treatment group; n=8) or AAV8-hSyn-DIO-mCherry (control group; n=4) into GABA/Gly vM cells of VGAT-Cre mice. Mice were instrumented with EEG and EMG electrodes, and sleep-wake behaviours analyzed for 2-hrs after administration of clozapine-*N*-oxide (CNO, 5mg/kg). Videography, EEG, and EMG were used to identify behavioral states. Glass-coated tungsten electrodes (125 µm, 0.3-0.5 MΩ) were used to record hM4D(Gi)-expressing GABA vM cells before and after CNO administration (n=4). Activation of hM4D(Gi) receptors in the vM by CNO rapidly and significantly attenuated discharge activity of vM cells in mice (before CNO: 183 ± 10 action potentials/minute; after CNO: 2 ± 1 action potentials/minute; ANOVA, $p<0.001$) for 95-120min before returning to baseline levels (175 ± 24 action potentials/minute). Although inhibition of the GABA/Gly vM neurons had no effect on sleep-wake architecture compared to controls (*t*-tests: wakefulness, $p=0.1808$; NREM sleep, $p=0.1041$; REM sleep, $p=0.5373$), it prompted elevated muscle activity during REM sleep (hM4D(Gi): 0.99 ± 0.02 normalized ∫EMG; mCherry: 0.76 ± 0.07 normalized ∫EMG; *t*-test, $p=0.0019$). Muscle activity was increased to NREM levels, and on occasion to levels arising under wake. This increase in muscle activity was constrained to REM sleep. Despite elevations in muscle activity during REM sleep animals failed to rouse, showed typical REM sleep cortical activity, and maintained stereotypical sleeping positions. These data provide support for the hypothesis that GABA/Gly neurons of the vM promote REM sleep paralysis.

N34. NEUROGENESIS AND MEMORY: A COMPUTATIONAL APPROACH

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The continuous addition of new neurons to the adult hippocampal circuits has been shown to induce forgetting of previously learned memories corroborating the findings of computational models that predicted these effects. However, the precise mechanisms mediating these neurogenesis-dependent forgetting effects are unclear. Here we developed a three layer feed-forward neural network to represent the hippocampus, with the input, middle and output layers representing

the entorhinal cortex, dentate gyrus and CA3 regions, respectively. We trained the network on a set of patterns and then, to model ongoing hippocampal neurogenesis, we added new neurons to the middle layer. Consistent with our in vivo results[1], addition of new neurons reduced retrieval success for learned patterns. Furthermore, training the network on new but conflicting information led to a facilitation of learning in the neurogenesis network compared to the non-neurogenesis network, but no benefit in the case where new, non-conflicting information was presented. This could indicate that the addition of new neurons provides a benefit only in the case of reduced interference of new learning, which has also been shown behaviourally. The results from this model will provide a framework for investigating the dynamics of neurogenesis-mediated forgetting in the hippocampus.

N35. TRANSIENT RECEPTOR POTENTIAL MELASTATIN 7 (TRPM7) REGULATES AXONAL DEVELOPMENT AND MATURATION OF PRIMARY HIPPOCAMPAL NEURONS

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Transient Receptor Potential Melastatin 7 (TRPM7) is a calcium-permeable, non-selective cation channel that has been implicated in biological processes such as cell adhesion, migration, cytoskeleton regulation and survival. As these processes are necessary for neurite outgrowth during neuronal development, we investigated whether TRPM7 is involved in regulation of neurite outgrowth. In this study we demonstrate that TRPM7 is highly expressed in the growth cones of mouse cultured hippocampal neurons, and that both viral knockdown and pharmacological inhibition of TRPM7 preferentially enhanced axonal outgrowth of hippocampal neurons at multiple time points during development. We also showed that pharmacological inhibition of TRPM7 accelerated the progression of neurons into a higher developmental stage, as was evident by the formation of morphologically distinct axons and dendrites. Moreover, we found that TRPM7 co-immunoprecipitated and co-localized with F-actin and α-actinin-1, two major cytoskeletal proteins involved in actin-based growth cone protrusion. Based on these findings we proposed a model of TRPM7-mediated calcium-dependent cytoskeletal dynamics at the neuronal growth cone. Out findings highlight the importance of TRPM7 during neuronal development and suggest a therapeutic potential of TRPM7 blockers in neurodegenerative and neurodevelopmental disease.

N36. PARVALBUMIN-POSITIVE INTERNEURONS MODULATE HIPPOCAMPAL-CORTICAL COUPLING AND FEAR MEMORY CONSOLIDATION

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As a memory undergoes consolidation, it becomes less reliant on the hippocampus (HPC) and more dependent on the medial prefrontal cortex (mPFC) for retrieval. Rhythmic oscillations in the mPFC, including delta waves and spindles, coincide with oscillations in the HPC, called sharp-wave ripples, and this oscillatory coupling across brain regions is thought to facilitate memory consolidation. While parvalbumin-positive interneurons (PVNs) fire in synchrony with spindles and ripples, whether they contribute to mPFC-HPC coupling, and by doing so, modulate memory consolidation, is unclear. Here, we combine the designer receptor approach with behavior experiments and in vivo recording in freely-behaving mice, to manipulate PVNs, and investigate their roles in memory consolidation. PV::Cre mice were infused with Cre-recombinase-dependent virus carrying the designer receptor hM4Di, which allows PVNs to be silenced by the designer drug clozapine-N-oxide (CNO). After surgery, mice were trained using contextual fear conditioning, then we silenced the PVNs. When mPFC or HPC PVNs were selectively inhibited during the consolidation period following conditioning, mice showed memory deficits. Electrophysiologically, we found that fear learning enhanced the probability of coupling between ripples, and cortical delta waves and spindles. This enhancement was attenuated when mPFC or HPC PV+ cells were silenced. This suggests that PVNs help coordinate mPFC-HPC co-activation, and interfering with their activity disrupts mPFC-HPC communication, and impairs memory consolidation.

