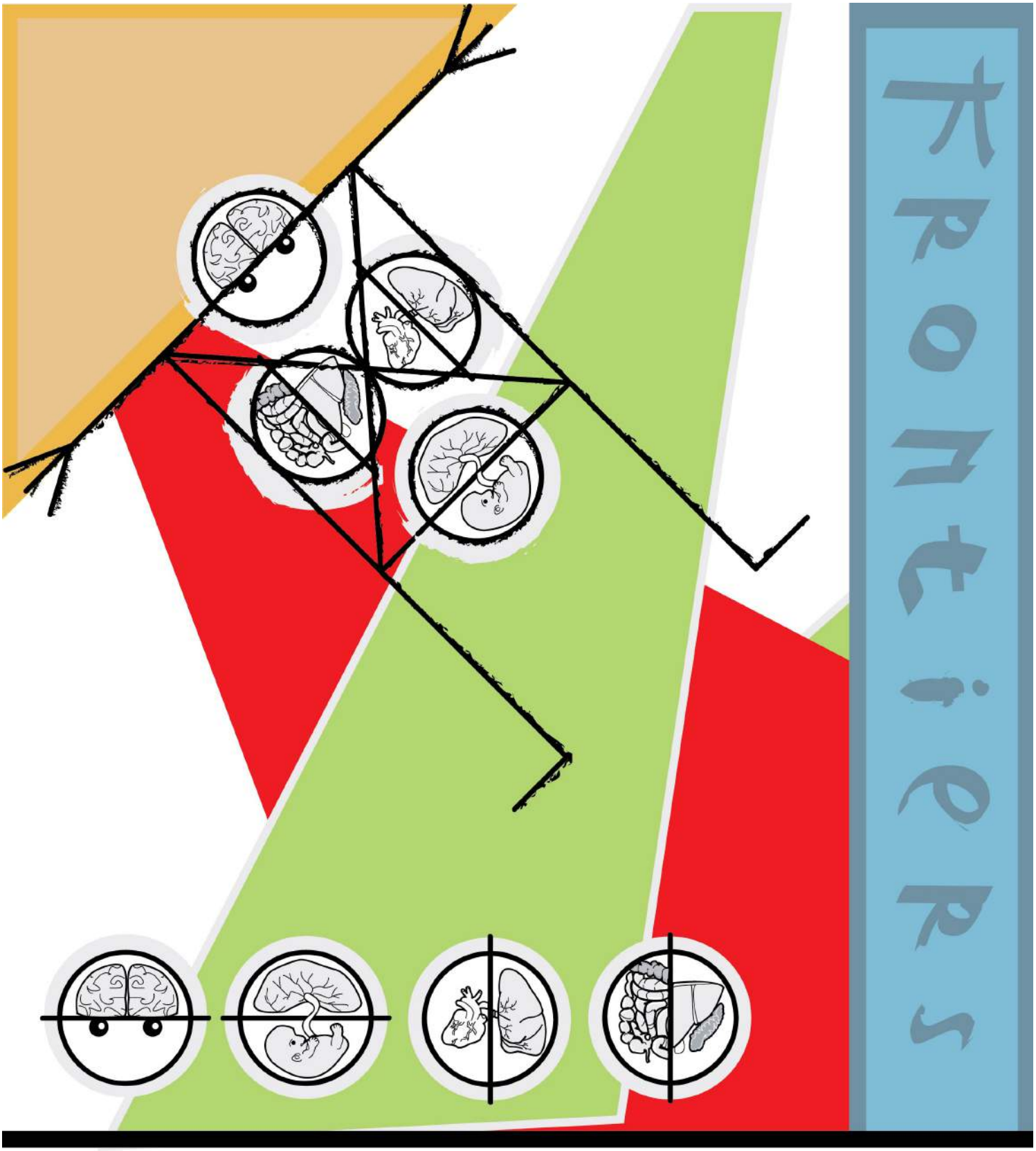

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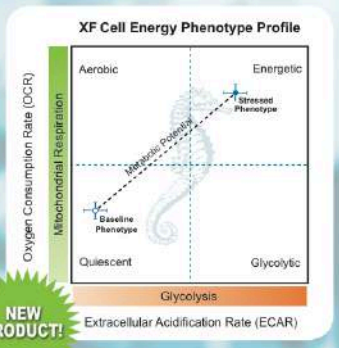

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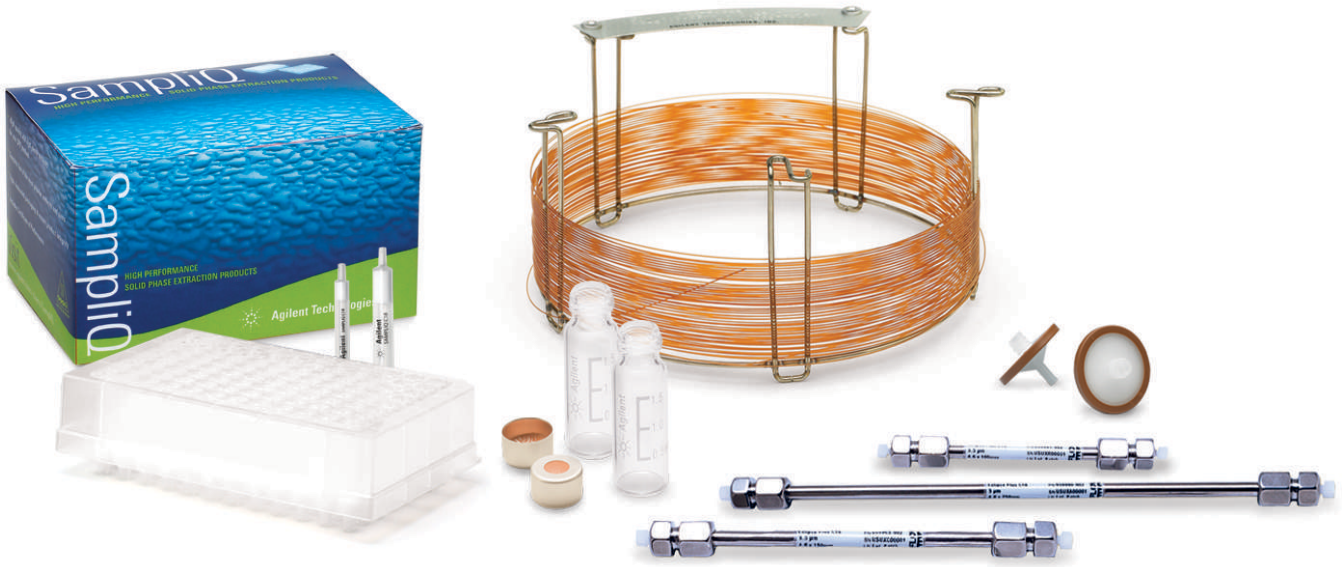

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Frontiers in Physiology Schedule

8:00 – 8:45 **Registration & Light Breakfast**
Medical Sciences Building – Stone Lobby

8:45 – 9:00 **Opening Remarks** – S. Ellis, H. Stacey and Dr. G. Collingridge
JJR Macleod Auditorium (MSB 2158)

9:00 – 10:05 **Inflammatory Processes & Degenerative Disease Oral Presentations**
JJR Macleod Auditorium (MSB 2158)

T. Nguyen	In situ localization of matrix metalloproteinase expression and activity in the rat myometrium during late gestation, term labour, and post-partum
H. Amatullah	Targeted deletion of DJ-1 attenuates morbidity and mortality in experimental sepsis through enhanced bacterial clearance
K. Chan	Palmitoleate reverses high-fat induced pro-inflammatory macrophage polarization via AMPK
E. Turlova	Transient receptor potential melastatin 7 (TRPM7) regulates axonal development and maturation of primary hippocampal neurons

10:05 – 10:20 **Break**

10:20 – 11:20 **KEYNOTE LECTURE**
JJR Macleod Auditorium (MSB 2158)

Integrating Metabolism Around the Clock

Dr. Mitchell Lazar
Sylvan Eisman Professor of Medicine
Chief, Division of Endocrinology, Diabetes, Metabolism
Perelman School of Medicine at the University of Pennsylvania

11:20 – 11:30 **Break**

11:35 *sharp* **Departmental Photo**
Medical Sciences Building Steps, 1 King's College Circle
(Outside Tim Horton's)

12:00 – 12:45 **Lunch**
JJR Macleod Lobby (just outside MSB 2158)

12:45 – 1:55 **Poster Viewing & Judging (All Platforms)**
Medical Sciences Building – Stone Lobby

2:00 – 3:00 **Cellular Adaptations & Protein Identification Oral Presentations**
JJR Macleod Auditorium (MSB 2158)

N. Dong	Time course of intrinsic plasticity during aversive long-term memory formation in *Lymnaea stagnalis*
M. Markovic	Connection between insulin-like growth factor-1 receptor and intestinal form and function in mice
J. Cosme	Label-free quantification of hypoxia-induced changes of the cardiac fibroblast secreted proteome
F. Wong	Transient and dynamically regulated EPCAM+ and CDCP1+ trophoblast populations present in the early human placenta

3:00 – 3:15 **Break**

3:15 – 4:15 **Signaling & Transmission Oral Presentations**
JJR Macleod Auditorium (MSB 2158)

M. Wong	Statin mediated modulation of pulmonary rhoa/rock signaling in experimental chronic neonatal pulmonary hypertension
M. Vu	TASK channels on basal forebrain cholinergic neurons modulate electrocortical signatures of arousal by histamine
M. LaPierre	Glucagon signaling in the dorsal vagal complex is sufficient and necessary for high-protein feeding to regulate glucose homeostasis
V. Moisiadis	Antenatal glucocorticoids lead to multigenerational programming of behavior and HPA function via paternal transmission

4:15 – 6:30 **Awards Ceremony & Reception**
Medical Sciences Building – Stone Lobby

Keynote Speaker

Dr. Mitchell A. Lazar, MD, PhD

Perelman School of Medicine at the University of Pennsylvania

Dr. Mitchell Lazar is the Sylvan Eisman Professor of Medicine and Genetics, the Chief of the Division of Endocrinology, Diabetes, and Metabolism, and the Director of the Institute for Diabetes, Obesity, and Metabolism at the University of Pennsylvania. He received his undergraduate degree in Chemistry from the Massachusetts Institute of Technology, then received a PhD in Neurosciences and an MD from Stanford University. He trained in Internal Medicine at Brigham and Women's Hospital and in Endocrinology at the Massachusetts General Hospital before joining the University of Pennsylvania faculty in 1989.

Dr. Lazar's identification of the nuclear heme receptor Rev-erba and its corepressor complex, and his pioneering studies of PPARg including discovery of the adipocyte hormone resistin, have linked basic mechanisms of gene transcription to physiology and metabolic diseases. He has given named lectures throughout the world, and has served as a member of the Board of Scientific Councilors of the NIDDK as well as many editorial and scientific advisory boards. He has been elected to the American Society for Clinical Investigation and the Association of American Physicians, and received two NIH Merit Awards, the Van Meter Award of the American Thyroid Association, the BMS Freedom to Discover Award, the Richard Weitzman Award, Edwin B. Astwood Lecture Award and Gerald D. Aurbach Lecture Award from The Endocrine Society, and the Stanley Korsmeyer Award of the American Society for Clinical Investigation. He was elected to the Institute of Medicine of the National Academy of Sciences in 2006, and to the American Academy of Arts and Sciences in 2008.

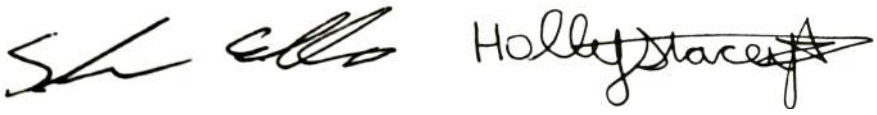
MESSAGE FROM THE FRONTIERS IN PHYSIOLOGY CO-CHAIRS

On behalf of the Graduate Association of Students in Physiology (GASP), we are thrilled to welcome you to the 35th annual Frontiers in Physiology (FIP) research symposium! Our symposium showcases the cutting-edge research currently being conducted by the graduate students (and undergraduate students) in the four independent yet collaborative platforms within the Department of Physiology at the University of Toronto. We are confident that the day will facilitate the exchange of scientific ideas amongst students and faculty members, promote inter-departmental 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 distinguished speaker to present a keynote lecture at FIP. This year, we are privileged and honoured to welcome Dr**. Mitchell Lazar**, a pioneer in endocrine research; discovering the hormone resistin and largely contributing to our understanding of transcriptional regulation of metabolism. Dr. Lazar is an endocrinologist and physician-scientist and serves as the Chief of the Division of Endocrinology, Diabetes, and Metabolism at Perelman School of Medicine (University of Pennsylvania). The lecture that Dr. Lazar will deliver is entitled "*Integrating Metabolism Around the Clock*." We are certain that Dr. Lazar will deliver an inspirational, educational and unforgettable talk.

Today's event would not be possible without the contributions of many generous individuals. Firstly, we would like to acknowledge the members of the FIP Planning Committee and GASP; whose efforts and dedication have ensured the success of FIP. Additionally, we are truly grateful for the continued support of the Department of Physiology, particularly **Dr. Steffen-Sebastian Bolz** (interim Chair), **Dr. Graham Collingridge** (Chair), the Graduate Coordinators and Academic Chairs: **Drs. Carin Wittnich, Martin Wojtowicz, Denise Belsham** and **Scott Heximer;** and **Jenny Katsoulakos, Colleen Shea, Paula Smellie** and the rest of the Departmental administrative staff. 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 in both oral and poster presentations. Finally, we would like to extend our must sincere gratitude to our institutional and commercial sponsors for their financial support; without them the event would not be possible.

We are delighted to have you with us today and we hope you will enjoy this stimulating and inspirational day.



Shane Ellis and Holly Stacey
Vice-Presidents, Graduate Association of Students in Physiology
Co-Chairs, Frontiers in Physiology

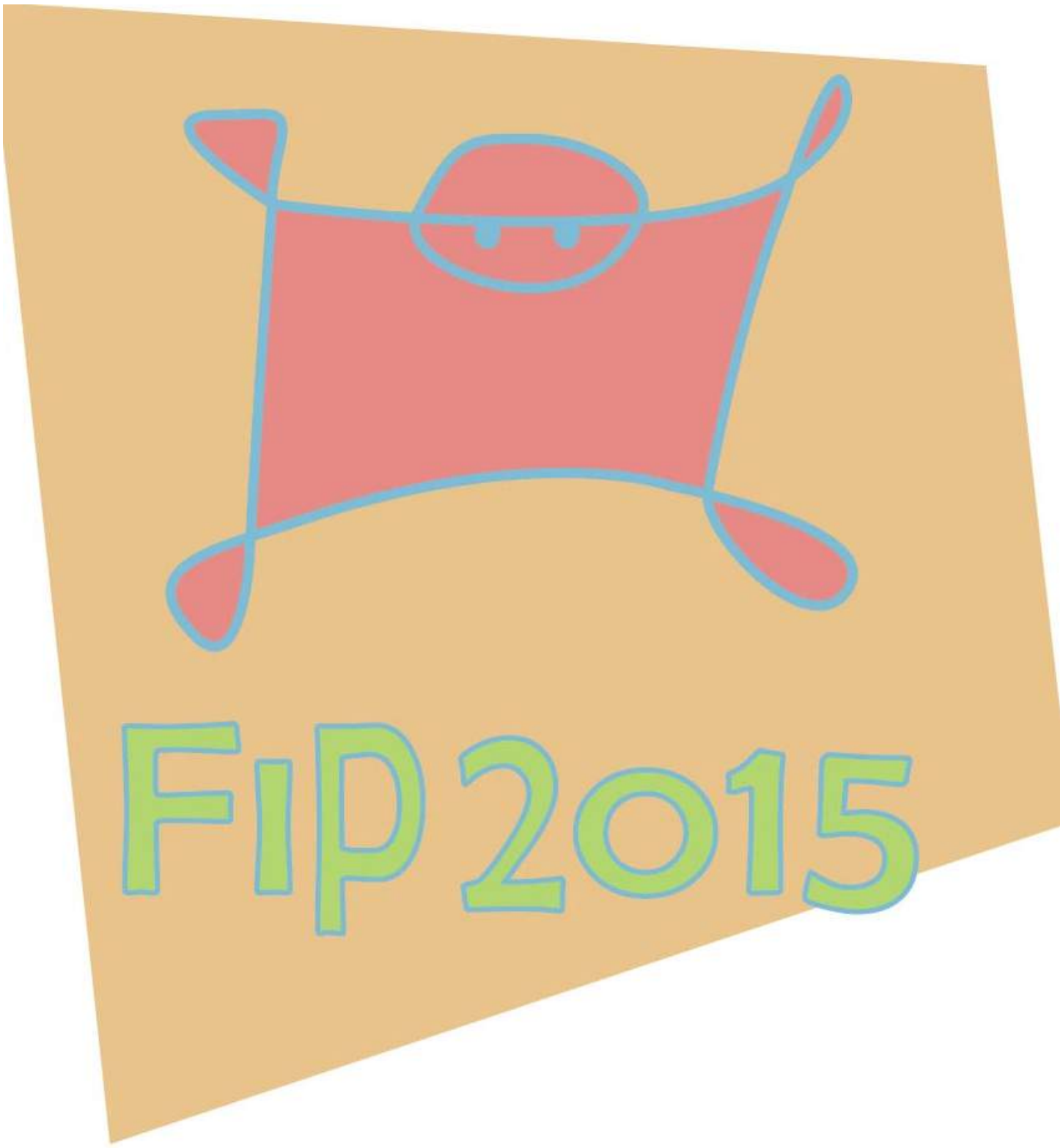
MESSAGE FROM THE CHAIR OF THE DEPARTMENT OF PHYSIOLOGY

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 35 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 **Dr. Mitchell Lazar** from the University of Pennsylvania, this year's FIP Keynote Speaker. Special thanks and acknowledgement is due today to **Ms. Holly Stacey** and **Mr. Shane Ellis,** FIP co-Chairs and Vice-Presidents of the Graduate Association of Students in Physiology (GASP). Holly and Shane's team have done a brilliant job in spearheading the process to ensure the success of this day. A special thank you also to M**r. Joobin Sattar,** 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,

Graham L. Collingridge, FRS, FMedSci, FSB, FBPhS
Ernest B. and Leonard B. Smith Chair, Department of Physiology
Faculty of Medicine
University of Toronto



ORAL PRESENTATIONS

Oral Session #1: Inflammatory Processes & Degenerative Disease

IN SITU LOCALIZATION OF MATRIX METALLOPROTEINASE EXPRESSION AND ACTIVITY IN THE RAT MYOMETRIUM DURING LATE GESTATION, TERM LABOUR, AND POST-PARTUM.