N37. SOMATOSTATIN CIRCUITS REGULATING RHYTHMIC BREATHING AND MOTOR OUTPUT IN VIVO: OPTOGENETICS AND PHARMACOLOGICAL STUDIES

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Breathing is an autonomic behaviour generated by a complex respiratory network in the brainstem. At its core is the preBötzinger Complex (preBötC), a population of neurons producing rhythmic breathing by driving the respiratory network and respiratory muscles. The preBötC contains interneurons expressing the inhibitory peptide somatostatin (SST) and generates rhythmic breathing. Importantly, SST-expressing neurons also project to the hypoglossal premotor area to activate the genioglossus muscle, an upper airway respiratory muscle. The preBötC therefore regulates two distinct

components of respiratory activity: rhythmic breathing and motor output. Using pharmacological and optogenetics approaches, we aimed to identify the roles of SST preBötC neurons in modulating rhythmic breathing and motor output. To determine the role of SST and its cognate receptors, we microperfused selected pharmacological agents into the preBötC of adult anesthetized rats while recording diaphragm and genioglossus muscle activity. Using a cre-lox strategy, we also expressed the excitatory channelrhodopsin ChETA in SST-cre mice. We injected the adeno-associated viral vector ChETA-mCherry into the preBötC of SST-cre mice. After 3 weeks to allow expression of ChETA, we inserted an optical fiber into the preBötC and stimulate SST-expressing neurons while recording respiratory muscle activity. SST (200 μM) decreased rhythmic breathing and genioglossus muscle activity. SST inhibition of rhythmic breathing, but not genioglossus muscle activity, was blocked by the SST2A receptor antagonist CYN-154806 (20 μM). Genioglossus muscle activity, but not rhythmic breathing, was decreased by the SST4 agonist NCC- (10μM). Using optogenetics, we then showed that excitation of SST neurons increased respiratory rate but did not increase genioglossus muscle activity in anesthetized mice. We dissected the neural circuits mediating rhythmic breathing and respiratory motor output in vivo. We identified two SST receptor subtypes mediating rhythm and motor output. Using optogenetics we found that SST neurons in the preBötC mediate rhythmic breathing.

Reproduction and Development Posters

R1. DISRUPTION OF JMJD6-MEDIATED HISTONE DEMETHYLATION OF *VHL* IN PREECLAMPSIA

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Persistent hypoxia is implicated in the pathogenesis of preeclampsia. Evidence implicates Jumonji C domain containing histone demethylases as oxygen sensors and regulators of hypoxic gene expression. We recently reported that the expression of the lysyl hydroxylase, JMJD6 (Jumonji domain containing protein 6) is regulated by oxygen in the developing placenta. Given that JMJD6 plays a key role in the histone code by demethylating arginine residues on histones 3 (H3R2me2) and 4 (H4R3me2), we sought to examine its epigenetic histone demethylase function in controlling the gene expression of *VHL* (von Hippel Lindau tumour suppressor), a regulator of the hypoxic response, in the human placenta in physiological and pathological conditions. Placentae were obtained from pregnancies complicated by preeclampsia (PE; n=45), and normotensive preterm (PTC; n=26) and term (TC; n=20) controls. JMJD6 overexpression in JEG3 cells significantly increased *VHL* mRNA in 20%, but not in 3% O_2. Chromatin Immunoprecipitation analysis of H4R3me2 following JMJD6 overexpression in JEG3 cells revealed >10 fold enhancement in H4R3-bound *VHL* relative to controls. *In vitro* demethylation of bulk histones and histones isolated from primary cytotrophoblasts revealed a striking decrease in both targets in 8% and 20% O_2, but not in 3% O_2, suggesting optimal JMJD6 demethylase activity in normoxia. qPCR analysis showed a significant decrease in *VHL* mRNA in PE placentae. Notably, Western blotting showed that both histone marks were elevated in whole cell lysates and histones isolated from PE placentae. Incubation of histones with recombinant JMJD6 reduced both marks in PTC and TC, but not in PE placentae, indicating an inability to demethylate its substrates in pathological conditions. This study provides novel evidence of oxygen-dependent *VHL* epigenetic regulation by JMJD6 through site-specific histone arginine methylation. We propose that the hypoxic environment in PE inhibits JMJD6 demethylase activity, thereby contributing to the decreased *VHL* observed in this pathology.

R2. PROGRAMMING OF MULTIDRUG RESISTANCE AT THE BLOOD-BRAIN BARRIER BY ANTENATAL GLUCOCORTICOID TREATMENT

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P-glycoprotein (P-gp; encoded by *Abcb1*) and breast cancer resistance protein (BCRP; encoded by *Abcg2*) expressed by brain endothelial cells (BEC) efflux an array of drugs, hormones and toxins at the blood-brain barrier (BBB). Our lab has characterized the developmental expression of P-gp, which is dramatically increased in late gestation, coincident with the endogenous cortisol surge. In cases of preterm birth, synthetic glucocorticoids are administered to mimic the cortisol surge and mature the fetal lungs. The long-term impact of this treatment on BBB transport is unexplored. Our lab has shown P-gp function is increased by corticosteroids in cells derived from gestation day (GD) 50, 65 and post-natal day (PND) 14 guinea pigs. Most recently, we discovered increased hypothalamic P-gp expression in young guinea pigs 55 days after exposure to sGC *in utero*. Another study found juvenile traumatic brain injury to suppress P-gp for up to 6 months. Together, these findings suggest that early exposures can lead to long-term changes (or 'programming') of drug resistance/sensitivity at the BBB. We therefore hypothesize that maternal sGC treatment increases P-gp and BCRP expression and function at the juvenile and adult BBB, resulting in reduced drug penetration into the brain. We will use a guinea pig model as this species has similar neurodevelopmental profile as humans, and give birth to neuroanatomically mature offspring. Levels of *Abcb1* and *Abcg2* mRNA and P-gp and BCRP protein will be measured by qRT-PCR and Western blot, respectively. BECs will also

be derived from microvessels and maintained in primary culture to assess of P-gp and BCRP function, by fluorescent functional assays and by a cytotoxicity bioassay using a toxic P-gp substrate colchicine. This project will advance understanding of how fetal exposures can lead to long-term changes in brain protection and drug sensitivity. These findings will have clinical ramifications, particularly in the neonatal brain where neurotoxicity of P-gp substrates has been reported. Prenatal programming of P-gp at the BBB may also form a link to neurodegenerative diseases such as Alzheimer's disease.

R3. COMPARATIVE CHARACTERISTICS OF MYOMETRIAL AND DECIDUAL CHEMOKINES RESPONSIBLE FOR THE INFILTRATION OF PERIPHERAL LEUKOCYTES DURING HUMAN LABOUR

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Infiltration of leukocytes into the uterine tissues is an essential step in parturition. In this study we examined (1) cytokine/chemokine profiles secreted by primary human myometrial and decidual cells before and during term labour (TL), and (2) the effect of myometrial and decidual secreted factors on peripheral neutrophil transendothelial migration (TEM). Myometrial biopsies and decidua samples were collected from elective caesarean section (term not in labour, TNL) or TL following informed consent. Tissues underwent collagenase digestion to obtain primary cells. To generate myometrial (MCM) and decidual conditioned media (DCM), cells were grown until confluence in DMEM supplemented with 20% FBS and then incubated in serum-free DMEM for 48 hours. TNL DCM, TNL MCM and TL MCM were analyzed by 67-plex Luminex assay for chemokine/cytokine protein expression. For TEM assays, a monolayer of primary human uterine microvascular endothelial cells (UtMVEC) formed in 3µm inserts was primed with MCM, DCM or serum-free DMEM (control). Peripheral blood neutrophils were isolated from whole blood of pregnant women via Histopaque density gradient, fluorescently-labelled, added to the insert and co-cultured with the CM-primed endothelial monolayer for 1 hour. Fluorescence of migrated cells collected from the lower well was measured with a plate reader. TNL DCM expressed higher levels of 67 cytokines compared to TNL MCM and TL MCM. TL MCM showed higher expression of all chemokines in comparison to TNL MCM. In preliminary experiments (n=2), MCM and DCM-primed neutrophils showed enhanced TEM compared to control neutrophils, with MCM priming eliciting a greater response compared to DCM. Cytokines involved in chemotaxis of peripheral leukocytes were detected in media conditioned by myometrial and decidual primary cells, implicating their potential role in leukocyte migration. Further experiments will investigate the effects of MCM and DCM on leukocyte activation, adhesion and migration.

R4. PROBIOTIC LACTOBACILLUS RHAMNOSUS GR-1 SUPERNATANT CAN ALTER LIPOPOLYSACCHARIDE-INDUCED CYTOKINE SECRETION BY HUMAN MYOMETRIAL CELLS