T. Nguyen[1,3], O. Shynlova[2,3], S. J. Lye[1,2,3]
Departments of Physiology[1] and Obstetrics and Gynecology[2], Faculty of Medicine, University of Toronto, Ontario, Canada; Lunenfeld Tanenbaum Research Institute[3], Mount Sinai Hospital, Ontario, Canada.

Term labour is an inflammatory process, where uterine smooth muscle (myometrium) cells (SMCs) and activated immune cells produce extracellular matrix-degrading proteases (matrix metalloproteinases, MMPs). This induces modulation of cellular barrier function and cellular behavior before term labour and during the postpartum (PP) period. We hypothesize that during late pregnancy, uterine MMPs' expression and activity increases in preparation for two major events: 1) term labour and 2) the postpartum uterine involution. To address this hypothesis, uterine tissues from pregnant rats were collected throughout gestation (GD), during labour and PP to examine MMP tissue localization using 1) immunohistochemistry and 2) immunofluorescence in conjunction with 3) in situ zymography. Immunohistochemical analysis of matrilysin (MMP7) and stromelysin (MMP11) revealed a steady increase throughout gestation, culminating during GD23 and PP. Specifically, visual analysis of MMP7 indicated that 1) during gestation, positive staining was localized mostly intracellularly in the perinuclear cytoplasmic region of the myometrial smooth muscle, however 2) during GD23 and the early PP period, MMP7 expression was detected extracellularly in the myometrial parenchyma. However, expression of MMP11 remains intracellular during gestation and labour, and is only secreted during PP uterine involution. Similarly, in situ zymography revealed increased extracellular MMP expression and activity typically during PP. Co-immunostaining for CD68 indicated that myometrial macrophages also expressed gelatinases (MMP2 and 9), and collagen I and collagen IV-degrading proteases. Localization analysis confirmed expression of MMP proteins at late gestation, during term labour and the PP period by SMCs and macrophages. Increased extracellular expression of MMP7 during labour suggests an association with the labouring process, potentially its role in promoting the leukocyte infiltration into the uterus. Furthermore, as functional protease activity was only found in the extracellular space during the PP period, it may suggest that the major role of MMPs is in promoting tissue remodelling during uterine involution.

TARGETED DELETION OF DJ-1 ATTENUATES MORBIDITY AND MORTALITY IN EXPERIMENTAL SEPSIS THROUGH ENHANCED BACTERIAL CLEARANCE

Hajera Amatullah[1,2], Yuexin Shan[2], Patricia Gali[2], Tatiana Maron-Gutierrez[2], Dun Yuan Zhou[2], Jennifer Tsang[2], Shirley Mei[3], Tak W. Mak[4], John Marshall[2], Patricia Liaw[5], Duncan Stewart[3], W. Conrad Liles[6], Claudia C. dos Santos[2]

[1]Department of Physiology, University of Toronto, Toronto, ON, CA. [2]Keenan Research Centre of the Li Ka Shing Knowledge Institute of St. Michael's Hospital, Toronto, ON, CA. [3]The Ottawa Hospital Research Institute, Ottawa, ON, CA. [4]Department of Medical Biophysics and Immunology, University Health Network, Toronto, ON, CA. [5]Department of Medicine, McMaster University, Hamilton, ON, CA [6]Department of Medicine, University of Washington, USA

Sepsis remains a leading cause of mortality in critically ill patients. Mesenchymal stem cells (MSC) have been shown to attenuate sepsis-induced organ injury and mortality in mice. Gene expression profiles in target organs from MSC- vs. saline-treated mice in caecal ligation and perforation (CLP) model of sepsis identified mitochondrial pathways related to the Parkinson disease (autosomal recessive, early onset) 7 [PARK-7] or DJ-1 gene as significantly altered in response to MSCs. The role of DJ-1 in sepsis has not been previously investigated. Wild-type (WT) C57Bl/6J and DJ-1 deficient (DJ-1 KO, knockout) mice were randomized to sham or CLP surgery. Organ dysfunction was assessed (i) histologically, (ii) functionally and (iii) biochemically. DJ-1 deficiency in mice resulted in a surprisingly marked decrease in CLP-induced mortality at both 48 hours and 7 days despite increased ROS and pro-inflammatory response compared to WTs. In addition, DJ-1 KO mice had lower levels of multi organ failure markers (serum lactate, bilirubin, troponin) as well as attenuated myocardial depression. Bacterial clearance and phagocytosis was determined *in vivo* and in primary macrophages. Bacterial counts in blood and tissues were markedly decreased in DJ-1 KOs and this was in keeping with increased phagocytosis observed in DJ-1KO bone marrow derived and peritoneal macrophages. DJ-1 KO mice and macrophages

displayed an enhanced M1 macrophage phenotype with increased levels of iNOS and NAPDH oxidase expression. Lastly, serum and neutrophils were obtained from human sepsis and control patients to determine clinical relevance of DJ-1. Elevated DJ-1 levels were observed in serum of septic patients compared with healthy patients; higher DJ-1 levels were associated with increased mortality and higher MODS. In conclusion, our study revealed DJ-1 as an important contributor to innate immune response in sepsis. Our current investigations explore the parenchymal cell specific contribution of DJ-1 in the pathology of sepsis.

PALMITOLEATE REVERSES HIGH FAT-INDUCED PRO-INFLAMMATORY MACROPHAGE POLARIZATION VIA AMPK

K.L. Chan[1,2], N.J. Pillon[1], D.M. Sivaloganathan[1], S.R. Costford[1], Z. Liu[1], A. Klip[1,2]
Cell Biology Program, The Hospital for Sick Children, Toronto, ON, Canada[1]; Department of Physiology, University of Toronto, Toronto, ON, Canada[2]

Type 2 diabetes (T2D) is a systemic disease predicted to affect 4 million Canadians and cost our healthcare system $16 billion/year by 2020. Preceding T2D is a pre-diabetic state characterized by obesity, insulin resistance, and as shown over the last decade, by chronic, low-grade inflammation. Immune cells, most notably macrophages, infiltrate metabolic tissues during obesity. *Ex vivo*, saturated fats initiate pro-inflammatory polarization of macrophages, which in turn confers insulin resistance to muscle cells. Conversely, unsaturated fat consumption has beneficial effects on whole-body insulin sensitivity, but it is unknown if this is mediated by skewing macrophage polarization. We hypothesize that unsaturated fats, particularly monounsaturated, induce an anti-inflammatory macrophage phenotype. To test this hypothesis, bone marrow-derived macrophages (BMDM) from diet-manipulated C57BL/6 mice were cultured and treated with BSA-conjugated saturated fatty acid palmitate (PA), unsaturated fatty acid palmitoleate (PO), or BSA alone. To evaluate dietary effects, BMDMs were isolated from mice fed high-fat diet (60% kcal) or low-fat diet (10% kcal) for 18 weeks. Macrophages from high-fat-fed mice displayed elevated pro-inflammatory cytokine expression and secretion, revealing *ex vivo* memory of their *in vivo* environment. Moreover, 6h treatment with PO *ex vivo* reversed this phenotype. PA-treatment of macrophages from chow-fed mice increased NFκB activation, pro-inflammatory gene expression, and cytokine secretion. Conversely, PO-treatement elevated anti-inflammatory genes, AMPK phosphorylation, and oxygen consumption, characteristic of anti-inflammatory macrophages. Interestingly, co-incubation with PO counteracted the PA-induced pro-inflammatory consequences. Furthermore, AMPK inhibition or knockout diminished the anti-inflammatory effects of PO, suggesting that PO activates AMPK to antagonize pro-inflammatory macrophage polarization. These results demonstrate the plasticity of macrophages in response to distinct lipids. In summary, saturated and unsaturated fats differentially regulate macrophage polarization, respectively conferring reversible pro- or anti-inflammatory phenotypes. Our findings demonstrate a novel mechanism by which the monounsaturated fat palmitoleate attenuates the metabolic inflammation that contributes to insulin resistance.

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

E. Turlova[1,2],CYJ Bae[2], M. Deurloo[2], W. Chen[1,2], A. Barszczyk[2], F.D. Horgen[5], A. Fleig[6], ZP Feng[2] and HS Sun[1,2,3,4]
Departments of Physiology[1], Surgery[2] and Pharmacology[3], Institute of Medical Science[4], Faculty of Medicine, University of Toronto, 1 King's College Circle, Toronto, Canada
College of Natural and Computational Sciences[5], Hawaii Pacific University, Kaneohe, HI 96744, USA
Center for Biomedical Research[6], The Queen's Medical Center, Honolulu, HI 96720, USA

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.

Oral Session #2: Cellular Adaptations & Protein Identification

TIME COURSE OF INTRINSIC PLASTICITY DURING AVERSIVE LONG-TERM MEMORY FORMATION IN LYMNAEA STAGNALIS

N. Dong, Z. P. Feng
Department of Physiology, University of Toronto, 1 King's College Circle, Toronto, Ontario, Canada

Studies of the mechanisms underlying learning and memory formation have largely focused on synaptic plasticity. However, there is growing evidence in a wide range of species and learning paradigms that the intrinsic membrane properties of a neuron can also be modified in an activity-dependent manner, a process termed intrinsic plasticity. Nevertheless, our understanding of the mechanisms and the functional relevance of intrinsic plasticity in memory formation remains in its infancy. The aversive operant conditioning paradigm of aerial respiration in the mollusc *Lymnaea stagnalis* provides an ideal model for studying learning-induced changes in intrinsic neuronal properties. The respiratory pacemaker neuron RPeD1 in which long-term memory (LTM) formation occurs is capable of modulating its excitability independent of synaptic drive. In this study, we employed intracellular sharp electrode recording to characterize the learning-induced changes in intrinsic properties of the RPeD1 during aversive LTM formation. We first established that aversive LTM formation 24 hours after conditioning is indeed associated with enhanced RPeD1 excitability, as reflected by depolarized membrane potential, increased spontaneous firing frequency, and enhanced gain of firing. These changes were observed along with reduced input resistance of the RPeD1. Subsequently, we demonstrated that learning-induced neuronal plasticity in RPeD1 exhibits a biphasic profile. The early component occurs during the first two hours immediately after conditioning and decays completely before the onset of the late component, which persists until 24 hours later. Our findings provide insights into the potential molecular mechanisms mediating intrinsic plasticity and open the way to further studies of its role in memory formation.

CONNECTION BETWEEN INSULIN-LIKE GROWTH FACTOR-1 RECEPTOR AND INTESTINAL FORM AND FUNCTION IN MICE

M. Markovic[1], C. Ackerley[3], P.L. Brubaker[1,2]
Departments of Physiology[1] and Medicine[2], University of Toronto, Ontario, Canada; Department of Paediatric Laboratory Medicine[3] The Hospital for Sick Children, Ontario, Canada

The intestinal microvilli are tiny, finger-like structures located on the apical end of epithelial cells. They function to aid in the catabolism and absorption of nutrients. A key characteristic of microvilli is uniformity. This allows for microvilli to increase the intestinal absorptive surface area by 9-16-fold. It is poorly understood, however, how microvilli achieve this and how this affects functionality. The intestinal epithelial insulin-like growth factor-1 receptor (IE-IGF-1R) is important in the signaling mechanism of the intestinotrophic drug, glucagon-like peptide-2 (GLP-2). Its role in epithelial cell structure and function in unknown. It is also unclear if the IE-IGF-1R is required for the GLP-2 induced growth response on microvilli morphology. The hypotheses are that GLP-2 increases microvillus morphology in an IE-IGF-1R-dependent pathway, and the IE-IGF-1R knockout alone alters microvillus form and function. Transmission and scanning electron micrographs of mouse jejunum showed that the E-IGF-1R plays a key role in microvillus morphology. Expression of mRNA transcripts for apical proteins (n=8-15) showed that GLP-2 treatment increased myosin-1a ($p<0.05$) and decreased sucrase ($p<0.01$) in control animals only, but increased insulin receptor tyrosine kinase substrate-1 in both control and knockout animals ($p<0.05$). In contrast, harmonin was significantly decreased by IE-IGF-1R KO ($p<0.05$). β-actin, myosin-5b and tropomyosin mRNA showed no difference by genotype or treatment. Villin mRNA also showed no difference; however, immunofluorescence suggested an up-regulation of villin protein expression with GLP-2 treatment in an IE-IGF-1R-dependent manner. Transmission electron micrographs suggest changes in lipid absorption in IE-IGF-1R knockout mice. An oral fat tolerance test, however, found no difference (n=5-6). Collectively, these findings suggest that GLP-2 affects intestinal epithelial morphology in both an IE-IGF-1R-dependent and -independent manner. Furthermore, the IE-IGF-1R plays an important role in the regulation of microvilli morphology and intestinal epithelial function.

LABEL-FREE QUANTIFICATION OF HYPOXIA-INDUCED CHANGES OF THE CARDIAC FIBROBLAST SECRETED PROTEOME

J.Cosme[1,2], A Ignatchenko[3], VIgnatchenko[3], T Kislinger[3,4], and AO Gramolini[1,2]

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Cardiac fibroblasts (CF) play central roles in the outcomes of heart failure after ischemia and fibrosis. Cardiomyocyte and CF intercellular communication can occur through paracrine and ECM interactions. In addition to soluble factors, cardiac cells secrete exosomes (EXO), with evidence suggesting CF EXO modulate pathophysiology *in vitro*. Herein, we propose proteomics of the fibroblast secretome in normal and stressed conditions will offer insights into the role of CF in heart disease. Primary mouse CF were cultured for 24h in 21% (normoxic) or 2% (hypoxic) O_2 for 24h in serum-free media. Conditioned media was differentially centrifuged and ultracentrifuged to obtain EXO and EXO-depleted secretome (SEC) fractions. Successful EXO isolation was indicated by CD81 enrichment via immunoblot, density measurements of 1.17-1.24 g/mL via sucrose gradient, and stereotypical morphology and size via electron microscopy. 6-step MuDPIT was performed with a LTQ-Orbitrap Discovery. Data was searched using XTandem!, OMSSA, MyriMatch, and Comet. Protein relative abundance was calculated using QSpec. Proteomic analysis identified 1760 unique proteins in total, with 1366 and 647 in normoxic EXO and SEC, respectively, and 1314 and 895 in hypoxic EXO and SEC, respectively. QSpec analysis identified 501 proteins differentially expressed between normoxic fractions, 152 proteins between hypoxic fractions, 150 proteins between normoxic and hypoxic SEC, and 439 proteins between normoxic and hypoxic EXO. Gene Ontology revealed hypoxic conditions increase expression of secreted proteins associated with ECM and signalling, suggesting an activated secretory phenotype. Proteins enriched in EXO and in SEC were associated with cytoskeleton and glycoprotein annotations, respectively. For functional assessment, we subjected cardiomyocytes pretreated with either CF EXO or SEC for 24h, to 60 μM H_2O_2 for 24h to mimic oxidative stress. MTT assays suggest a reduced viability due to CF-derived secreted factors.CF secretome proteomics reveal differential expression based on mode of secretion and oxygen-levels *in vitro*.