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Preterm labour (PTL) affects approximately 11% of pregnancies worldwide. While the majority of PTL are idiopathic, intrauterine infection is a key contributor to PTL through the activation of inflammatory pathways. Probiotic *Lactobacillus rhamnosus* GR-1 is an innate species that confers health benefits by interfering with pathologic bacterial and yeast growth in the vagina. Recently, it has been shown that GR-1 supernatant (GR-1SN) can delay LPS-induced PTL in pregnant CD-1 mice and decrease concentrations of key pro-inflammatory cytokines in maternal plasma, uterine muscle (myometrium) and fetal gestational tissue (amniotic fluid and placenta). The objective of this study is to examine the mechanisms behind this preventative phenomenon imposed by GR-1SN in human myometrial cells. Human myometrial cells (immortalized cell line hTERT-HM) were cultured in DMEM/F-12 supplemented with 10% FBS. When 75% confluent, myometrial cells were serum-starved and pre-treated with GR-1SN for 24h, followed by a stimulation with LPS (100ng/mL) or vehicle. Cell conditioned media was collected and analyzed for pro-inflammatory cytokines IL-8 and MCP-1 using ELISA. LPS stimulus induced 4-fold increase in IL-8 ($P<0.001$) and 4.5-fold increase in MCP-1 secretion ($P<0.05$) by human myometrial cells. GR-1SN pre-treatment prior to LPS stimulus suppressed both IL-8 and MCP-1 secretion in hTERT-HM by 2-fold ($P<0.01$). Levels of IL-8 and MCP-1 secreted in the cultured media by myometrial cells pre-treated with GR-1SN were not different from the control vehicle-treated cells. Alternatively, pre-treatment with low dose LPS (0.1 ng/mL) also suppressed hTERT-HM cytokine secretion as compared to a stimulation with high-dose LPS (100ng/mL). We hypothesize that there are biologically active components within GR-1SN that can desensitize TLR4 receptors or function as TLR4 antagonists thus suppressing activation of inflammatory pathways and subsequent pro-inflammatory cytokine secretion. Further experiments are in progress to elucidate the mechanisms by which GR-1SN interacts with cellular processes to influence cytokine secretion.

R5. OOCYTE-SPECIFIC PDSS2 DEFICIENCY LEADS TO EMBRYO DEVELOPMENT ARREST AND ALTERATIONS IN MITOCHONDRIAL CONTENT AND FUNCTION

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CoQ, a lipophilic molecule important for proper mitochondrial function, is synthesized through the cooperation of several different enzymes. One of the enzymes, polyprenyl diphosphate synthase, is a heterotetramer of subunits Pdss1 and Pdss2. Previously, it was shown in our lab that Pdss2-deficient female mice displayed similar characteristics as aged female mice; they had decreased ovarian reserves and reduced ATP synthesis. In our study, we sought to fully characterize the cellular consequences of Pdss2 deficiency in murine oocytes and study the role of Pdss2 in embryogenesis. For our studies, we focused on GV, MII, and 2-cell embryos for assessment. In addition to chromosomal anomalies, there was a general decrease in the markers of mitochondrial content and function. There was, however, an unexpected increase in the respiring mitochondrial pool in the 2-cells from Pdss2-deficient females. Developmental arrest was also observed in Pdss2-deficient embryos, as only ~10% were able to make it to the late blastocyst stage. We tried to rescue this phenotype by supplementing females from birth with coQ in their drinking water. However, coQ supplementation was not able to ameliorate developmental arrest seen in Pdss2-deficent embryos. A recently published study regarding Pdss2 mutants demonstrated that the dysfunction in these mutants could be due to autophagic and translational issues rather than coQ deficiency. Therefore, our next step is to assess markers of autophagy and lysosomal function to test whether such dysfunction may underlie phenotypes seen in Pdss2-deficient oocytes.

R6. A SYSTEMATIC REVIEW OF STUDIES ON SPERM TELOMERE LENGTH (SPTL) AND MALE FERTILITY DEMONSTRATES THE MAJOR IMPACT OF STUDY DESIGN ON FINAL RESULTS

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The cause of infertility remains unknown in 30% of cases. When a male factor is identified, the underlying cause is unexplained in 25% of cases. To improve diagnosis and treatment of idiopathic infertility, clinical testing of the male requires further development. Telomeres are non-coding sequences of DNA (TTAGGGn) located at the terminus of linear chromosomes. Chromosomes flanked by short telomeres are less stable and more susceptible to reactive oxygen species-induced damage. Infertile men often exhibit high levels of unexplained sperm DNA damage. In recent years, numerous publications have related sperm telomere length (spTL) with male fertility outomes, but generated conflicting results. Here, we conduct a comparative analysis of studies to determine the cause. We searched PubMed using the keywords 'telomere length', 'sperm' and 'human'. The search returned 12 primary articles that conducted direct comparisons between mature spTL and male fertility potential (i.e. clinical tests of sperm health, fertility outcomes, and/or offspring health). We compared final conclusions to determine the level of concordance. Next, we compared sample size (n), fertility status and age of participants, as well as semen fraction, DNA isolation method (when applicable), and TL assay used in the analysis. The results were summarized into 9 categories, and each study characterized as 'in agreement' or 'disagreement'. There was consensus (100% concordance) that spTL distributions demonstrate inter-individual variability, spTL does not correlate with morphology, and intra-individual spTL is greater than somatic cell TL. Discordant findings (66.7%-75% concordance) included: longer spTL is associated with improved semen analysis parameters (motility and concentration), and more prevalent in fertile men. Studies that compared spTL and DNA fragmentation were divergent (50% concordance). To investigate the cause of variation, our second analysis revealed three major differences in design across studies: (1) evaluation of different semen fractions, (2) evaluation of different male populations with variable fertility status and age; and (3) utilization of TL assays that differ in upstream DNA processing. Overall, we have shown that opposing findings in the literature are disparate due to important differences in study design and assay selection, and that these factors can dramatically alter final results of spTL.

R7. DECIDUA-DRIVEN DIFFERENTIATION OF ANGIOGENIC PHENOTYPE IN SECOND TRIMESTER PERIPHERAL BLOOD NEUTROPHILS FROM HEALTHY AND PREECLAMPTIC WOMEN

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Previously, we identified a novel population of 2nd trimester decidual neutrophils possessing a distinct angiogenic phenotype compared to peripheral blood neutrophils (PBN). In TGFβ-dominant environments, eg. tumours, traditional N1

inflammatory neutrophils differentiate to an angiogenic cancer-promoting N2 phenotype. We hypothesize that the decidua similarly differentiates PBN into an N2-like phenotype. To investigate our hypothesis, PBN isolated from women at 16 wk, 26 wk and women suffering from preeclampsia (PE) were split into 6 treatment groups: 1) RPMI; 2) TGFβ; 3) TGFβ + IL8; 4) 2nd trimester decidua-conditioned media (DCM); 5) DCM + DMSO; & 6) DCM + anti-IL8 antibody + 10uM SB431542 (TGFβ inhibitor). After 5h, coculture was performed with uterine endothelial cells (UtMVEC), umbilical vein endothelial cells (HUVEC) or HTR8 trophoblast cells for 24h to compare functional angiogenic ability via tube formation assay. Total tube length was significantly enhanced by DCM-PBN coculture in all cell lines and abrogated by inhibitor pre-treatment. RNA was also isolated for RT-qPCR of N1/N2 factors, which indicate that DCM can significantly enhance N2 marker expression. Notably, DCM-PBN show significantly different expression of 5 genes across the 3 groups (16 vs. 26 vs. PE, one-way ANOVA), of which 3 (CMYC, CXCR4, FAS) can be used to reliably distinguish PE from 26 wk samples (via binary logistic regression), suggesting that PE neutrophils may be resistant to decidual differentiation. Further experiments in progress will aim to elucidate the specific differences between healthy and PE PBN and why neutrophil differentiation might be defective in preeclamptic pregnancies.

R8. EPIGENETIC REGULATION OF PLACENTAL GENE EXPRESSION IN TRANSCRIPTIONAL SUBCLASSES OF HUMAN PREECLAMPSIA

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Preeclampsia (PE) is a heterogeneous, hypertensive disorder of pregnancy, with no robust biomarkers or effective treatments. We hypothesize that this heterogeneity is due to the existence of multiple subclasses of PE driven by different molecular pathways. In support of this hypothesis, we recently identified five clusters of placentas within a large microarray dataset (N=330), of which three (clusters 1, 2, and 3) contained clinically distinct subgroups of PE samples. However, while transcriptional analysis of placentas can classify patients, the addition of epigenetic information should reveal gene regulatory mechanisms behind the pathology. We therefore subjected 43 of our samples from transcriptional clusters 1, 2, and 3 to Infinium Human Methylation 450K arrays, and investigated relationships between the gene expression and methylation data. In comparison to transcriptional cluster 1 placentas, the healthiest samples in our dataset, lower methylation was found to be associated with upregulated genes involved in epithelial differentiation and glycolysis in cluster 2 samples, including the known PE markers FLT1, LEP, and INHBA. Additionally, a significant downregulation of electron transport and ATP synthesis genes were identified in cluster 2 samples without an increase in methylation. Also in contrast to cluster 1, cluster 3 revealed increased expression of genes associated with defense/immune response, for which corresponding epigenetic changes were confirmed. However, we also identified a large number of immune-associated upregulated genes in cluster 3 that could not be explained by methylation, some of which are not normally expressed by the placenta. Overall, we have established epigenetic mechanisms behind the development of "canonical" PE in cluster 2 and "immunological" PE in cluster 3. Currently, we are exploring alternative explanations for the changes in gene expression with poor correlation to DNA methylation, such as knockdown by microRNAs of respiration-related genes in cluster 2, and possible infiltration of maternal immune cells in cluster 3.

R9. THE INFLUENCE OF PHYSICAL ACTIVITY IN A MOUSE MODEL OF EPITHELIAL OVARIAN CANCER

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Only 45% of women diagnosed with epithelial ovarian cancer (EOC) survive five years after diagnosis. The high mortality is attributed to a lack of early detection biomarkers and indistinct symptoms. The disease is detected when EOC cells have exfoliated from the primary ovarian tumor site to transform the intraperitoneal fat depots into solid tumors. In addition to the metabolic shift in the tumor microenvironment, there is a progressive loss of skeletal muscle that is associated with changes in metabolic pathways in cancer patients. The host-energy balance can influence disease progression, as caloric excess is tumor promoting, while caloric restriction delays progression. Energy expenditure, by physical activity could provide an intervention to delay EOC progression and onset of the molecular mechanisms that govern tumor growth and cachexia. Female C57BL/6 mice were exercised (n=21) on a motorized treadmill (21m/min, 1hr, 3 times/week) and compared to sedentary animals (n=18). Five-weeks after the start of the exercise regimen, mice were injected with mouse ovarian surface epithelial cancer cells (MOSEC) under the ovarian bursa in a syngeneic mouse model. Injection of MOSEC cells results in the formation of intraperitoneal tumors and accumulation of ascites, reminiscent of EOC progression in women. Mice were assessed for changes in body composition (echoMRI). At end-point, tumors and the gastrocnemius muscle were harvested and were used to assess the differences in metabolic signaling cascades and cachexia markers by qPCR and western blot between exercise and sedentary animals. Physical activity prolongs survival of mice ($p<0.05$) and

reduces tumor burden (p<0.002) compared to sedentary animals. Despite exercise treatment, there is no difference in body composition throughout the progression of the study. While there were no significant changes to the cachexia markers in the muscle, metabolic pathways such as the IGF-axis were influenced by physical activity in both the tumors and muscle.