TRANSIENT AND DYNAMICALLY REGULATED EPCAM+ AND CDCP1+ TROPHOBLAST POPULATIONS PRESENT IN THE EARLY HUMAN PLACENTA

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The vascular exchange region of the human placenta, called the villus, facilitates nutrient and gas exchange between maternal and fetal circulations. Placental vascular defects lead to pathologies in up to 10% of all pregnancies, resulting in life long chronic disease in the mother and child. We propose our limited knowledge of human villus development is preventing advances in technologies to counter pregnancy complications. As most developmental processes involve transitory populations of cells that drive and organize developing cellular networks, I hypothesize a cellular analysis of the human placenta will identify transitory cell populations which direct villus development. Trophoblast enriched fractions of single cells were enzymatically isolated from human chorionic villi at week 6 and 10 of gestation. Cells were analyzed in duplicate by a high throughput flow cytometry assay for 370 CD antigens. Markers of transient cell subpopulations were selected as those strongly expressed at week 6 and decreased by week 10. Candidate markers and cell populations were confirmed thorough immunohistochemistry of placental sections and gene expression analysis. Using the most stringent filtering criteria, we confidently identified 21 developmentally regulated populations in both replicates of the screen. We selected EpCAM (CD326) and CDCP1 (CD318) for further validation because both markers consistently show decreasing expression and are known to label populations of early mouse trophoblast cells. Immunohistochemistry and flow cytometry analysis confirmed EpCAM and CDCP1 transiently label trophoblasts but interestingly, EpCAM and CDCP1 are expressed by mutually exclusive sub-populations as EpCAM+ cells are predominatly clustered in the base of the proximal column with CDCP1 expressed by discrete cells in the distal column. Genome wide gene expression suggest EpCAM may contribute to cytotrophoblast cells while CDCP1 develop into extravillous trophoblasts. My work has developed strong evidence for heterogeneity among early trophoblast cells and supports the existence of transient subpopulations

Oral Session #3: Signaling & Transmission

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

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Chronic pulmonary hypertension (PHT) is a lethal disease in newborns. The activity of RhoA and its downstream mediator Rho-Kinase (ROCK) is critical to the pathogenesis of experimental chronic neonatal PHT. Systemic ROCK-specific inhibitors have been shown to prevent and reverse experimental chronic neonatal PHT, but at the cost of severe systemic hypotension. The statin drug class is used in the prevention of cholesterol related cardiovascular disease. Recent studies have also shown pleiotropic benefits 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 limit pulmonary vascular RhoA activity and thus prevent experimental chronic neonatal PHT. Our model chronically exposed neonatal Sprague-Dawley rats to hypoxia (13% O_2) and had treated with Simvastatin (0.1, 2, or 10 mg/kg) or vehicle (20% DMSO in PBS) by daily intra-peritoneal injection from birth for 14 days. Lung RhoA and activity was quantified by Western blot analysis of GTP-RhoA and phosphorylated MYPT1 content. Arterial medial wall area (MWA) was measured on elastin-stained lung sections. Smooth muscle was measured on α-smooth muscle actin stained lung sections. Pulmonary arterial acceleration time (PAAT) was measured by pulse wave Doppler as an inverse marker of pulmonary vascular resistance(PVR). All doses of Simvastatin led to significantly ($p < 0.05$) decreased MWA with peak effect at 2 mg/kg/d. Simvastatin 10 mg/kg/d had a significant reduction in fully muscularization of pulmonary arteries compared to vehicle. GTP-RhoA was significantly reduced by 10 (but not 0.1) mg/kg/d Simvastatin. ROCK activity was significantly reduced by 10 mg/kg/d of Simvastatin. PVR was partially decreased by 0.1 and completely normalized by 2 and 10 mg/kg/d Simvastatin. The significance of this work may provide a safe and effective alternative for therapeutic inhibition of the RhoA/ROCK pathway in the neonate without causing systemic hypotension.

TASK CHANNELS ON BASAL FOREBRAIN CHOLINERGIC NEURONS MODULATE ELECTROCORTICAL SIGNATURES OF AROUSAL BY HISTAMINE

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Basal forebrain cholinergic neurons are the main source of cortical acetylcholine, and their activation by histamine elicits cortical arousal. TWIK-like acid sensitive K^+ (TASK) channels modulate neuronal excitability and are expressed on basal forebrain cholinergic neurons, but the role of TASK channels in the histamine-basal forebrain cholinergic arousal circuit is unknown. We first expressed TASK channel subunits and Histamine Type-1 receptors in HEK cells. Application of histamine (20 μM) *in vitro* inhibited the acid-sensitive K^+ current, indicating a functionally coupled signaling mechanism. We then studied the role of TASK channels in modulating electrocortical activity *in vivo* using freely-behaving wild-type (n=12) and ChAT-Cre:TASK$^{f/f}$ mice (n=12), the latter lacking TASK-1/3 channels on cholinergic neurons. TASK channel deletion on cholinergic neurons significantly altered endogenous electroencephalogram oscillations in multiple frequency bands. We then identified the effect of TASK channel deletion during microperfusion of histamine (1 mM) into the basal forebrain. In NREM sleep, TASK channel deletion on cholinergic neurons significantly attenuated the histamine-induced increase in 30-50Hz activity, consistent with TASK channels contributing to histamine action on basal forebrain cholinergic neurons. In contrast, during active wakefulness, histamine significantly increased 30-50Hz activity in ChAT-Cre:TASK$^{f/f}$ mice but not wild-type mice, showing that the histamine response depended upon the prevailing cortical arousal state. In summary, we identify TASK channel modulation in response to histamine receptor activation *in vitro*, as well as a role of TASK channels

on cholinergic neurons in modulating endogenous oscillations in the electroencephalogram and the electrocortical response to histamine at the basal forebrain *in vivo*.

GLUCAGON SIGNALLING IN THE DORSAL VAGAL COMPLEX IS SUFFICIENT AND NECESSARY FOR HIGH-PROTEIN FEEDING TO REGULATE GLUCOSE HOMEOSTASIS

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High-protein feeding acutely lowers the postprandial glucose response compared to low-protein feeding, despite a dichotomous stimulation of glucagon secretion. This apparent discrepancy suggests the existence of another mechanism to counteract the effects of hepatic glucagon action during high-protein feeding in order to maintain glucose homeostasis. In fact, a negative-feedback system has been revealed wherein glucagon action in the hypothalamus suppresses glucose production (GP). The dorsal vagal complex (DVC) is another brain region which integrates hormonal signals to regulate glucose homeostasis, though a role of glucagon signalling in this region has yet to be identified. A glucagon receptor (Gcgr)–PKA–Erk1/2 cascade has been observed *in vitro*, and DVC insulin activates Erk1/2–K_{ATP} channel signalling to lower GP. Thus, we hypothesised that DVC glucagon lowers GP via Gcgr–PKA–Erk1/2–K_{ATP} channel signalling and that this glucagon action lowers plasma glucose levels during high-protein feeding. DVC glucagon administration during pancreatic euglycemic clamps in rats lowered GP, while Gcgr inhibition abolished this glucagon effect. The ability of DVC glucagon infusion to lower GP was also lost when PKA, Erk1/2, or K_{ATP} channels were inhibited. The directionality of the signalling cascade was assessed by Western analyses; Gcgr antagonist blocked the DVC glucagon-induced phosphorylation of PKA, and the PKA inhibitor Rp-cAMPS negated the activation of Erk1/2 by DVC glucagon. Together with our previous findings that DVC Erk1/2 signalling triggers K_{ATP} channels, these data reveal a DVC Gcgr–PKA–Erk1/2–K_{ATP} channel cascade that lowers GP. During fasting-refeeding experiments, blocking DVC Gcgr signalling negated the acute ability of high- vs. low-protein feeding to reduce the plasma glucose rise, indicating that the elevated circulating glucagon during high-protein feeding acts in the DVC to lower plasma glucose. Collectively, these data unveil a novel location of glucagon signalling and introduce a physiological role of postprandial brain glucagon action.

ANTENATAL GLUCOCORTICOIDS LEAD TO MULTIGENERATIONAL PROGRAMMING OF BEHAVIOUR AND HPA FUNCTION VIA PATERNAL TRANSMISSION

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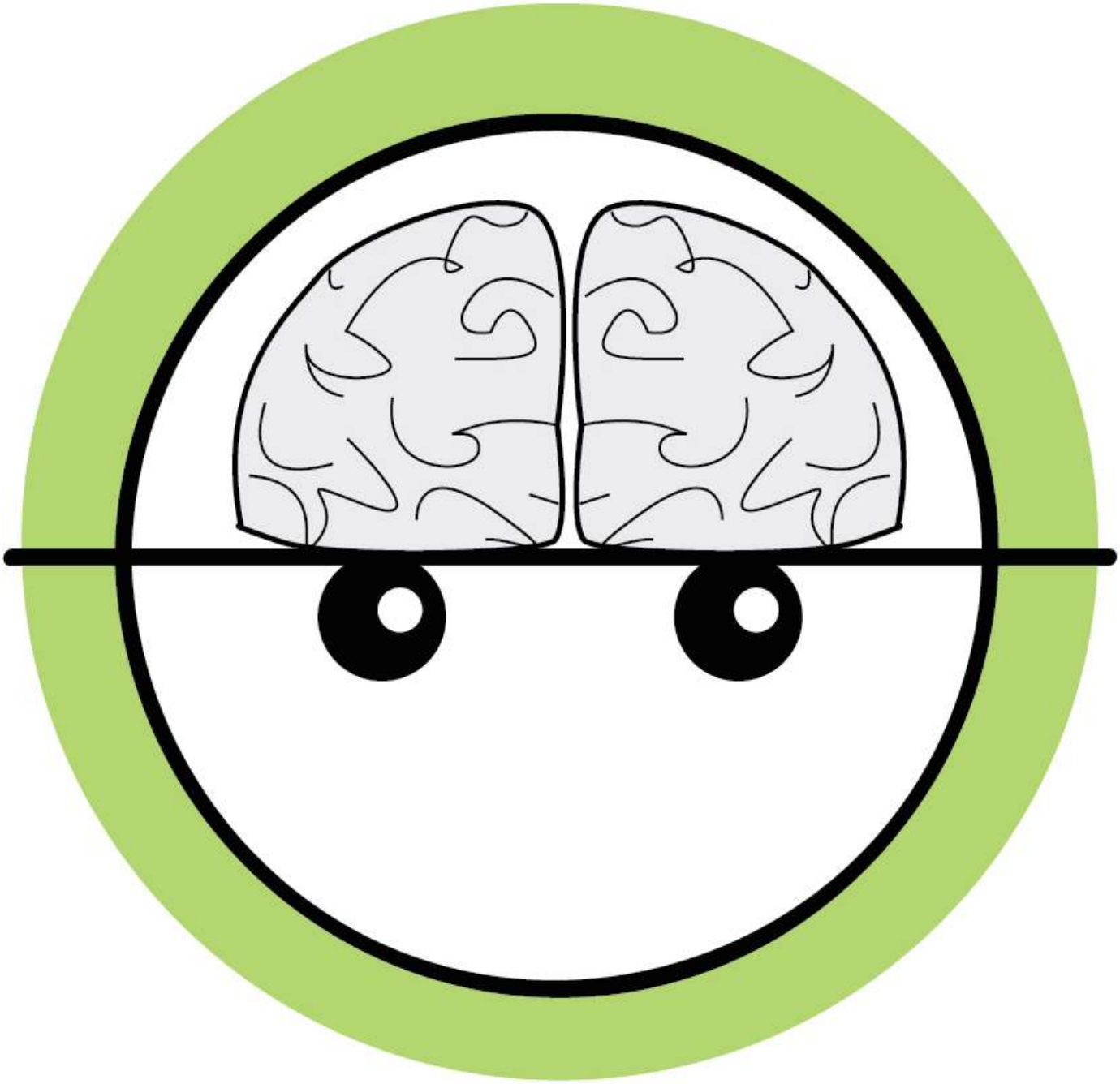
Glucocorticoids are powerful molecules that are required for development of the fetal brain and lungs. Animal studies have shown antenatal treatment with synthetic glucocorticoids (sGCs) to modify the fetal brain epigenome, and to have multigenerational effects on behaviour and hypothalamic-pituitary-adrenal (HPA) function via maternal transmission. Emerging data indicate that there may be multigenerational effects of sGC in humans. It is currently not known whether multigenerational influences of sGC on behaviour and HPA function can be transmitted via the paternal germ line. We hypothesized that prenatal treatment with sGC would result in paternal transmission of: increased locomotor activity, reduced attention and suppressed HPA function in adult F_2 guinea pig offspring. Pregnant guinea pigs received 3 courses of either saline or betamethasone (Veh or sGC) at 75% gestation (term ~69 days). Adult F_1 male offspring were mated with control females to produce F_2 offspring. Adult male and female F_2 offspring underwent behavioural and HPA testing: open field (OF)-locomotor activity; 24-hour locomotor activity in the home cage; prepulse inhibition (PPI)-attention; saliva: HPA function. In F_2 males, sGC significantly modified the profile of locomotor activity in the OF. There was a one-hour phase advance in 24h-activity in the home cage in sGC males, and in F_2 females sGC resulted in increased 24h-activity with significant interaction with reproductive cycle. In F_2 females and males, sGC resulted in increased PPI. All results: $P<0.05$. In females, sGC resulted in an increased HPA response to stress. Antenatal sGC treatment resulted in paternal transmission of altered stress-related behaviours to adult F_2 offspring following. The stage-of-cycle-dependent effects of sGC on 24hr-activity in F_2 females indicate that sGC programming interacts with sex hormones. Unexpectedly, sGC resulted in an increase in attention in 2nd generation males and females. This study suggests germ-line transmission of the effects of sGC across generations.



B.R.A.I.N
POSTERS

THE NEUROPROTECTIVE EFFECT OF VRAC INHIBITION ON THE NEONATAL HYPOXIC-ISCHEMIC BRAIN INJURY

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Perinatal and neonatal hypoxic-ischemic (HI) brain injury has become one of the major causes of acute mortality and chronic morbidity in infants and children. However, the mechanism of HI is still largely unclear. One of the non-glutamate mechanisms that have been identified to play a role in stroke is the volume-regulated anion channel (VRAC). VRAC is responsible for mediating the swelling-induced chloride current and plays an important role in the regulation of cell volume during osmosis. VRACs are widely expressed in the brain, however, their role in HI brain injury is not clear. In this study, we hypothesize that blockade of VRAC reduces the neonatal hypoxic-ischemic brain injury in 7 day-old mice, using a selective VRAC blocker 4-(2-butyl-6,7-dichloro-2-cyclopentyl-indan-1-on-5-yl) oxobutyric acid (DCPIB) at different timelines; 20 minutes prior to ischemia, 20 minutes prior to hypoxia and 5 minutes after hypoxia. To study the effect of VRAC on brain damage, we will use 2,3,5-triphenyltetrazolium chloride (TTC) and Nissl staining to assess the infarct volume, and whole brain imaging to assess the morphological changes in the brain. Furthermore, neurobehavioral testing to assess sensorimotor functions 1, 3 and 7 days following the hypoxic-ischemic injury. In conclusion, preliminary data showed reduction of infarct volume in DCPIB treated group compared to vehicle-treated, which may be considered as a potential agent for drug development for HI.

DIFFERENTIAL ROLES OF THE MEVALONATE PATHWAY IN THE DEVELOPMENT AND SURVIVAL OF MOUSE PURKINJE CELLS IN CULTURE

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The cerebellum is an important locus for motor learning and higher cognitive functions, and Purkinje cells constitute a key component of its circuit. Biochemically, significant turnover of cholesterol occurs in Purkinje cells, causing the activation of the mevalonate pathway. The mevalonate pathway has important roles in cell survival and development. In this study, we investigated the outcomes of mevalonate inhibition in immature and mature mouse cerebellar Purkinje cells in culture. Specifically, we found that the inhibition of the mevalonate pathway by mevastatin resulted in cell death, and geranylgeranylpyrophosphate (GGPP) supplementation significantly enhanced neuronal survival. The surviving immature Purkinje cells, however, exhibited dendritic developmental deficits. The morphology of mature cells was not affected. The inhibition of squalene synthase by zaragozic acid caused impaired dendritic development, similar to that seen in the GGPP-rescued Purkinje cells. Our results indicate GGPP is required for cell survival and squalene synthase for the cell development of Purkinje cells. Abnormalities in Purkinje cells are linked to motor-behavioral learning disorders such as cerebellar ataxia. Thus, serious caution should be taken when using drugs that inhibit geranylgeranylation or the squalene-cholesterol branch of the pathway during development.

THE MODULATION OF THALAMIC BETA POWER DURING MOTOR LEARNING IN ESSENTIAL TREMOR AND PARKINSON'S DISEASE

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Beta oscillations are commonly observed in the major motor structures of the brain, including the basal ganglia, motor cortex, thalamus and cerebellum. In healthy subjects, the power of beta oscillations is suppressed during movement execution but is high during rest and postural maintenance. Recent studies have shown that the magnitude of this movement-related beta suppression correlates positively with improved performance during motor learning. Conversely, we hypothesized that low beta suppression is a marker for poor motor learning in essential tremor (ET) and Parkinson's disease (PD) patients. In order to test this hypothesis, we designed a motor task that requires the patients to adapt their motor output to novel and unexpected visual feedback. The patients were asked to perform a centre-out task which involved moving a cursor on a computer screen from a central starting point to equidistant targets to the left or right (baseline condition - NORMAL). The display was then horizontally inverted so that leftward movements produced rightward deflections of the cursor on the screen and vice versa (experimental condition- INVERTED). The centre-to-out reaction time was measured for first 5 and last 5 trials in the INVERTED condition to assess visuomotor adaptation in the interim. Improvements in reaction time were then compared against the magnitude of beta power suppression in the cerebellar thalamus. Beta power was measured from spike and local field potential recordings that were obtained during microelectrode-guided mapping procedures in deep brain stimulation surgery. For ET patients, recording were obtained

from the cerebellar area of the thalamus (ventral intermediate nucleus) whereas for PD patients, recordings were obtained from the subthalamic nucleus of the basal ganglia.

MECHANISTIC INSIGHTS INTO THE ROLE OF MUNC18 PROTEIN IN MAST CELL SECRETION

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The molecular actions of Munc18 toward syntaxins and/or Soluble N-ethylmaleimide-sensitive factor Attachment protein REceptor (SNARE) protein complex have been studied for its importance in neurotransmitter releases in great depth; however, Munc18's precise roles and its functional contributions to exocytosis in other cell-types remain largely unknown. Here, using RBL-2H3 cells, well-known cell-line of mast cells, we demonstrate the importance of Munc18 and its structural determinants in the degranulation of mast cells. Lentivirus-mediated stable double knockdown of Munc18-1/2 in mast cells cause devastating reduction in both IgE- and ionomycin-dependent degranulation accompanied with decreased expressions in syntaxins-3, a syntaxin isoform which is known to be interacting with Munc18. Additionally, we found there was a severe perturbation in intracellular localization of syntaxin-3 in Munc18-1/2 double knockdown mast cells, implying Munc18 works as a molecular chaperone which maintains expression level and trafficking of syntaxin-3 in mast cells. The reduction in degranulation as well as decreased expressions of syntaxin-3 were restored upon re-introduction of wildtype Munc18, suggesting the specificities of phenotypes observed. However, point mutations in domain-1 of Munc18; namely K46E and E59K, each displayed complete lost in its ability to rescue the defective degranulation and decreased expression of syntaxin-3. Using yeast two-hybrids, we found K46E and E59K mutants exhibit completely abolished binding to syntaxin-3. Finally, independent stable and strong knockdown of syntaxin-3 in mast cells show defective IgE- and ionomycin-dependent degranulation. Collectively, our data demonstrate the importance of Munc18 in mast cell degranulation and that it participates in exocytosis via regulation of syntaxin-3.

NEW INSIGHTS ON TLR4 SIGNALLING IN A MOUSE MODEL OF SUBARACHNOID HEMORRHAGE

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Subarachnoid hemorrhage (SAH) from a ruptured cerebral aneurysm is a severe form of stroke that is associated with death in about 35% of cases and permanent morbidity in the majority of survivors. Morbidity and mortality is due mainly to the initial effect of the hemorrhage and to delayed cerebral ischemia (DCI). Many experimental studies have been published investigating large artery angiographic vasospasm as a cause of DCI over the last several decades. However, reduction of large artery angiographic vasospasm in randomized placebo-controlled clinical trials has had no significant effect on clinical outcomes after SAH. Recent studies have investigated other aspects of SAH pathophysiology to uncover alternative therapeutic targets. 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 brain injury and poor outcome after experimental SAH. We hypothesize that excessive signaling through TLR4 contributes significantly to a potent pro-inflammatory response to subarachnoid blood, resulting in further delayed deterioration after the initial SAH ictus. Our preliminary data demonstrates that abolishment of TLR4 signalling results in improved outcome, as measured by behavioral testing and neuronal cell death assays. We hope to delineate the underlying mechanisms using a combination of pharmacological and genetic manipulations of the TLR4 signaling pathway.

EXHAUSTED PRESYNPATIC GLUTAMATE RELEASE IMPLICATED IN SEIZURE TERMINATION IN AN IN VITRO MODEL OF EPILEPSY

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Given that uncontrolled seizures can lead to long term neurological consequences, our understanding of how the brain spontaneously exits from these conditions offers much therapeutic advantage for those who suffer from these intractable epileptic states. An excessive excitatory drive to cortical pyramidal cells has been observed as a key component during these ictal events. The mechanism through which the network excitation ends, thereby terminating seizures has yet to be determined. A recent study implicates exhaustion of GABA release, the primary inhibitory neurotransmitter in the central nervous system, as a mechanism of ictal initiation. The present study hypothesizes a similar mechanism at glutamateric terminals that leads to ictal termination. In order to test this hypothesis, spontaneous epileptiform events were recorded in vitro from layers 2 and 3 of the mouse somatosensory cortex after a hyperexcitable state was induced using a low Mg ACSF.

Local field potentials and whole cell recordings from visually identified pyramidal neurons were made from these slices. The cells were voltage clamped at -70mV to isolate the actions of the excitatory postsynaptic currents while local stimulation protocols were applied to these slices at various intervals along the time period of the seizure like events. At ictal termination the following observations have been made: 1) an increase in charge transfer of large spontaneous excitatory currents approaching seizure termination, which is suppressed by Gabazine application; 2) an increase in membrane conductance at -70mV; 3) a loss of opto-evoked excitatory postsynaptic potentials with a reduction in electrically evoked responses, locally and projected from deep layers; 4) no change in glutamate evoked excitatory currents; and 5) a loss of sucrose evoked excitatory currents. (This study was funded by the Canadian Institute for Health Research.)

MODELLING OF LOCAL FIELD POTENTIALS IN THE HIPPOCAMPUS

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Information about neural spiking can be extracted from the high-frequency band (>500 Hz) of extracellular potentials. The low-frequency part (<500 Hz) is called the local field potential (LFP) and is a widely available signal in many recording configurations. LFPs are associated with a number of cognitive processes and physiological functions in health and disease and complement the information obtained by analysis of spikes. Because multiple neuronal processes contribute to the LFP, the signal is inherently ambiguous and more difficult to interpret than spikes. While existing spiking models effectively capture the firing patterns of biological networks they are limited in their ability to reproduce LFPs recorded experimentally. Fortunately, the biophysical origin of LFPs is well understood in the framework of volume conductor theory in which extracellular potentials are generated by transmembrane currents passing through cellular membranes in the vicinity of the electrode. We focus on the construction of a pyramidal model which generates computational LFPs. This pyramidal model is being designed to receive inhibitory synaptic input from a number of CA1 interneurons comprising a previously reconstructed interneuron network which has properties derived from the literature and patch clamp recordings. This network was built using four different types of interneurons and has been used to understand how they could affect the power of CA1 theta oscillations. Our developing LFP models will help us constrain these interneuron network parameters so as to move closer to experimentally recorded LFPs. In addition, we will move toward simulation of LFPs generated by larger networks and incorporate mechanisms underlying the generation of CA1 theta oscillations previously developed in our lab. Constructive coupling of these network models with our LFP simulations will bring us closer to understanding the recorded electrical activity of CA1. For our simulations we use LFPy, a ''Python'' package for calculation of extracellular potentials.

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 the active zone (AZ). Based on single-channel gated fusion we predicted that SVs are attached to N type (CaV2.2) calcium channels by a molecular tether (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). SV tether-like fibers have been observed in presynaptic terminals by cryo-electron tomography (Siksou et al. 2007, Fernandez-Busnadiego et al. 2013) but are obscured in conventional transmission electron microscopy (EM) by the dense cytoplasm. We showed that these tethers could be revealed by imaging synaptosomes (SSM) ghosts in which the cytoplasm had been ejected by osmotic rupture (Wong et al. 2014). Two classes of links were observed that were related to the distance of the SV from the AZ: multiple-short (less than 45 nm) or single-long (45-175 nm) tethers. Based on its amino acid backbone we estimated that a channel 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 an anti-CaV2.2 antibody (Khanna et al. 2006) and EM immunogold-labelling to localize the channel C terminal-tip. 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 and for the G-L tether model of SV capture.

GTF2I COPY NUMBER ALTERS SEVERITY OF HYPOXIC-ISCHEMIC BRAIN INJURY IN NEONATAL STROKE 7Q11.23 DISORDER MODELS

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Departments of Physiology[1], Medicine[2], Molecular Genetics[3], Surgery[4], University of Toronto

Hypoxic-ischemic encephalopathy and following secondary brain damage is a major cause of neuropsychological handicaps in infants. Gtf2i is a strong candidate for neurocognitive development. Gtf2i is one of the key genes deleted or duplicated in Williams Beuren Syndrome (WBS) or 7q11.23 Duplication syndrome respectively. Both are chromosomal disorders characterized by neurodevelopmental, cardiovascular and connective tissues abnormalities and intellectual disability. Many of the cardiovascular abnormalities that can be seen in WBS are known or suspected risk factors for stroke, including hypertension, cardiac disease and arterial stenosis. Stenosis within the cerebral vasculature is most probably a major determinant of stroke in WBS. Significant aortic dilation was observed in 7q11.23 Duplication syndrome suggesting a possible causal relationship. Gtf2i protein product TFII-I inhibits intracellular calcium levels, which may affect Ca2+ level dependent axonal development but also suggest a potential neuro-protective effect during HI insult due to its negative regulation of Ca2+ levels. In this study we investigated the role of Gtf2i in hypoxic-ischemic (HI)-induced brain injury in postnatal 7 day old mutant pups. We found that Gtf2i copy number altered infarct volume, cell death and inflammatory response; furthermore we assessed the neurobehavioral recovery. Bradykinin induced deregulated intracellular Ca2+ levels and differential expression of the Bradykinin B1 receptor before and after HI in our mutants could underlie the difference in infarct volume and neurobehavioral recovery. We conclude that the copy number of Gtf2i underlies cellular and molecular mechanisms that may contribute to the severity of neonatal
ischemic stroke and secondary brain damage.

EFFECT OF THETA BURST STIMULATION ON NEURONS IN THE MOTOR THALAMUS OF MOVEMENT DISORDER PATIENTS

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Tremor is a debilitating hyperkinetic symptom of many movement disorder patients seen as rhythmic oscillations of those body parts affected. There are two major types of tremor: rest tremor, as occurs in Parkinson's disease, and action tremor that is characteristic of essential tremor (ET) patients. Tremor is thought to be caused by a central rhythm generator. We have shown that a certain population of neurons within the GPi are capable of being induced into a theta oscillation following focal microstimulation, even in patients lacking tremor. Since GPi projects to the thalamocortical motor loop, we stimulated the motor thalamus with theta burst to determine what effect this will have on the intrinsic firing patterns of neurons within the motor thalamus. If the microstimulation-induced theta oscillations are responsible for inducing tremor in these patients, then stimulating the motor thalamus should entrain the neuronal firing to the tremor rhythm. Well isolated single units from 6 ET patients undergoing Vim DBS implantation surgeries were stimulated with theta-burst stimulation (100uA bursts of 4 pulses, 150μs pulse width, 200ms interburst interval, 3.3ms intraburst interval) for 5 seconds from a distal electrode ~ 1 mm away while neuronal activity was recorded from a focal electrode. An accelerometer was positioned on the hand and recorded tremor modification while cellular recordings were examined for neuronal firing alterations. Recordings from 13 single thalamic neurons have been made. Theta stimulation had no effect on one neuron, however, 12 thalamic neurons were inhibited following theta burst stimulation. Of these 12 neurons, 9 were initially driven by the theta stimulation before being inhibited near the end of the train, and 3 cells showed prolonged inhibition following the termination of theta stimulation. Accelerometer data shows evidence of tremor reduction from theta stimulation on 3 thalamic neurons. The results of this study support a role of the GPi and motor thalamus in tremorgenesis.

KCa3.1/IK1 CHANNEL REGULATION BY CGMP-DEPENDENT PROTEIN KINASE (PKG) VIA REACTIVE OXYGEN SPECIES AND CAMKII IN MICROGLIA: AN IMMUNE MODULATING FEEDBACK SYSTEM?

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The intermediate conductance Ca^{2+}-activated K^+ channel, KCa3.1 (IK1/SK4/KCNN4) is widely expressed in the innate and adaptive immune system. KCa3.1 contributes to proliferation of activated naïve and central memory T lymphocytes and, in CNS-resident microglia, it contributes to Ca^{2+} signaling, migration and production of pro-inflammatory mediators. KCa3.1 is under investigation as a therapeutic target, including for CNS disorders that involve microglial activation. However, it is post-translationally regulated, and this will determine when and how much it can contribute to cell functions. We previously found that KCa3.1 trafficking and gating both require binding of calmodulin (CaM), and that the current is inhibited by cAMP kinase (PKA). It contains a single consensus site for phosphorylation by cGMP kinase (PKG) and in some

cells, PKG can also increase Ca^{2+}, CaM activation and ROS. Therefore, this study addressed KCa3.1 regulation through PKG-dependent pathways. We focused on primary rat microglia and the MLS-9 microglia cell line, using perforated-patch recordings to preserve intracellular signaling. Elevating cGMP increased KCa3.1 current and intracellular ROS production, and both were prevented by the selective PKG inhibitor, KT5823. Evidence that regulation was indirect was that, in inside-out patches from transfected HEK293 cells, active PKG did not directly affect the single-channel activity. In MLS-9 microglia, the cGMP/PKG-evoked increase in KCa3.1 current was mediated by ROS, mimicked by applying hydrogen peroxide, inhibited by a ROS scavenger (MGP), and prevented by a selective CaMKII inhibitor (mAIP). Similar results were seen in alternative-activated primary rat microglia; their KCa3.1 current required PKG, ROS and CaMKII, and they had increased ROS production that required KCa3.1 activity. This regulation pathway is expected to have broad implications because the KCa3.1 channel is important in numerous cells throughout the body.

CALCIUM RESPONSES TO SINGLE ACTION POTENTIALS IN SPINAL CORD LAMINA I NEURONS ARE MEDIATED BY T-TYPE VGCCS

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Lamina I neurons of the spinal cord are critical for the integration of nociceptive information from the periphery and relay this information to the brain. Lamina I neurons exhibit hyperexcitability and decreased inhibition in chronic pain models. Voltage-gated calcium channels (VGCCs) have been implicated in the development of chronic pain symptoms, however their functionality in lamina I neurons is poorly understood. Here, we develop an approach to measure calcium responses evoked by individual action potentials (APs) in lamina I neurons. We made current-clamp recordings from the soma 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 allowed for analysis of calcium responses. Single APs induced robust $\Delta G/R$ increases in the soma (peak $\Delta G/R = 0.06$, n= 20 cells), nucleus (peak $\Delta G/R = 0.03$, n= 6 nuclei), dendrites (peak $\Delta G/R = 0.2$, n=20 dendrites) and dendritic spines (peak $\Delta G/R = 0.07$, n=5 spines). We found that calcium responses were prevented by the addition of tetrodotoxin. The effects of cadmium, nickel, and TTA-P2 demonstrated that calcium responses were mediated by T-Type VGCCs. These findings suggest that single APs are capable of opening T-Type VGCCs, and eliciting calcium entry into the entire dendritic arbour and nucleus. Calcium influx caused by AP firing could aid integration of inputs and alter gene transcription to upregulate excitability.

NMDAR AND mGluR CO-ACTIVATION INDUCED CHANGES IN NEURONAL EXCITABILITY IN A DEVELOPING CENTRAL SYNAPSE

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The development of high fidelity synaptic transmission at the calyx of Held-MNTB synapse requires the complex regulation of synaptic glutamate receptors and active intrinsic membrane properties. Previous studies have shown that patterned activity can concurrently activate NMDARs and mGluRs and increase the fidelity of neurotransmission at high rates by translational and post-translational modifications of glutamate receptors. It is however unknown whether or how activity drives the development and maturation in the intrinsic excitability of MNTB principal neurons. We used NMDA and DHPG (100 μM each) to selectively co-activate postsynaptic glutamate receptors of developing MNTB neurons from the auditory brainstem of prehearing mice. We show that co-activation of NMDAR and mGluR have time-dependent alteration of the action potential (AP) waveform and of the maximum AP frequency during a 100ms current step stimulus. Given that AP waveform is dependent on the biophysical properties of a number of voltage-gated ion channels, we performed whole cell voltage-clamp recordings to determine potential mechanisms underlying observed changes in AP waveform. We observed an increase in current density and an acceleration of kinetic properties of TEA-sensitive potassium current 30 minutes after application of NMDAR and mGluR agonists. These results suggest that selective co-activation of NMDAR and mGluR can regulate intrinsic membrane properties and play a role in shaping action potential waveform and the ability to spike at high-frequencies.

TRPM2-NULL MICE IS NEUROPROTECTIVE AGAINST NEONATAL HYPOXIC ISCHEMIC BRAIN INJURY

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Neonatal hypoxic-ischemic (HI) brain injury and its related diseases hypoxic-ischemic encephalopathy and cerebral palsy have emerged as leading causes of brain damage and neurological deficits in children. Recent advances in stroke research have identified non-glutamate mechanisms associated with ischemic neuronal death in adult. Among the molecules involved in non-glutamate mechanisms, transient receptor potential melastatin 2 (TRPM2), a nonselective calcium

permeable cation channel, is reported to mediate neuronal death following acute ischemic insults in adult mice. However, the role of TRPM2 channels in neonatal HI brain injury remains unknown. Since neonatal and adult mice may differ in their response and recovery to ischemic brain injury, understanding if the involvement of TRPM2 is age-dependent is fundamentally important. In this study, we hypothesize that TRPM2-null mice ($TRPM2^{-/-}$) reduces neonatal HI brain injury in postnatal 7-day-old mice *in vivo.* To study the effect of TRPM2 on neonatal brain damage, we used 2,3,5-triphenyltetrazolium chloride (TTC) staining to assess the infarct volume and whole brain imaging to assess morphological changes in the brain. In addition, we also evaluated neurobehavioral outcomes for sensorimotor function post 1, 3 and 7 days following HI injury. We report that the infarct volumes are significantly smaller and behavioral outcomes are improved in neonatal $TRPM2^{-/-}$ mice compared to that in the wild type mice. These findings suggest that TRPM2 channels play an essential role in mediating HI brain injury independent of age; genetically eliminating TRPM2 channels can provide neuroprotection against HI injury.

MOLECULAR PATHWAYS RESPONSIBLE FOR NMDA RECEPTOR-MEDIATED BEHAVIOURAL PLASTICITY.