R10. TRANSGENERATIONAL EPIGENETIC PROGRAMMING IN THE HIPPOCAMPUS BY ANTENATAL GLUCOCORTICOIDS

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Antenatal exposure to high levels of glucocorticoids (maternal stress, synthetic glucocorticoids [sGC]) alters brain development in young animals and children. In animals, prenatal sGC exposure modifies DNA methylation and gene expression in the brains of 2nd generation (F_2) offspring. We hypothesized that antenatal sGC exposure modifies DNA methylation in the hippocampi of F_3 offspring via paternal transmission. Pregnant guinea pigs (F_0) received 3 courses of sGC or saline (C). Adult F_1 & F_2 male offspring bred with control females to produce F_2 & F_3 offspring. F_1 & F_3 juvenile female hippocampi (C/sGC; n=6) were collected at day 40. Hippocampal DNA methylation was measured using Next-Gen bisulfite sequencing. The hippocampal transcriptome was assessed in the same tissues using RNA-seq. sGC resulted in differential methylation of 221 genes in F_1 and 191 in F_3 ($p \leq 0.001$, FDR 5%). Differential methylation occurred in genes related to calcium signaling pathways in F_1 ($p \leq 0.001$, Benjamini < 0.005), and neuron projection and long-term potentiation in F_3 ($p \leq 0.001$, Benjamini < 0.03). RNA-seq identified differential expression of 441 gens in F_1 and 709 in F_3 ($p \leq 0.0001$, FDR 5%). sGC down-regulated genes involved in neurotransmitter binding and receptor activity in F_1, and transmembrane transport in F_3 (NES > 1.6, $p \leq 0.01$, FDR= 25%). This is the first study to demonstrate that antenatal sGC exposure programs heritable changes in DNA methylation in the brain.

R11. METHIONINE-MEDIATED PROTECTION OF FETAL PROGRAMMING BY PRENATAL SYNTHETIC GLUCOCORTICOIDS

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Women at risk for preterm delivery receive treatment with synthetic glucocorticoids (sGC) in order to promote the maturation of fetal organs and reduce infant mortality associated with prematurity. However, sGC treatment also alters fetal brain development, and has been shown to modify behaviour and neuroendocrine function in children. We have previously demonstrated in guinea pigs that maternal sGC treatment profoundly modifies gene expression in the fetal hippocampus, and this is associated with reduced DNA methylation in gene promoters. Previous studies have shown that methyl-donor (e.g. methionine) composition of the maternal diet can influence DNA methylation. Increasing maternal intake of methyl-donor compounds influences the methionine cycle, a series of reactions involving the transfer of methyl groups that is vital to the process of DNA methylation, and promotes increased methylation. We hypothesize that sGC will result in DNA hypomethylation in the hippocampus through changes in the expression of key methionine cycle enzymes, and that maternal methionine supplementation will alleviate this hypomethylation and subsequent changes in patterns of gene expression. At gestational day (GD) 30, pregnant guinea pigs will be provided with methionine-enriched drinking water (0.4g/L) or untreated water. On GD 40|41 and 50|51, mothers will be subcutaneously injected with either sGC (betamethasone; 1mg/kg) or saline (vehicle). Liquid chromatography-mass spectrometry will determine the levels of key methionine cycle metabolites (e.g. methionine, homocysteine) in fetal hippocampi and fetal and maternal plasma. qRT-PCR will quantify mRNA levels of key methionine cycle genes (e.g. *Mat, Dnmt*). Transcriptional data will drive epigenetic analysis by pyrosequencing in order to determine the methylation status of genes of interest. This research is clinically relevant as 10% of births worldwide are preterm. This will be the first study to demonstrate protection against adverse effects of prenatal sGC treatment, an important step towards minimizing the life-long neuroendocrine outcomes of this life-saving therapy.