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Several neurodevelopmental disorders are thought to involve impairments in NMDA receptor signalling, including autism and schizophrenia. The pathophysiology of schizophrenia can be associated with NMDA receptor hypofunction leading to miswiring of neurons in the brain. Our laboratory has developed a new mouse line (NR1-IR, inducible resuce) with a 90% reduction in NMDA receptor levels. The mutation can be restored to normal by an inducible Cre recombinase. We have used this line to restore NMDA receptor levels and measure behavioural outputs. We discovered that some, but not all, behavioural abnormalities could be normalized in adult mice. My thesis will investigate the molecular events that occur in the process of this behavioural plasticity. We hypothesize that restoration of NMDA receptor levels produces a cascade of molecular changes. Changes in gene expression will reveal the mechanisms by which neurons respond to improved NMDA receptor signalling and synaptic connectivity. Gene expression profiles of wildtype, NR1-IR, and NR1-IR/Cre-transgenic mice will be performed using RNAseq and qPCR methodologies established in our laboratory. Functionally clustered genes showing altered expression levels following restoration of NMDA receptor levels in a mouse model of NMDA receptor hypofunction, will be mapped temporally into early, intermediate, late and resistant genes mediating plasticity. These studies will be performed in the hippocampus, a brain region that show variable levels of behavioural plasticity in our model. Using the NR1-IR mouse line to rescue NMDA receptors, we will be able to determine genes and molecular mechanisms mediating plasticity in the hippocampus.

ROLE OF GLYPICANS IN THE DEVELOPING CENTRAL NERVOUS SYSTEM

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Chondroitin Sulfate Proteoglycans (CSPG) are a class of proteoglycan molecules that play a wide range of roles in the developing and the adult central nervous system (CNS). Importantly, CSPGs have been implicated in inhibiting the regeneration of the optic nerve after injuries. Animal models with an injured optic nerve show upregulation of CSPGs in the surrounding region, which then exerts its inhibitory effect on the optic nerve. These molecules are normally present in the extracellular matrix where they function via RPTPσ, found on the growth cone of axons. Glypicans (GPCs) are GPI-anchored Heparan Sulfate Proteoglycans (HSPGs) that also interact with RPTPσ. As GPI-anchored molecules, GPCs are preferentially localized in the lipid rafts (microdomains on the plasma membrane rich in cholesterol and sphingolipids). Retinal explants cultured on CSPGs and treated with PI-PLC, an enzyme that removes GPI-anchors from proteins, showed rescue in neurite outgrowth on this inhibitory substrate. Hence, we hypothesize that RPTPσ is recruited into lipid rafts by GPCs, and that this recruitment is required for its mediation of CSPGs' growth inhibitory effects. Furthermore, this interaction could be targeted to neutralize the inhibitory effect of CSPGs. Here we show that GPCs 1 and 3 are expressed in the retinal ganglion cell layer of the developing E8 chick retina using *in situ* hybridization. Using this knowledge, short hairpin RNAs against these GPCs have been generated and their expression silenced in dissociated retinal ganglion cells (dRGCs). We predict that blocking the GPC-RPTPσ interaction will lead to enhanced neurite outgrowth of RGCs cultured on CSPGs. This study is relevant to the development of therapeutics for promoting optic nerve regeneration in those with damaged optic nerve, and could be further extended to other parts of CNS where CSPGs also play a role in regeneration.

THE ROLE OF PAK SIGNALING WITHIN THE ENTORHINAL CORTEX IN THE REGULATION OF SYNAPTIC PLASTIICTY AND SOCIAL MEMORY

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Social cognition is an extraordinarily complex memory process that shapes the formation and maintenance of social bonds essential for our physical and mental health. Autism spectrum disorders (ASD) are characterized by distinct impairments in communication and social engagement, and are associated with single gene mutations. PAKs (p21-activated kinases)1-3 are a family of serine/threonine protein kinases that are; target enzymes of Rho small family GTPases and central regulators of both actin cytoskeleton, neuronal morphology, and are involved in synaptic and behavioural plasticity. However, the molecular mechanisms and neural circuitry that underlies the role of PAK signaling in social memory and the pathophysiology of ASD remain elusive. We generated a transgenic mouse model of ASD where the spatiotemporal expression of a dominant negative PAK3 mutation in the entorhinal cortex is modulated. Unlike traditional knockout models, the tetracycline inducible system allows us to analyze the role of PAK3 in the regulation of both synaptic transmission and plasticity, and behavioural responses without potential developmental perturbations. We found that mutant PAK3 mice have impaired social memory but normal novelty recognition and olfaction. Furthermore, the memory deficits were fully rescued with the administration of a tetracycline analog that blocks the expression of the mutant PAK3 transgene, which suggests that the specific impairments are not perturbed at development and are caused by deficits in mature synapses. Through imaging, we identified the neural circuitry responsible for social memory which extends from the axons of layer II/III cells in the entorhinal cortex to the hippocampus and basolateral amygdala. Accordingly, the entorhinal-hippocampal and entorhinal-amygdaloid connections in mutant mice have reduced basal synaptic strength, plasticity, and enhanced paired pulse facilitation suggesting deficits in presynaptic neuronal transmission. Ultimately, PAK signaling in the entorhinal cortex regulates neuronal transmission, long-lasting synaptic plasticity, and social memory through the activation of the Rho signaling pathway and subsequent cofilin-dependent actin regulation.

SEX DIFFERENCES IN THE INVOLVEMENT OF SPINAL P2X4 RECEPTORS AND BDNF IN PAIN HYPERSENSITIVITY INDUCED BY PERIPHERAL NERVE INJURY

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Chronic neuropathic pain is characterized by mild to severe pain and results in significant human suffering and economic burden. The spinal mechanisms underlying neuropathic pain have been extensively investigated in male rodents, indicating an essential role for P2X4 receptor-driven release of brain-derived neurotrophic factor (BDNF) from spinal microglia in the maintenance of pain hypersensitivity. However, we recently demonstrated that microglia do not mediate pain hypersensitivity in female mice. The role of BDNF in mediating pain hypersensitivity in females remains unknown. Consequently, we investigated the role of spinal BDNF in neuropathic pain in female and male mice. Neuropathic pain was modeled in mice via spared nerve injury. Mechanical sensitivity was measured using von Frey fibers. Drugs were administered via intrathecal injection. First, we found that intrathecal application of BDNF (0.5µg/mouse) in naïve mice induces pain hypersensitivity in either sex. Second, we determined that pain hypersensitivity after spared nerve injury was reversed in males but not females through inhibition of TrkB functioning via spinal application of TrkB-Fc (0.5µg x3 days) and y1036 (5.0µg). Furthermore, we found that inhibiting P2X4 receptors with TNP-ATP (5.0µg) reversed pain hypersensitivity in males but not in females. Finally, we found that *P2rx4* gene expression was upregulated in male but not female mice after spared nerve injury. Our experiments provide evidence indicating that female mice do not use P2X4 receptors or BDNF to mediate neuropathic pain hypersensitivity. Taking into consideration sex differences in the spinal mediation of chronic pain may greatly improve future treatment development.

ENHANCED THALAMIC $GABA_AR$-MEDIATED SPILL-OVER INHIBITION ELICITS ANESTHETIC-LIKE CHANGES IN ELECTROCORTICAL ACTIVITY THAT DO NOT REQUIRE T-TYPE CA^{2+} CHANNEL ACTIVATION

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Modulation of thalamic GABAergic signaling can trigger state-associated changes in electrocortical activity. There are 3 types of $GABA_A$ receptor ($GABA_AR$)-mediated inhibition: tonic (*i.e.* extrasynaptic), phasic (*i.e.* synaptic), and spill-over, which requires both synaptic and extrasynaptic $GABA_ARs$. Importantly, the alterations in thalamic activity elicited by the general anesthetic etomidate require both synaptic and extrasynaptic $GABA_ARs$ *in vitro*. Here we test 2 hypotheses *in vivo*: 1) enhanced thalamic spill-over inhibition elicits changes in electrocortical activity that resemble those elicited by etomidate, such as increased 8-12Hz and 12-30Hz signaling, decreased 1-4Hz signaling, and increased spindle-like oscillations; and 2) thalamic T-type Ca^{2+} channels, which promote 1-4Hz signaling, do not mediate the changes in electrocortical activity elicited by enhanced spill-over inhibition. Microperfusion of the extrasynaptic $\delta GABA_AR$ positive

allosteric modulator DS2 (100μM), which promotes spill-over inhibition, into the thalamus effected electrocortical activity in wild-type mice, but not in mice lacking δGABA$_A$Rs. During NREM sleep, DS2: (i) increased 8-12Hz and 12-30Hz power, (ii) decreased 1-4Hz power, and (iii) increased spindle-like oscillations. The electrocortical effects of DS2 were unaffected by blocking T-type Ca^{2+} channels with TTAP2 (300μM). These results indicate that enhanced thalamic spill-over inhibition elicits changes in electrocortical activity that resemble those elicited by etomidate at the thalamus and do not require T-type Ca^{2+} channel activation.

EVALUATION OF A NEW TARGETED IRRADIATION TECHNIQUE BY STUDYING ITS EFFECTS ON NEUROGENESIS AND INFLAMMATION

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Radiation therapy is commonly used for the treatment of cancer and it is often associated with cognitive impairments. Furthermore, radiation is a powerful technique for studying adult neurogenesis, the generation of new neurons throughout adulthood. Reduction in neurogenesis in the hippocampus, an essential brain region for learning and memory, has been shown to play a major role in causing the cognitive impairments associated with radiation. Improving the accuracy of targeted irradiation methods has major implications for the fields of cancer therapy and neuroscience. In our study, we are evaluating the effectiveness and accuracy of a newly developed irradiation technology by assessing neurogenesis and inflammation in irradiated rats. Adult rats were irradiated with a dose of 5 Gy and sacrificed either 24 hours or 7 days after irradiation. 5 Gy is the dose previously shown, with less accurate targeting, to cause approximately 90% reduction in adult neurogenesis. Using immunohistochemistry, cells expressing DCX (doublecortin, a marker of neuroblasts and immature neurons) were quantified. The number of DCX+ cells was significantly reduced in the irradiated animals compared to the controls, confirming that the irradiation had been effective in targeting neurogenesis. However, this reduction was seen only in the more ventral regions of the hippocampus. This regional difference may have been due to the aiming technique (i.e. only the more ventral regions have been irradiated) or due to regional differences in sensitivity to irradiation. In order to tease these two possibilities apart, further tests will be done. Expression of GFAP (glial fibrillary acidic protein, a marker of astrocytes) will be quantified to evaluate the accuracy of the irradiation technology. In addition, GFAP expression may be different between the 24-hour and 7-day groups, in contrast to DCX expression, which was not significantly different between the two groups.

THE ROLE OF DISRUPTED-IN-SCHIZOPHRENIA 1 (DISC1) IN ACTIN-CYTOSKELETON REGULATION AND SYNAPTIC PLASTICITY

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Disrupted-in-Schizophrenia 1 (DISC1) has emerged as a strong genetic risk factor for psychological disorders such as schizophrenia, bipolar disorder, and major depression. This protein has been shown to be important in neurodevelopment through its involvement in neuronal proliferation and migration. In this study we find evidence that DISC1 may also play a role in the regulation of synaptic plasticity, a generally accepted mechanism for learning and memory. Using a line of DISC1 mutant mice with a single amino acid change, Q31L, we find that N-methyl D-aspartate (NMDA) receptor-dependent hippocampal long-term potentiation and depression are enhanced and reduced, respectively, in the Q31L mutants compared to the wildtype controls in field recordings at the CA1 dendritic region. In addition we observed behavioural deficits in the DISC1 mutants with respect to measures of prepulse inhibition and working memory. At the molecular level we found changes in the Ras-related C3 botulinum toxin substrate 1 (Rac1) signaling cascade, a well-established signaling pathway involved in the regulation of actin cytoskeleton and dendritic spines, which may mediate the observed changes in synaptic plasticity. We hypothesize that DISC1 plays a role in hippocampal synaptic plasticity through its actions on the Rac1 signaling pathway

USING MULTI-COMPARTMENT MODELS TO INVESTIGATE THE EFFECTS OF DENDRITIC DISTRIBUTIONS OF H-CURRENT IN SYNAPTIC INTEGRATION OF ORIENS-LACUNOSUM/MOLECULARE (O-LM) HIPPOCAMPAL INTERNEURONS

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Interneurons, or inhibitory cells, serve a primary role in the orchestration of information flow in hippocampus. One interneuron type in the hippocampus is the oriens/lacunosum-moleculare (O-LM) cell, which provides direct feedback inhibition and regulation of excitatory pyramidal cell activity in the CA1 region. To understand how O-LM cells carry out their role in CA1 information flow, it is necessary to study their intrinsic and synaptic properties. A challenging aspect of

such investigation is to determine the contributions of voltage-gated ion channels expressed in dendrites, which critically affect neuronal output. A prominent ion channel present in O-LM cells is the hyperpolarization-activated cyclic nucleotide-gated (HCN) channel, which underlies the I_h current. The dendritic distributions of this channel type are at present unknown in O-LM cells. Using previously developed multi-compartment computational models, we found that non-uniform dendritic distributions could better represent of O-LM cell output. In order to examine the potential functional significance of non-uniform dendritic distributions of I_h, we implemented multiple simulations of inhibitory inputs onto O-LM cells from the medial septum/diagonal band of Broca as well as local excitatory synaptic inputs. The inhibitory inputs were arranged along the dendrites of the O-LM models, preferentially grouped closer to the soma, according to recently acquired experimental data from our collaborators at the University of Montana. The ability of the O-LM cell models to generate back-propagating action potentials in the context of various delays of the onset of single excitatory inputs and dendritic inhibition was assessed. We found that the presence of I_h in dendritic locations allowed for the speeding up of back-propagating action potentials, compared to models with somatic I_h only. This effect on synaptic integration may have implications for long-term potentiation in O-LM cells.

NANOPARTICLES ARE RELEASED FROM HUMAN PRE-IMPLANTATION EMBRYOS AND THEIR SHORT RNA CONTENT CORRELATES WITH EMBRYO MORPHOLOGY

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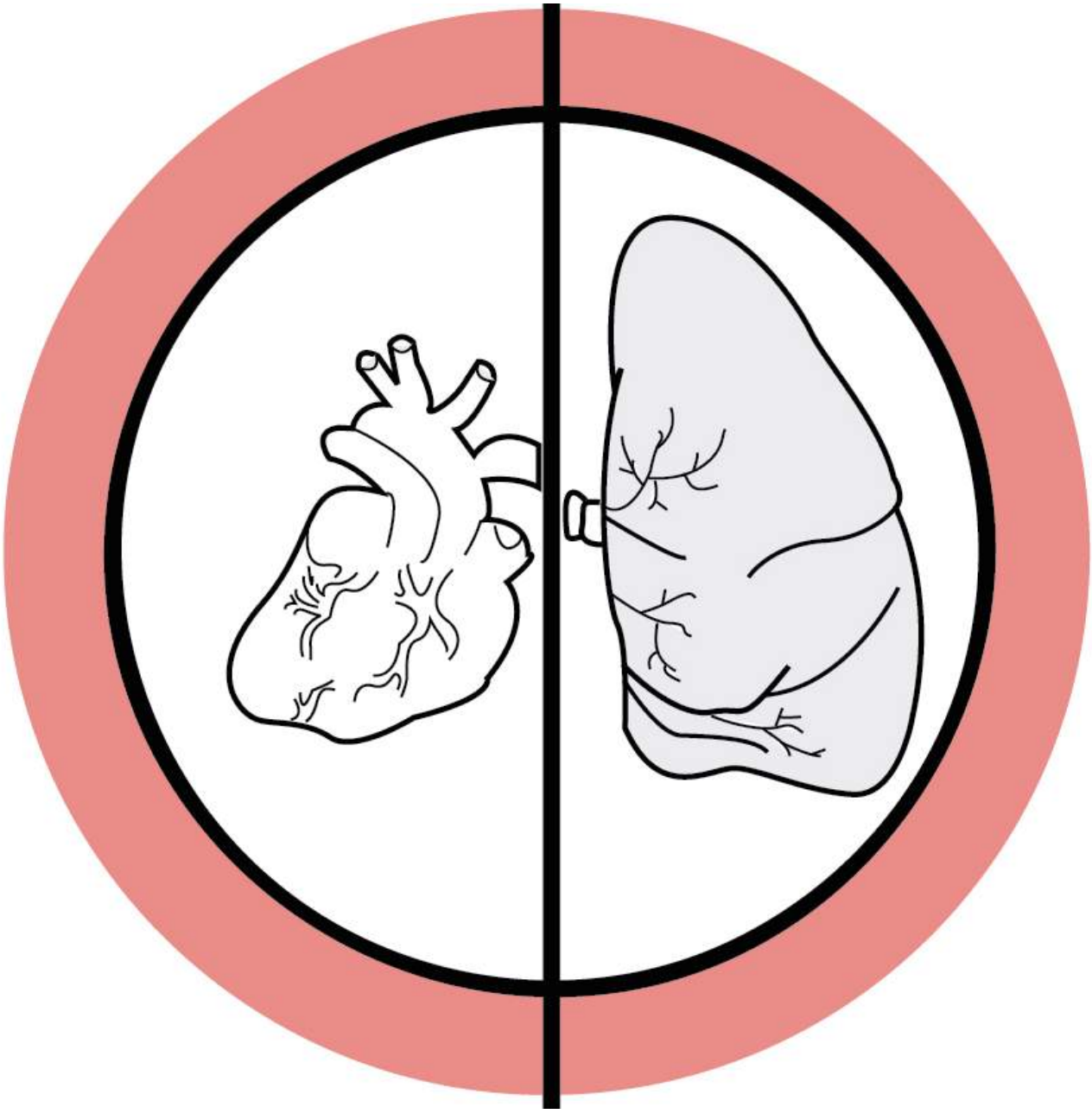
Extracellular vesicles (EVs) are small membrane bound vesicles that have been shown to be released from most cell types in the body, and are a form of cell-to-cell communication. They carry proteins, RNA, DNA and lipids to targeted recipient cells. Our objective was to determine whether EVs are released by pre-implantation embryos and whether their RNA content could inform on embryo quality. REB approval was obtained and informed consent was obtained for all donated samples. Donated abnormal embryos (3PN) were cultured at 5% O_2 and 6% CO_2 in 30uL of Global media™ or CCM media™ for three or five days. For microscopy, embryos were fixed with 0.15% gluteraldehyde and 4% paraformaldehyde in PBS. Embryos were labeled with CD9 primary antibody , followed by immunogold labeling of secondary antibodies. Sections were imaged with a Hitachi-H 700 TEM. RNA extraction was performed using the Total Exosome Isolation Kit™ and quantified using the NanoVue Spectrophotometer. CD9 positive EVs appeared to be released from the membrane of various stages of human pre-implantation embryos, including 1 cell zygote, 3 cell stage, morula and blastocyst. Preliminary data shows that exosome-associated small RNA concentration in conditioned medium of day 3 embryos with good morphology is 25% higher than that of day 3 embryos with poor morphology. Similarly, small RNA concentration in conditioned medium of day 5 embryos with good morphology is 31% higher than that of day 5 embryos with poor morphology. This is the first demonstration of EV appear release from the surface of human pre-implantation embryos. Preliminary data showed that the concentration of exosome-associated small RNA concentration in embryo-conditioned medium is higher with embryos exhibiting poor morphology. This could potentially be a new non-invasive marker of embryo competency.



CARDIOVASCULAR POSTERS

SLC6A14 ENHANCES CFTR CHANNEL FUNCTION THEREBY AFFECTING THE CYSTIC FIBROSIS PHENOTYPE

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Cystic fibrosis (CF) is an autosomal recessive genetic disorder affecting about 4000 Canadians. CFTR channel mediates apical fluid secretion, thereby keeping the surface hydrated. ΔF508 mutation in the CFTR gene is the most common CF one responsible for disease, mainly affecting the lung, intestine, pancreas and epididymis. However, patients homozygous for ΔF508 show a significant variation in their lung function at any age. It is proposed that this variation could be due to the presence of modifier genes, and a recent genome-wide association study (Sun et al, 2012) found SLC6A14 as a major modifier of the cystic fibrosis phenotype. SLC6A14 is an electrogenic Na^+ and Cl^- dependent, neutral/cationic amino acid transporter, expressed on the surface of epithelia. We hypothesized that SLC6A14 could affect the epithelial fluid secretory capacity. So in collaboration with Toronto Centre for Phenogenomics, we generated a SLC6A14 knock-out mouse. Using the colonic closed-loop assay to measure epithelial fluid secretion, we found that the SLC6A14 knock-out mice have a reduced fluid secretory capacity compared to controls. So to understand the mechanism by which this occurs, we used a BHK heterologous over-expression system, in which only SLC6A14 and F508del CFTR were being over-expressed. Interestingly, SLC6A14 enhanced F508del CFTR function in these cells. On the basis of studies of other related amino-acid transporters, we hypothesized that, this enhancement could be mediated by depolarization induced Ca^{2+} release. Using a cytosolic Ca^{2+} imaging method, we found that SLC6A14 stimulation with the amino acid lysine, resulted in increased intracellular calcium, which in turn is known to be an indirect trigger of CFTR function. Taken together, we discovered that SLC6A14 enhances CFTR channel function, making it a plausible drug target

INVESTIGATING FUNCTION OF TMEM65 VENTRICULAR CARDIOMYOCYTE PROTEIN THROUGH AAV9-MEDIATED TRANSDUCTION IN CD1 MICE HEARTS

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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 cardiomyocyte physiology. Here we investigate previously uncharacterized ventricular protein Transmembrane protein 65 (Tmem65), identified at the CM ICD in both mice and human cardiac tissue. Preliminary *in vitro* work on cultured mouse neonatal cardiomyocytes using lentivirus shRNA knockdown, showed altered expression of gap junction functionality and Ca^{2+} dynamics; both of which are essential for electrical propagation. We hypothesize decreased expression of Tmem65 orchestrates arrhythmogenic conditions by destabilizing gap junction proteins, primarily Connexin 43. Administered through the tail vein, AVV9 targets specifically cardiomyocytes and therefore allows Tmem65 treatments to reflect whole animal physiology. AVV9 will contain either Tmem65 shRNA (knockdown) or Tmem65 human cDNA (overexpression). To subclone the most effective shRNA in AAV9, lentiviral transduction on CM cultures were performed and then compared by immunoblotting. Wild-type CD1 neonatal mice (1-3 days) were used for neonatal CM cultures, transduced with lentivirus shRNA and compared. The most effective construct is selected to be subcloned into AAV9 and injected into mice (8-10 weeks). After a minimum of 4 weeks, hearts were removed to confirm viral transduction in cardiac tissue using qPCR, and sectioned and stained for confirmation of Tmem65 overexpression. Future studies will analyze Tmem65 protein complex using novel biotinylation techniques, followed by mass spectrometry analysis to evaluate possible interacting partners of Tmem65. This work will identify Tmem65's physiological role in cardiomyocytes, cardio-pathobiology, and ICD protein interactions, and potentially provide insight into underlying mechanisms of arrhythmogenic diseases.

RECOVERY OF SKELETAL MUSCLE MICROVASCULAR MYOGENIC REACTIVITY AFTER MAJOR BLOCKADE

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Resistance arteries play a critical role in regulating tissue perfusion, vascular resistance, and mean arterial pressure (MAP). This is achieved through the myogenic response (MR): the innate ability to adapt vascular diameter to perfusion pressure. Our laboratory has previously determined that smooth muscle cell-derived TNFá is a critical regulator of MR. Discrete elimination of smooth muscle cell TNFα (using tamoxifen-inducible smooth muscle cell-specific TNFα knockout mice; SM-TNFá-KO) reduces cremaster skeletal muscle resistance artery (sRA) TNFá mRNA expression by ~50%

(n=3 untreated, n=4 tamoxifen-treated). This is associated with an abrogation of sRA MR and a reduction in MAP by ~10%. Unexpectedly, both MR and MAP recover within 7 days post-knockout. This recovery is not associated with a recovery in sRA TNFá mRNA expression (n=2). S3ubsequent *in vitro* TNFá scavenging (etanercept) abolishes MR in sRAs from both untreated and recovered SM-TNFá-KO mice. Interestingly, the MR in sRAs of recovered SM-TNFá-KO mice is stronger at physiological pressures, despite a smaller increase in intracellular Ca^{2+}. Collectively, these data suggest that TNFα-signaling is amplified in recovered SM-TNFá-KO mice to compensate for the genetic deletion of smooth muscle cell TNFα, ultimately resulting in increased sensitivity to intracellular Ca^{2+} and augmented MR at physiological pressures. These findings suggest that the MR is flexibly regulated and can "escape" pharmacological inhibition to re-establish MAP.

THERAPEUTICALLY TARGETING THE CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR (CFTR) IN CEREBROVASCULAR DYSFUNCTION ASSOCIATED WITH SUBARACHNOID HEMORRHAGE (SAH).

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Subarachnoid hemorrhage (SAH) is a devastating type of stroke, in which an intracranial bleed initiates delayed cerebrovascular constriction and subsequent ischemia. Current therapeutic strategies attempt to alleviate the cerebrovascular constriction; however, their lack of specificity disrupts cerebral autoregulation and consequently, limits their overall efficacy. We have identified an inflammatory mechanism that pathologically augments myogenic vasoconstriction (an intrinsic mechanism of resistance arteries that matches flow resistance to the prevalent transmural pressure): at its core, tumor necrosis factor α (TNFα) down-regulates the cystic fibrosis transmembrane conductance regulator (CFTR) and thereby enhances pro-constrictive sphingosine-1-phosphate (S1P) signaling. We demonstrate that SAH down-regulates CFTR protein expression in cerebral arteries by a TNFα-dependent mechanism; CFTR mRNA expression is unaltered, indicating the involvement of a post-translational effect. As our model predicts, reducing CFTR activity in control cerebral olfactory arteries (100nM CFTR(inh)-172 *in vitro*) enhances myogenic tone and thus, mimics the SAH phenotype. Therapeutically increasing microvascular CFTR expression *in vivo* (3mg/kg/day C-18) normalizes the elevated myogenic tone in SAH; olfactory arteries from sham-operated mice are not affected. Since CFTR is more highly expressed in cerebral arteries relative to skeletal muscle arteries, C-18's effects may be localized to the cerebral microcirculation. We conclude that C-18 specifically remedies the enhanced vasoconstriction in SAH. CFTR therapeutics may have clinical application for preventing ischemic injury in SAH.

ELUCIDATING THE ROLE OF STATIN-INDUCED RGS5 UPREGULATION IN VASCULAR SMOOTH MUSCLE CELLS

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Statins are a type of lipid-lowering drugs used in treating atherosclerosis that has pleiotropic effects independent of its inhibition of cholesterol synthesis, namely inhibiting the recruitment of vascular smooth muscle cells (VSMCs). This recruitment in atherosclerosis relies on heterotrimeric G-protein signalling, which is regulated by a class of proteins known as regulators of G-protein signalling (RGS). One member, RGS5, was shown in a microarray analysis to be significantly upregulated following statin treatment. With this in mind, we hypothesized that RGS5 serves as a mediator of the pleiotropic effects of statins during atherosclerosis. The cell culture model was established from aortic VSMCs subjected to 1, 10, 100 μM fluvastatin or vehicle-control for 24 hours. qRT-PCR to assess mRNA changes revealed an increased RGS5 mRNA with fluvastatin treatment (highest with 10 μM). In contrast, *ex vivo* attempts at altering RGS5 expression through statins was unsuccessful at reproducing the upregulation. This may be attributed to the differential contextual states of VSMCs in cell cultures versus *ex vivo*, or an environmental influence such as a circulating factor or other non-target cells (e.g. endothelial cells). Future retrials will involve assessing the effect of growth factor PDGF-BB on the statin-induced RGS5 upregulation, using positive control genes such as matrix metalloproteinase-1. The mechanism by which the statin-induced RGS5 upregulation occurs was investigated by assessing levels of a potential RGS5 regulator peroxisome proliferator-activated receptors (PPARs). qRT-PCR for their mRNA levels demonstrated an increase in PPARs following fluvastatin treatment, implicating their role in RGS5 upregulation. This analysis will be further extended by assessing pharmacological modulation of PPAR activity (GW0742, a PPARδ agonist; rosiglitazone, a PPARγ agonist) on RGS5 expression levels. In conclusion, the preliminary data suggests an inhibitory role for RGS5, and that its upregulation mediates statins' pleiotropic effects.

PROGRESS TOWARDS DEFINING THE BINDING SITE FOR THE FIRST DRUG TARGETING A CYSTIC FIBROSIS CAUSING MUTANT

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Cystic fibrosis (CF) is a disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The gene encodes for the CFTR protein, a PKA-activated and ATP-gated anion channel expressed on the apical surface of epithelial cells. Ivacaftor (Kalydeco) is currently the only drug that has been approved for the treatment of CF (nine CF-causing mutations to date). It works as a potentiator where it enhances the activity of mutant CFTR on the cell surface following endogenous PKA phosphorylation of the protein. The mechanism of action and binding site of Ivacaftor, however, still remain unknown. Here, we investigate the structure-function relationship of Ivacaftor by comparing the activity of Ivacaftor derivatives in a fluorescence-based assay. Baby hamster kidney (BHK) cells are loaded with SPQ, a fluorescent and halide-sensitive dye. Upon stimulation of CFTR channel activity, chloride efflux dequenches SPQ fluorescence. The subsequent increase in fluorescence is used as a readout of CFTR potentiation by Ivacaftor analogues. Our studies revealed structural components of Ivacaftor that are necessary for its activity, as well as a position on the molecule that can potentially be modified without affecting its properties. A biotin tag was incorporated at this position, and this chemical probe is currently being used as a tool to develop a binding assay. We are optimizing a protocol with the Octet system, which utilizes biosensors to characterize protein-small molecule interactions. Preliminary results suggest that it is feasible to quantify the binding of purified Wt-CFTR to Ivacaftor-biotin immobilized on streptavidin-coated biosensors. The development of binding assays will be useful for identifying the Ivacaftor binding site on CFTR. Knowledge of the binding site will ultimately provide valuable insight into its mechanism of action and enable the development of more effective therapeutic drugs in the future.

HUMAN UMBILICAL CORD-DERIVED PERIVASCULAR CELLS (HUCPVCS) DEMONSTRATE GREATER ANGIOGENIC POTENTIAL THAN TERM OR BONE MARROW MSCS USING A NOVEL RAT AORTIC RING ASSAY

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To compare the angiogenic properties of first trimester-derived (FTM) and term HUCPVCs (two young sources of mesenchymal stromal cells (MSCs)), and bone marrow-derived MSCs (BMSCs) by investigating their effects on and physical interactions with endothelial networks, as well as their ability to differentiate into endothelial like cells. We developed a novel functional angiogenic assay utilizing ex-vivo tissue culture of rat aortic rings. For this assay, adult rat aortas were sectioned and embedded into Matrigel™. CellTrackerGreen™-labeled MSCs were added after 5 days of aortic rings in culture. MSC integration, tube formation and network augmentation were monitored by microscopy. Quantification of endothelial networks and fluorescently- labeled MSC was performed using the ImageJ™ software. After one week in culture, both rat cells and human MSCs were isolated from aortic ring co-cultures and the cells were sorted by fluorescence activated cell sorting (FACS) using the human antigen-specific marker TRA-1-85 and analyzed for MSC markers (CD105, CD90), pericyte markers (NG2, CD146) and endothelial markers (CD31, CD34). The sorted cells were lysed and isolated RNA was stored for next generation sequencing (NGS). FTM HUCPVCs migrated towards and integrated more efficiently into the developing tubular network where they developed elongated morphology, and also increased network growth when compared to term HUCPVCs and BMSCs. Phenotyping analyses, currently in progress, will give a better insight into whether FTM, term HUCPVCs and BMSCs are differentiating by expressing endothelial markers after a week in culture. An NGS panel will be applied to evaluate critical changes in cellular expression under these culture conditions. FTM HUCPVCs display increased chemotaxis, affinity for developing vasculature and pericyte-like morphologies when compared to term counterparts or BMSCs. These behavioral characteristics support their potential for tissue regeneration after ischemic or traumatic injury, such as MI, stroke and brain injuries.

CHANGES IN MULTIPLE BREATH WASHOUT MEASURES AFTER RAISED VOLUME RAPID THORACOABDOMINAL COMPRESSION MANEUVERS IN INFANTS

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Multiple breath inert gas washout (MBW) measurements in infants are performed supine and often under sedation in conjunction with other lung function tests such as raised volume rapid thoracoabdominal compression (RVRTC). Recent

data has suggested that RVRTC may influence MBW measurements. This study sought to determine whether indeed RVRTC might influence the subsequent measure of LCI, to determine whether the timing procedures of these tests ought to be standardized. Infants underwent baseline MBW testing using 4% sulfur hexafluoride, analyzed using an AMIS 2000 mass spectrometer (Innovision A/S, Odense, Denmark). This was followed by RVRTC, and repeated MBW. All measures were performed in triplicate, to achieve a minimum of two acceptable trials. Statistical analysis was performed using SAS 9.3, and R 3.1.1. 23 healthy children, and 34 with respiratory disease (cystic fibrosis or recurrent wheeze) underwent the entire protocol with repeatable trials. The RVRTC maneuvers had no effect of the LCI of healthy children (-0.13±0.39, p=0.12), however, they did influence scores in those children with respiratory disease (-0.27±0.57, p=0.01). When corrected to z-scores to account for the height-dependence of LCI, the difference between groups was even more pronounced, with no significant change in healthy children (-0.25±0.73, p=0.1), and a large decrease in those with respiratory disease (-0.44±0.92, p=0.001). RVRTC maneuvers were shown to reduce LCI in infants with respiratory disease, but not in healthy infants. This is likely due to the RVRTC procedure improving ventilation of relatively poorly ventilated lung units, which previously contributed significantly to the overall ventilation inhomogeneity. Therefore, the timing of RVRTC and MBW procedures must be standardized.

AGED ALLOGENEIC PLATELETS TRIGGER ANTIBODY-INDEPENDENT TRANFUSION RELATED ACUTE LUNG INJURY VIA A SPHINGOLIPID-DEPENDENT MECHANISM

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At present lung injury is the leading cause of morbidity and mortality following transfusions. Transfusion related acute lung injury (TRALI) is associated with endothelial leak and neutrophil associated pulmonary damage. TRALI can be classified as either antibody dependent or non-antibody dependent. Antibody mitigation strategies have lowered the prevalence of TRALI but have not eliminated its incidence. Remaining cases may be non-antibody dependent TRALI which at present have no prevention strategies nor treatments beyond supportive care. To investigate non-antibody dependent mechanisms a novel two hit murine stored platelet TRALI model was created involving a proinflammatory priming hit of intraperitoneal lipopolysaccharide (2 mg/kg LPS) two hours prior to transfusion of 10 ml/kg of stored (1 to 7 days) allogeneic C57Bl6 male platelets into recipient male BALBc mice who were monitored for 6 hours prior to necropsy to assess end points of acute lung injury. Aged platelet transfusions after LPS led to recipient mice with elevated lung wet-to-dry weight ratios, bronchoalveolar lavage (BAL) fluid protein content, BAL MIP2 levels, lung tissue myeloperoxidase activity and histologic changes in keeping with increased lung injury when compared with mice transfused fresh platelets (stored 1 day) or sham controls. Sphingolipid analysis in platelets showed a storage-time dependent increase in the levels of ceramide, a well-documented mediator of lung injury. Consistent with a pathogenic role of ceramide accumulation in aged platelets, donor platelets from mice deficient in the ceramide-forming enzyme acid sphingomyelinase (asMase; *smpd1*$^{-/-}$) induced significantly less injury in recipient mice following transfusion as compared to WT platelets. Ceramide accumulation in aged donor platelets constitutes a key mechanism in non-antibody dependent TRALI, thus identifying sphingolipid metabolism as an important target in order to increase the safety and longevity of stored blood products.