R12. IS IT PRA OR PRB DOMINANT? PROGNOSTIC POTENTIAL OF PROGESTERONE RECEPTOR POSITIVE BREAST TUMOURS

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In normal mammary tissue two major isoforms of progesterone receptor (PRA and PRB) are expressed in equal ratios. However, in the incidence of tumour formation, their expression is altered. PRB is known to have a proliferative role in the breast whereas there is controversial evidence surrounding the actions of PRA. From the studies in other reproductive

tissues it can be deciphered that PRA may have functions contrasting to PRB. We hypothesize that PRB expressing breast cancer (BC) cells will have a different proliferative behaviour compared to those expressing PRA. We aim to compare PRB *vs* PRA expressing BC cell lines for their proliferation rate *in vitro* and to determine if PRB and PRA differentially regulate the PI3K/AKT/mTOR signalling pathway. Western blot analysis of six BC cell lines (MCF7, BT474, MDA231, MDA453, MFM223 and T47D) was performed to determine the expression of PR isoforms and status of PI3K/AKT/mTOR pathway after treatment with progesterone (P4, 100nm) or vehicle. Proliferation rate of these cell lines was compared via MTT Assay. Western profiling showed that MCF7 and T47D cell lines express both PRA/B receptors, BT474 and MDA231 express PRA alone, MFM223 expresses PRB alone and MDA453 is PR negative. MTT assay confirmed that PRB dominant MFM223 has the highest proliferation rate among all cell lines studied. The analysis of PI3K/AKT/mTOR pathway showed that MFM223 cells expressed high levels of phospho-4EBP1 protein, indicative of high mTOR activity; whereas PRA dominant BT474 and MDA231 both have low level of AKT and 4EBP1 phosphorylation. Stimulation with P4 did not affect cell proliferation and PI3K/AKT/mTOR pathway activation. Our data suggests that the proliferative potential of breast tumours depends upon the type of PR isoform expressed. Therefore the details regarding PR isoform expression can prove valuable in the prognostic assessment of breast tumour biopsies.

R13. DISRUPTION OF SPHINGOLIPID HOMEOSTASIS AFFECTS MURINE PLACENTAL DEVELOPMENT

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Sphingolipids are a class of bioactive lipids that have recently been shown to play an important role in regulating successful pregnancy and embryo development. Acid sphingomyelinase (aSMase) is a lysosomal enzyme that regulates sphingolipid production in a variety of cell types. The objective of this study is to investigate how acid sphingomyelinase regulates murine placental development. Fetal and maternal phenotypes of acid sphingomyalinsase-deficient (KO) mice were analyzed during pregnancy and compared to wild-type mice of the same background. We observed a ~20% reduction in KO fetal weights (n=7) compared to WT (n=8) at E18.5 ($p=0.008$). Similarly, there was an approximate 20% reduction of KO neonate weights compared to WT ($p<0.001$). Microscopic inspection of midline KO placenta sections revealed a less branched labyrinth region. Sphingolipid level analyses of KO (n=4) and WT (n=4) placentae showed an increase in unmetabolized sphingomyelin ($p<0.005$), and a decrease of sphingosine-1-phosphate (S1P, $p<0.005$). Western blot analysis revelaed that after serum starvation, S1P treated placenta precursors cells known as trophoblast stem cells (TSC's) expressed high levels of phospho-p70S6 kinase (p-p70S6k, downstream target of mTOR) while untreated control TSC's lacked expression of p-p70S6K. These outcomes suggest that placental development or function may be compromised in aSMase deficient mice, where sphingolipid homeostasis is disrupted. Mass spectometry results indicate that a build up of unmetabolized sphingomyelin and/or a reduction in S1P in the placenta may be responsible for this placental phenotype. Finally, western blot analysis showed that S1P treated TSC's had increased levels of downstream mTOR targets such as p-p70S6K responsible for promoting protein synthesis in nutrient poor conditions. Together, these results provide insights into the possible molecular mechanisms underlying pregnancy disorders that are primarily due to imporper placenta development and function.

R14. OUTCOMES OF PLACENTAL DERIVED SEROTONIN FROM HIGHLY SEASONAL PREGNANT WOMEN

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Neurodevelopmental disorders have a major impact at a personal, familial and societal level, yet our ability to prevent them remains highly limited. Preclinical studies have demonstrated that during pregnancy, serotonin derived from maternal tryptophan at the placenta is an important signal for establishing fetal brain circuitry. Abnormal serotonin signaling during critical periods of brain development represents a major risk factor for various neurodevelopmental disorders. One plausible risk factor is changes in mood and appetite in women during fall and winter seasons. Individuals that are highly seasonal experience low mood, increase in carbohydrate consumption, weight gain and hypersomnia. Thus, pregnancies spanning fall and winter can be vulnerable to these lifestyle changes. Interestingly, numerous population studies have found an excess of births in spring associated with schizophrenia, especially in Northern countries. No studies to date have examined how the placental serotonin system in highly seasonal women differs from healthy pregnant women unaffected by winter. Also, no studies to date have examined whether seasonal changes in mood, weight and energy level influence maternal circulating tryptophan levels during pregnancy. We will study pregnant women taking part in the Ontario Birth Study, a large longitudinal pregnancy cohort being recruited at Mount Sinai Hospital. We hypothesize that, 1) Maternal tryptophan levels during pregnancy spanning fall and winter will be reduced and 2) placentae from highly seasonal women will undergo compensatory processes to maximize fetal serotonin when maternal precursor availability is sub-optimal for spring births. Levels of maternal plasma tryptophan levels will be measured at different time points in pregnancy utilizing

mass spectrometry. Placental tissue collected at birth will be analyzed for measures of placental serotonin synthesis using reverse transcriptase q-PCR and western blot. This study will generate cutting edge data on placental serotonin metabolism of fundamental importance to understanding early brain development and neurodevelopmental disorders.