THE EFFECTS OF EXERCISE ON CARDIAC ELECTRICAL REMODELLING

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Cardiac remodeling is a term used to describe the changes in cardiac biochemistry, gene expression, structure and function of the heart in response to stress, which themselves can be categorized into physiologic (i.e.exercise) and pathophysiologic (i.e.disease). Our lab has identified distinct remodeling patterns between the atria and ventricles of the mouse heart in response to endurance exercise (Nature Commun,2014). In particular, the atria developed fibrosis, inflammation and atrial fibrillation(AF), whereas the ventricles showed improved contractile function with reduced vulnerability to induced arrhythmia. Also, our microarray studies showed significant increase in the activation of the TNFa-NFkB pathway, a central regulator of cardiac inflammation and cardiomyocyte's survival, in exercise-associated atrial remodeling. In addition to the differential electrical remodeling, we have also shown differential levels of NFkB activity in exercised ventricle vs. atria, with an elevation of NFkB activity in the atria, while no significant difference in the ventricles. There may also be intrinsic differences in the TNFa-NFkB signaling activity between the chambers, causing these differential responses to exercise. I hypothesize that the pro-arrhythmic properties exhibited in endurance exercised left atria result from changes in NFκB-

associated gene expression(i.e. ion channel expression) and structural changes(i.e. fibrosis), leading to increased conduction velocity heterogeneity acting as a substrate for arrhythmias. To test this, I have performed deep RNA-sequencing to obtain full atrial/ventricular transcriptomes for 4 mice groups: exercised and sedentary CD1s and TNFa knockout mice. Using RNA-Seq results, I will identify additional genes or pathways with modified expressions using gene ontology and pathway analysis. In conclusion, together with preliminary data from our lab, my study will allow in-depth examination of mRNA and protein expression levels of different ion channels as well as genes related to cardiac inflammation and fibrosis, to examine the underlying mechanism behind differential exercise-induced electrical and structural cardiac remodeling in the atria/ventricle.

MECHANISMS OF ATRIAL FIBRILLATION INDUCED BY ENDURANCE EXERCISE

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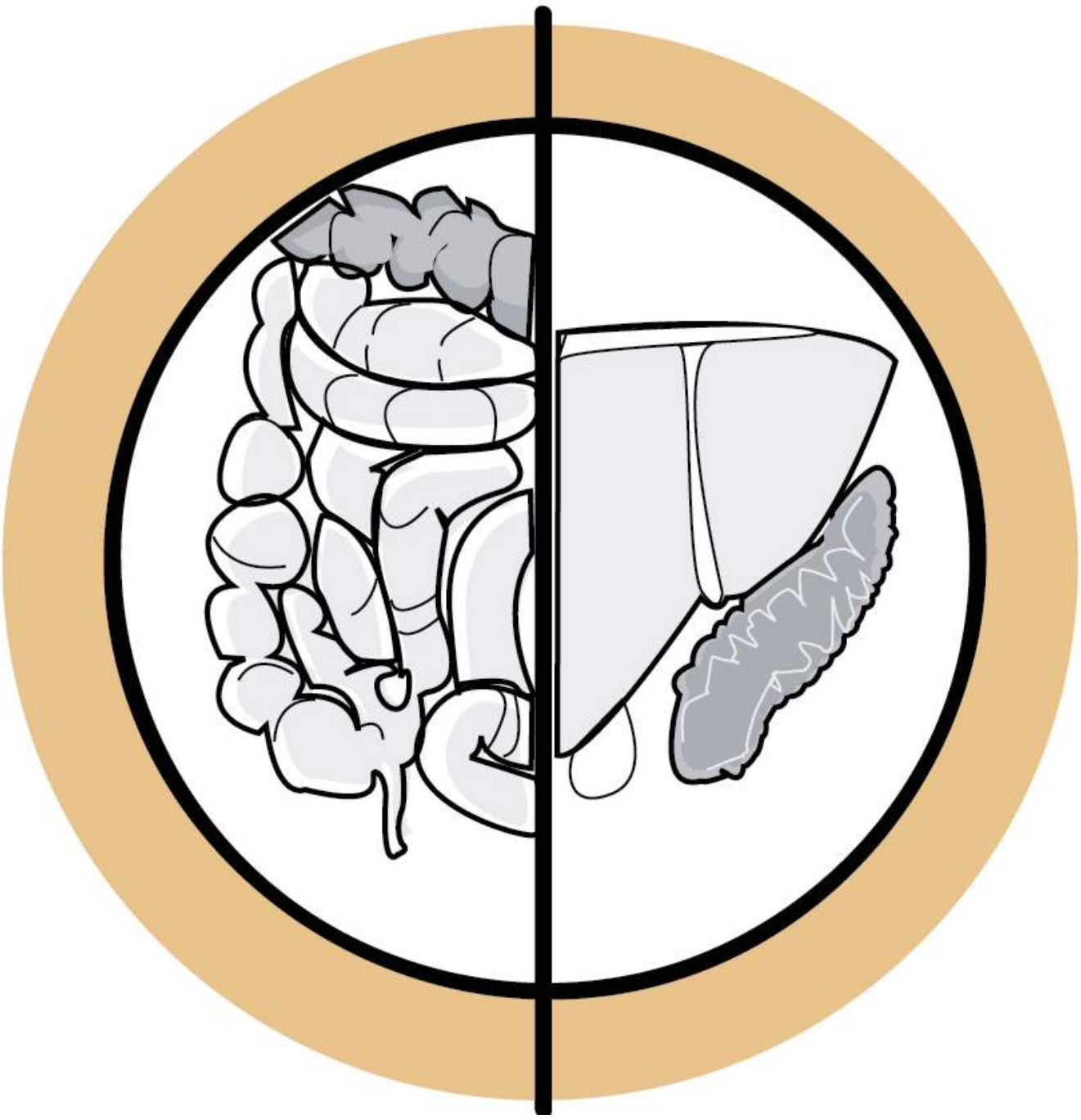
Atrial fibrillation (AF) is the most common supraventricular arrhythmia. Long-term endurance exercise causes a 2-10 fold increase in an athlete's risk of AF. The molecular mechanisms underlying endurance exercise mediated AF remain largely unknown. Our laboratory has developed three 6-week endurance exercise mouse models (swim, treadmill and free wheel), which demonstrate increased vulnerability to AF. Swim and free wheel exercise induce increased vulnerability to AF by distinct mechanisms involving structural and electrical remodeling respectively. Microarray analysis of the swim model indicates exercise-induced activation of TNFα mediated inflammatory pathways. Results from TNFαKO mice indicate that despite different mechanisms underlying increases in AF vulnerability induced by different exercise models, TNFα plays a role in both swim mediated structural remodeling and free wheel mediated electrical remodeling. All three models demonstrate increased parasympathetic nerve activity (PNA). Based on current evidence, we hypothesize that the arrhythmogenic shortening of the atrial effective refractory periods (AERPs) and action potential durations (APDs) induced by free wheel exercise is mediated by TNFα signaling and increased PNA. Whole-cell patch clamp recordings in isolated atrial cardiomyocytes will be used to characterize changes to individual ion channel currents underlying free wheel exercise mediated shortening of APDs and AERPs. The role of TNFα in mediating changes in ion channel currents underlying free wheel exercise-induced electrical remodeling will be studied. Patch clamp recordings and western blot analysis will be used to understand the role of exercise-induced increases in PNA and/or changes in downstream muscarinic cholinergic signaling cascades in free wheel exercise mediated electrophysiological changes. This will provide novel insight into the molecular mechanisms underlying electrical remodeling in free wheel exercise-induced AF. Gaining a more through understanding of the mechanisms underlying exercise-induced AF will provide potential therapeutic targets and a basis for more effective treatment of AF in endurance athletes.



ENDOCRINE & DIABETES POSTERS

THE ROLE OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-4 IN GLUCAGON-LIKE PEPTIDE-2-INDUCED GROWTH IN THE MURINE INTESTINE

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Glucagon-like peptide-2 (GLP-2) is an enteroendocrine hormone released in response to nutrient ingestion that plays an important role in stimulating growth and function of the intestinal epithelium (IE). A degradation-resistant analog of GLP-2 is an approved treatment for patients with short bowel syndrome. The GLP-2R is expressed on the intestinal subepithelial myofibroblasts, enteric neurons, and some enteroendocrine cells but not on the IE where the growth effects of GLP-2 are observed. Thus, GLP-2 is thought to act indirectly through other growth factors. Previous work has established that GLP-2 stimulates insulin-like growth factor-1 (IGF-1) in intestinal cells, and that both IGF-1 and the IE-IGF-1R are essential for GLP-2-enhanced crypt-villus growth. In addition, insulin-like growth factor binding protein-4 (IGFBP-4), a modulator of IGF-1 bioactivity, has been shown to be integral for GLP-2-induced intestinal growth. Thus, we hypothesized that IGFBP-4 increases IGF-1 bioactivity on the IE and that GLP-2 regulates the intestinal IGFBP-4 expression. Chronic GLP-2 treatment in normal C57Bl/6 mice significantly increased IGFBP-4 mRNA expression in mucosal scrapes compared to vehicle-treated mice ($p<0.05$, 0.1μg/g for 10d). Consistently, GLP-2 treatment significantly increased IGFBP-4 mRNA expression in fetal rat intestinal cells ($p<0.05$, 10^{-6}M for 2hrs). In order to assess the role of IGFBP-4 in modulating the bioactivity of IGF-1 on the IE, IEC-6 cells were treated with IGF-1(100ng/ml) and/or IGFBP-4 and changes in proliferation were assessed by thymidine incorporation. Unexpectedly, IGFBP-4 significantly decreased IGF-1-induced proliferation with x0.5, x1, and x2 molar concentration of the IGF-1 ($p<0.05$). The results of this study suggest that while IGFBP-4 expression is regulated by GLP-2, it inhibits IGF-1-stimulated-IE growth. The role of IGFBP-4 is likely dependent on cell type within the IE and its growth promoting role under GLP-2-stimulated

DEFICIENCY OF A NOVEL ADAPTOR PROTEIN, XB130, IS ASSOCIATED WITH THE DEVELOPMENT OF SEVERE MULTINODULAR GOITER

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Xb130 is an adaptor protein predominantly expressed in thyroid glands. It participates in the regulation of cell proliferation, survival and migration by modulating PI3K/AKT pathway. Clinical studies suggest that Xb130 is involved in tumorigenesis. Investigation of Xb130's role in spontaneous tumorigenesis using old mice accidently revealed that Xb130 knockout (KO) mice develop multinodular goiter (MNG) late in life. Development of MNG is closely associated with hypothyroidism caused by dysfunctional thyroid hormonogenesis. Therefore, we hypothesize that Xb130 plays an essential role in the regulation of thyroid hormone synthesis. Aged Xb130 KO mice developed significantly enlarged thyroids that are approximately 8-fold larger than those of Wt mice. Histological analysis indicated that the aged mice developed varying degrees (moderate to severe) of MNG, which is characterized by asymmetrically enlarged glands containing dilated colloid-rich follicles with flattened epithelium. Most (91%) of the Wt mice exhibited normal sized thyroids with normal histology, in contrast to only 18% of the KO mice ($p<0.01$). Although the moderate form of MNG were detected in both groups, the severe form of MNG were only found in the KO mice (45%; $p<0.0001$). We then found that younger (6-month-old) KO mice were hypothyroid with significantly elevated (2.8-fold) TSH and relatively lower (1.1-fold) T4 in serum. In contrast, the old (18 month) KO mice had euthyroid MNG with similar serum levels of TSH and T4 to those of Wt mice. Moreover, we showed defective thyroglobulin iodination in Xb130 KO mice through western blotting for iodinated-thyroglobulin and using ^{125}I radioisotope study. Lastly, we determined that Xb130 is localized in apical membrane of thyrocytes and that Xb130 KO mice have problems in forming microvilli, in which thyroglobulin iodination occurs. As a future study, the main goal is to uncover the molecular mechanism of XB130 in maintaining the thyroid homeostasis for thyroid hormone synthesis.

conditions may only occur in a subset of cells within the IE.

THE ROLE OF SYNTAXIN-1a IN THE SECRETION OF GLUCAGON-LIKE PEPTIDE-1 FROM THE PRIMARY, ADULT MOUSE L CELL

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Glucagon-like peptide-1 (GLP-1) is a proglucagon-derived gut hormone secreted by the intestinal L cell, an enteroendocrine cell located predominantly in the distal small intestine. GLP-1 is one of the key mediators of the incretin effect, whereby oral glucose ingestion significantly increases the secretion of insulin as compared to isoglycemic intravenous glucose administration. The insulinotrophic effects of GLP-1 have made it a key target for the development of type 2 diabetes treatments with both GLP-1 receptor agonists and GLP-1 degradation inhibitors currently in use, however an alternate potential approach would be to increase endogenous GLP-1 secretion. While the signaling pathways for GLP-1 secretion

have been well characterized, the precise mechanism by which GLP-1 is secreted has remained elusive. Prior research by the Brubaker lab using the GLUTag model L cell line has demonstrated that the core soluble NSF attachment protein receptor (SNARE) protein vesicle-associated membrane protein 2 (VAMP2) is essential for GLP-1 secretion, and that VAMP2 interacts solely with the SNARE protein syntaxin-1a. Therefore, we hypothesize that syntaxin-1a and GLP-1 are co-expressed in the primary, adult mouse intestinal L cell. To establish the adult mouse intestinal cell culture (AMIC) model, wild-type adult (~8-12 weeks) mouse intestines were cultured and imaged via immunocytochemistry. The AMIC model was then used on syntaxin-1a (flox/flox) mice, which demonstrated a clear co-expression of GLP-1 and syntaxin-1a. These results were further supported by immunohistochemistry of wildtype mouse jejunal samples. In order to demonstrate a functional role for syntaxin-1a in the secretion of GLP-1 syntaxin-1a (flox/flox) AMICs were infected with a Cre recombinase-expressing adenovirus, resulting in an approximately two-fold decrease in syntaxin-1a expression relative to control. Currently we are developing an *in vivo* surgical model for adenoviral knockdown of syntaxin-1a. In conclusion, this data demonstrates that GLP-1 and syntaxin-1a are coexpressed in the primary, adult mouse L cell.

THE ROLE OF SIRT1 IN FAT-INDUCED BETA CELL DYSFUNCTION

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Sirtuin 1 (SIRT1) is a protein deacetylase that has been shown to be beneficial to β cell function by increasing insulin secretion. In obesity, high levels of circulating free fatty acids (FFA) are known to induce β-cell lipotoxicity and consequently β-cell dysfunction. Several studies have found a strong association between low SIRT1 activity and fat-induced β-cell dysfunction. In our previous studies, using hyperglycaemic clamps, we found that activation of SIRT1 by resveratrol infusion in rats partially protected against β-cell dysfunction induced by 48-h oleate infusion, compared with 48-h oleate infusion alone. In addition, we found that BESTO transgenic mice, which overexpress SIRT1 specifically in β-cells, were partially protected against β-cell dysfunction induced by 48-h oleate infusion, whereas wild type mice (C57BL/6) were not. As SIRT1 is NAD+-dependent, we explored the possibility that NAD+ deficiency is responsible for the decreased SIRT1 activity observed in our preliminary data in islets of oleate-infused rats. NAD+ levels in the islets were measured across all rat treatment groups; however, no difference between the groups was observed. However, a previous study1 has shown that the NAD+ precursor, nicotinamide mononucleotide (NMN), prevented fat-induced β-cell dysfunction, suggesting that stimulation of SIRT1, however induced, counter-acts the decreased SIRT1 activity in the presence of oleate. I have performed experiments in which adult, male C57BL/6 mice were infused for 48h with saline, oleate, oleate+NMN, or NMN alone. I have found that the co-infusion of NMN with oleate resulted in a significant and dramatic improvement of beta cell function, in contrast to beta cell dysfunction caused by the infusion of oleate alone. This may be due to the beneficial effects of increased SIRT1 activity, which include decreasing oxidative stress and increasing ATP production in the beta cells. In addition, I found that the co-infusion of NMN with oleate completely prevented an elevation in plasma FFA levels. This could be due to anti-lipolytic effect of NMN, including increased FFA uptake by skeletal muscles. I will be performing additional experiments to increase the reliability of my data. In addition, I will be measuring the activity of SIRT1 in islets of rat infused for 48h with saline, oleate, oleate + resveratrol, or resveratrol alone, in order to investigate potentially altered SIRT1 activity in my lab's previous experiments. Thus far, my lab has found significant and substantial evidence supporting that SIRT1 activation, whether by resveratrol, genetic over-expression, or metabolite administration (NMN), protects against fat-induced beta cell dysfunction.