R15. LOCAL ESTROGEN THERAPY MODULATES THE EXPRESSION OF ECM REMODELLING & STRUCTURAL GENES IN VAGINAL TISSUE OF POSTMENOPAUSAL WOMEN WITH SEVERE PELVIC ORGAN PROLAPSE.

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Pelvic Organ Prolapse (POP) affects almost half of postmenopausal women. Current treatment options for POP include reconstructive pelvic surgeries with various vaginal implants and optional Local Estrogen Therapy (LET). We aim to analyze the effect of LET on the expression of extracellular matrix (ECM) components and genes participating in collagen/elastin biogenesis in vaginal tissues of postmenopausal women with severe POP. Postmenopausal women undergoing reconstructive pelvic surgery (POPQ=3-4) were recruited. Vaginal biopsies were collected from patients treated with LET (average duration 7.5 months, N=15) and patients not using LET (N=17) and frozen in liquid nitrogen for biochemical study. Total RNA was extracted and the expression of ECM structural and remodeling genes was analyzed by RT-qPCR. We examined transcript levels of major vaginal collagens (COL1,3,4,5) and ECM maturation enzymes: Lysyl Oxidase (LOX), LOXL1-3, ADAMTS2/PNP, and BMP1/PCP. The mRNA levels of main structural proteins of vaginal collagen fibers COL1,3,5 and maturation enzyme BMP1 were significantly elevated (3-4 fold increase, $p<0.05$) in vaginal tissues of POP patients treated with LET as compared to untreated POP patients. LOX, LOXL1, ADAMTS2 genes were also up-regulated but not significantly. Gene expression level of ECM remodeling enzymes matrix metalloproteases (MMP1-3, 7-9, 10, 11, 14) and their tissue inhibitors (TIMP1-4) was also examined. Several genes (MMP2, MMP14 and TIMP2, TIMP4) were significantly ($p<0.05$) up-regulated by LET, in particular major gelatinase MMP2 (7.5-fold increase) and TIMP2 (5-fold increase), while the mRNA levels of MMP3 (main tissue stromelysin) showed a significant ($p<0.05$) 5-fold decrease in estrogen-treated tissue. Altogether, we observed an increase in MMPs/TIMPs – known regulators of tissue repair and regeneration. These changes in combination with observed increases in the expression of structural COL1,3,5 and proteins involved in the production of mature collagen/elastin fibers support a beneficial role of estrogen in the remediation of pelvic floor tissue in POP patients.

R16. DYNAMICALLY REGULATED TROPHOBLAST SUBPOPULATIONS ISOLATED FROM EARLY HUMAN PLACENTA

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The placenta arises from a network of trophoblast and mesoderm cells but many questions about early cellular development remain: how heterogeneous are trophoblast cells? Is there a stem cell population analogous to the ones isolated from mouse models? What signals drive differentiation into various cell lineages? I aim to identify transitory populations of cells within the human placenta that organize and direct organogenesis. Candidate trophoblast progenitor populations were first identified through a high throughput flow cytometry screen. Expression of 370 CD antigens were quantified in trophoblast enriched fractions isolated from either week 6 or week 10 placentas, the critical period of villus specification and vascularization. Flow cytometry data was read into R for automated and data directed gating using flowCore and OpenCyto packages. 37 dynamic subpopulations were identified between the two timepoints and 6 were selected for further validation through immunohistochemistry and targeted flow cytometry. Among these, populations expressing either EpCAM (Epithelial Cell Adhesion Molecule) or CDCP1 (CUB Domain-Containing Protein 1) were selected for detailed investigation because their expression significantly decreased as the cells differentiated into complex villus structures and they are known to mark discrete trophoblast subpopulations in the mouse. Targeted flow cytometry and immunohistochemistry analysis confirmed distinct EpCAM+ and CDCP1+ populations become restricted to discrete cellular subpopulations. The gene profiles of these subpopulations suggest EpCAM+ cells contribute to the villous cytotrophoblast lineage while CDCP1+ cells become extravillous trophoblasts (EVTs). More importantly, gene set enrichment suggests both subpopulations remain mitotically active. This is particularly interesting for the CDCP1+ population because EVTs are believed to be a terminally differentiated lineage. My work provides strong evidence to support the existence of transient populations of trophoblast cells in the human placenta and contributes to our understanding of how trophoblast populations communicate and organize into a mature, multicellular tissue.

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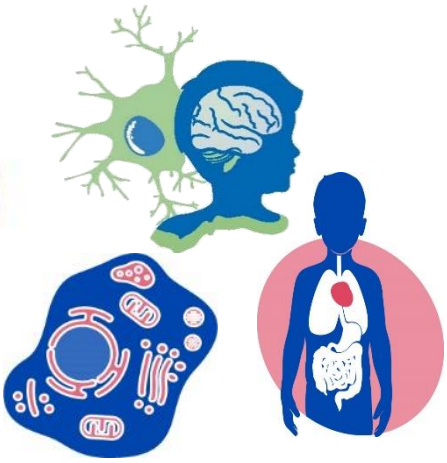

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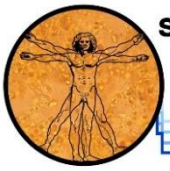

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