THE GROWTH AND PROLIFERATIVE EFFECTS OF GLUCAGON-LIKE PEPTIDE-2 ON THE IRRADIATED MURINE SMALL INTESTINE

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Glucagon-like peptide-2 (GLP-2) is an enterotrophic hormone secreted by intestinal L-cells in response to nutrient ingestion. GLP-2 promotes the expansion of the intestinal epithelium by inhibiting villus cell apoptosis and stimulating crypt cell proliferation, leading to increased crypt-villus height and improved intestinal function. However, it is unknown which intestinal stem cell (ISC) population is activated by GLP-2 to promote its proliferation. It has been demonstrated that treatment of degradation-resistant GLP-2 pre- and post-ionizing radiation (IR) increases clonogenic crypt survival at radiation doses resistant in position 4 reserve ISCs, marked by the gene expression of B-cell specific Mo-MLV insertion region-1 homolog (Bmi-1). Thus, we hypothesize that GLP-2 promotes small intestinal epithelial growth in irradiated Bmi-$1^{GFP/+}$ C57Bl/6 mice by stimulating the proliferation of Bmi-1^{+} ISCs. With a GFP-coding region knocked in to one Bmi-1 gene, this transgenic mouse model allows a Bmi-1^{+} ISC to exhibit GFP fluorescence. Subcutaneous injections of h(Gly^2)-GLP-2 (0.2 μg/g body weight, b.i.d.) were administered to Bmi-$1^{+/+}$ and Bmi-$1^{GFP/+}$ for 14d pre- and 4d or 5d post-10Gy-IR (n = 7 per group). Small intestinal weight normalized to body weight increased in Bmi-$1^{+/+}$ and Bmi-$1^{GFP/+}$ mice 4d and 5d post-IR ($p < 0.01$). As no crypts were observed in all intestinal sections, villus height and submucosal thickness were measured to characterize mucosal growth. PBS-treated Bmi-$1^{GFP/+}$ mice 5d post-IR showed a greater submucosal thickness compared to Bmi-$1^{+/+}$ mice ($p < 0.05$). Additionally, GLP-2-treated Bmi-$1^{+/+}$ and Bmi-$1^{GFP/+}$ mice exhibited taller villi compared to

controls ($p < 0.01$). As co-GFP fluorescence and EdU immunofluorescence revealed proliferating cells between and within villi, the number and localization of Bmi-1$^+$ ISCs is being quantified. Collectively, these results suggest that GLP-2 administration pre- and post-IR exerts a protective gravimetric and morphometric effect on the irradiated murine small intestine, likely by stimulating the proliferation of reserve Bmi-1$^+$ ISCs.

LEPTIN ACTIVATES A JEJUNAL PI3K-DEPENDENT AND STAT3-INDEPENDENT SIGNALING PATHWAY TO LOWER GLUCOSE PRODUCTION IN UNCONTROLLED DIABETES

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The adipocyte-derived hormone leptin acts on the leptin receptor in the hypothalamus and activates PI3K- and STAT3-dependent signaling to regulate glucose homeostasis. Additionally, central leptin administration lowers plasma glucose levels in the STZ-induced insulin-deficient uncontrolled diabetic rodents in association with a drop in glucagon levels. Leptin is also synthesized in the stomach and activates intestinal leptin receptors to maintain mucosal integrity. Given that jejunal nutrient sensing is necessary for duodenal-jejunal bypass (DJB) to lower glucose production (GP) and glucose levels in uncontrolled diabetic rodents, we sought to determine whether jejunal leptin action lowers GP through leptin receptor-PI3K and/or STAT-3 dependent mechanisms in normal, STZ-insulin deficient diabetic and high fat diet (HFD)-insulin resistant rodents. We then evaluated whether jejunal leptin action mediates the glucose-lowering effect of DJB in insulin-deficient uncontrolled diabetes. In rats and mice, we administered leptin into the jejunum for 50 min and evaluated changes in GP during the pancreatic clamps *in vivo*. Chemical loss-of-function approaches targeting intestinal leptin receptor-mediated signaling were utilized to assess the underlying mechanisms involved. Intrajejunal leptin infusion activated jejunal PI3K and STAT3 and lowered GP in normal rats and mice independent of changes in circulating leptin, insulin and glucagon levels. The GP-lowering effect was negated in leptin receptor deficient fa^k/fa^k rats and db/db mice, or upon co-infusion with a leptin receptor antagonist. Interestingly, blockade of jejunal PI3K and not STAT-3 signaling negated jejunal leptin to lower GP in normal rats, while the metabolic effect of leptin was also seen in STZ-induced uncontrolled diabetic (independent of changes in glucagon levels) and HFD rodents. Lastly, blockade of jejunal leptin action disrupted glucose homeostasis during refeeding in uncontrolled diabetic rodents that received DJB. These data unveil a glucoregulatory site of leptin action and suggest that enhancing leptin-PI3K signaling in the jejunum lowers plasma glucose levels in diabetes.

STIMULATION OF QUIESCENT STEM CELLS BY GLUCAGON-LIKE PEPTIDE-2 (GLP-2) IN THE MURINE SMALL INTESTINE

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The endocrine hormone, glucagon-like peptide-2 (GLP-2), is secreted from intestinal L-cells upon nutrient ingestion whereupon it stimulates the growth of the intestinal epithelium. However, the proliferative crypt cell population upon which GLP-2 acts is unknown. It has been shown that chronic treatment with degradation-resistant GLP-2 increases clonogenic crypt survival at irradiation (IR) doses that are lethal to the Lgr5-, but not the quiescent position-4- stem cells marked by Bmi-1 (B-cell specific Mo-MLV insertion region-1 homolog). We therefore tested the hypothesis that GLP-2-induced proliferation involves the Bmi-1$^+$ stem cells in the small intestine, assessed using the Bmi-1$^{GFP/+}$ mouse model. h(Gly^2)GLP-2 (0.1µg/g, q.d., sc) was administered chronically, followed by BrdU at t=-1h. Jejunal villus height and crypt depth increased with GLP-2 treatment, by 32 and 24%, resp. ($p<0.01$-0.001, N=6) in Bmi-1$^{+/+}$ mice. Although villus height was increased by GLP-2 in Bmi-1$^{GFP/+}$ mice, by 25% ($p<0.01$, N=7-8), crypt depth was not affected. Furthermore, the GLP-2 response for the area-under-the-curve of the proliferation index for positions 7-14 showed a higher response in the Bmi-1$^{+/+}$ compared to Bmi-1$^{GFP/+}$ mice ($p<0.01$, N= 5-8). To address the possibility that the Bmi-1$^{GFP/+}$ mice could respond to a higher dose of GLP-2, mice were treated with 0.2ug/g h(Gly^2)GLP-2 at t=-6 and -3h, followed by EdU at t=-1h. Positional analysis of EdU immunofluorescence indicated an increased area-under-the-curve of proliferation within crypt positions 6-11 in the Bmi-1$^{GFP/+}$ mice ($p<0.05$, N=3). Lastly, to definitively activate Bmi-1$^+$ stem cells, 0.2ug/g h(Gly^2)GLP-2 was administered b.i.d. to Bmi-1$^{+/+}$ and Bmi-1$^{GFP/+}$ mice for 14d pre- and 4- or 5d post-10Gy-IR (N=7). Villus heights 4d post-IR were significantly increased in both genotypes, due to GLP-2 treatment ($p<0.01$, N=7). Proliferation analyses are ongoing. Taken together, these findings indicate that a full genetic complement of Bmi-1 is required for the chronic, but not the acute proliferative effects of GLP-2.

SYNTAXIN-1A PLAYS AN ESSENTIAL ROLE IN GLUCAGON-LIKE PEPTIDE-1 SECRETION FROM THE ADULT MOUSE INTESTINAL L-CELL

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Glucagon-like Peptide-1 (GLP-1) is an incretin hormone secreted from distal intestinal L-cells. Due to the glucose-lowering actions of GLP-1, GLP-1 receptor agonists and GLP-1 degradation inhibitors have been successfully implemented in the clinic for the treatment of Type II Diabetes. Another potential therapeutic approach lies in enhancing endogenous GLP-1 release. Although signaling pathways promoting GLP-1 secretion have been extensively characterized, little is known about the precise downstream mechanism of GLP-1 exocytosis. We hypothesized that Syntaxin-1a, a core SNARE protein, is essential for GLP-1 exocytosis from primary murine intestinal L-cells. Using immunocytochemistry, 1.1±0.1% of adult mouse intestinal cells (AMIC) in culture expressed GLP-1. AMIC cultures were also found to widely express Syntaxin-1a, with 96.7±0.2% of cells expressing Syntaxin-1a. Furthermore, Syntaxin-1a and GLP-1 were found to co-localize, as 100% of primary L-cells also expressed Syntaxin-1a. Immunohistochemical analysis further demonstrated co-localization of GLP-1 and Syntaxin-1a. AMICs responded appropriately to several known secretagogues, with enhancement of GLP-1 secretion up to 2.3-fold in response to 10 – 50μM forskolin/IBMX ($p < 0.05 – 0.01$), 1.7-fold ($p < 0.05$) in response to 1μM glucose-dependent insulinotropic peptide and 3.5-fold ($p < 0.01$) following treatment with 15μM oleoylethanolamide (OEA). To specifically examine the role of Syntaxin-1a in GLP-1 secretion, AMIC cultures were generated from Syntaxin-1a$^{fl/fl}$ mice and subsequently infected with adenovirus-cre-recombinase (AdV-iCre) or empty adenovirus (AdV-RFP) for 2 days, followed by analysis of Syntaxin-1a expression and GLP-1 release. qRT-PCR analysis demonstrated an 83.9±6.1% knockdown of Syntaxin-1a expression in AMICs infected with AdV-iCre as compared to AdV-RFP. ICC confirmed the knockdown of Syntaxin-1a at the protein level. Syntaxin-1a-cre L-cells exhibited a 1.3-fold increase in basal secretion, but the response to 15μM OEA was significantly abrogated
($p < 0.05$). Thus, Syntaxin-1a plays an essential role in GLP-1 secretion from the primary, adult mouse ileal L-cell.

THE OMEGA-3, POLY-UNSATURATED FATTY ACID DOCOSAHEXAENOIC ACID (DHA) REGULATES GNRH1 GENE EXPRESSION IN A NON-CLONAL, GNRH-SYNTHESIZING NEURONAL CELL MODEL

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Although the links between nutrition and reproductive function are widely accepted, the specific effects of nutrients on the reproductive axis are not well understood. The reproductive axis is coordinated by hypothalamic neurons expressing and secreting gonadotropin-releasing hormone (GnRH). Nutrients such as fatty acids may regulate these neurons; however, the complex organization of the hypothalamus and the dispersed distribution of GnRH neurons make it difficult to study the molecular mechanisms regulating GnRH neuronal cell biology *in vivo*. To circumvent these issues, our lab has generated a novel, immortalized GnRH-synthesizing cell line. This line was generated from primary hypothalamic culture from a 2-month-old, female, transgenic GnRH-GFP mouse. The neurons were immortalized and then subsequently fluorescence-activated cell-sorted to obtain a non-clonal population of GnRH neurons, the mHypoA-GnRH/GFP cell line. Using this model, we studied how docosahexaenoic acid (DHA), an omega-3, polyunsaturated fatty acid with well-documented health benefits, affects *Gnrh1* gene expression. We treated mHypoA-GnRH/GFP cells with 100 μM DHA. Two, four, and eight hours of DHA increased *Gnrh1* gene expression. To explore the potential molecular mechanisms through which DHA upregulated *Gnrh1* gene expression, we used western blot analysis to study the activation of intracellular signaling pathways. DHA can activate the G protein-coupled receptor 120 (GPR120), which is expressed by mHypoA-GnRH/GFP cells, and induce PI3K/Akt and PKC/ERK1/2 signaling. The PKC inhibitor K252c and MKK1/2 inhibitor U0126 blocked DHA-mediated increases in *Gnrh1* gene expression while the PI3K inhibitors wortmannin and LY294002 did not. In addition, the transcriptional inhibitors actinomycin D and DRB blocked DHA-mediated changes in *Gnrh1* gene expression. Using promoter and transcription factor databases, we identified potential downstream, ERK1/2-modulated transcriptional regulators of *Gnrh1*: C/EBPβ, Elk-1, and PPAR-γ. Although the PPAR-γ agonist rosiglitazone increased *Gnrh1* gene expression, the PPAR-γ antagonist T0070907 did not block DHA-mediated changes in *Gnrh1* gene expression. Here, we demonstrate the polyunsaturated, omega-3 fatty acid DHA may have direct regulatory effects on GnRH neurons, suggesting nutritional inputs may affect the reproductive axis at the level of the hypothalamus.

THE SATURATED FATTY ACID PALMITATE REGULATES AGOUTI-RELATED PEPTIDE (AgRP) GENE EXPRESSION AND IMPAIRS INSULIN SIGNALING IN A NEURONAL CELL MODEL DERIVED FROM ADULT MOUSE HYPOTHALAMUS, mHypoA-NPY/GFP

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Obesity results from the loss of balance between food intake and whole body energy expenditure. Recent evidence suggests a role for hypothalamic insulin resistance in obesity pathogenesis, and that obesity-associated hypothalamic inflammation underlies this resistance. This neuroinflammation is mediated, at least in part, through activation of the classic IKKβ/NF-κB inflammatory signaling pathway. Palmitate, a non-esterified saturated fatty acid which exists at high levels in the plasma of obese individuals, has received considerable attention due to its ability to activate TLR4/NF-κB signaling. Indeed, icv injection of palmitate has been shown to attenuate hypothalamic insulin signaling and increase IKKβ activity, which may contribute to the overall function of individual hypothalamic cell types linked to energy homeostasis. We therefore hypothesize that palmitate will induce neuroinflammation, impair neuronal insulin signaling and alter the gene expression of hypothalamic neuropeptides involved in regulating feeding. Using an immortalized, hypothalamic, neuronal cell line expressing the orexigenic neuropeptide Y (NPY) and agouti-related peptide (AgRP), we have studied the effects of palmitate on AgRP gene expression, inflammatory status and neuronal insulin signaling. mHypoA-NPY/GFP neurons were first treated with 25 uM palmitate and qRT-PCR was used to measure AgRP transcript levels over the course of 24 hours. We found that 25 uM palmitate significantly upregulated AgRP mRNA levels at 24 hours, and increased pro-inflammatory gene expression (NF-κB and IκBα) within the first 4 hours of treatment. We then assessed the impact of 24 hour palmitate pre-treatment on neuronal responsiveness to insulin. Using Western Blot analysis, we determined that 24 hours of palmitate pre-treatment significantly decreased insulin-mediated Akt phosphorylation in mHypoA-NPY/GFP neurons. Thus, palmitate induces inflammation, regulates AgRP gene expression and impairs insulin signaling in a specific population of hypothalamic neurons involved in regulating energy homeostasis. This alteration in AgRP gene expression may be linked to increased feeding and obesity.

GAMMA-AMINOBUTYRIC ACID PROTECTS AGAINST OBESITY INDUCED HEPATIC INSULIN RESISTANCE IN HIGH FAT DIET FEED MICE BY INHIBITING KUPFFER CELL ACTIVATION.

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Obesity induced hepatic insulin resistance is a major contributor to the development of type 2 diabetes. The excess accumulation of intrahepatic fats and the subsequent activation of Kupffer cells and production of tumor necrosis factor (TNF)-α is a candidate mechanism for the pathogenesis of this disorder. Potential strategies capable of targeting this underlying inflammation would greatly improve hepatic insulin sensitivity and glucose homeostasis. In our previous studies with diabetic mouse models, we showed gamma-aminobutyric acid (GABA) treatment exerted immune modulatory actions, particularly on inhibiting T-cell activation and reducing the circulating pro-inflammatory cytokine profile. Given GABA's anti-inflammatory capabilities we investigated the effectiveness of oral GABA treatment on protecting against obesity induced hepatic insulin resistance in high fat diet feed mice and also the mechanism by which GABA mediates this effect. Our results demonstrated GABA treatment significantly improved glucose tolerance and insulin sensitivity. Examination of the livers revealed significantly reduced TNF-α positive Kupffer cells and hepatic TNF-α mRNA expression in GABA treated mice. Furthermore, there was evidence of reduced intrahepatic fat deposits and visceral adipose mass. To address the potential mechanism by which GABA exerts is effect on macrophages we treated RAW 264.7 macrophages, a mouse peritoneal macrophage cell line, with palmitate and GABA. Palmitate alone significantly increased TNF-α expression but was attenuated with GABA co-treatment. This effect was observed to be mediated by the down-regulation of toll-like receptor 4 (TLR4), where GABA treatment resulted in significant reductions in TLR4 expression under basal and palmitate-stimulated conditions. Collectively, this study provides strong support for GABA as an effective anti-inflammatory agent capable of protecting against obesity induced hepatic insulin resistance. Given GABA is safe for human consumption it may be beneficial as a potential therapy to improve glucose homeostasis in obesity and an intervention for type 2 diabetes.

THE EFFECTS OF SODIUM SALICYLATE AND METFORMIN ON TUMOUR NECROSIS FACTOR ALPHA INDUCED INFLAMMATION IN A HYPOTHALAMIC NEUROPEPTIDE Y/AGOUTI-RELATED PEPTIDE NEURONAL CELL MODEL

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Neuropeptide Y/Agouti-related peptide (NPY/AgRP) secreting neurons in the hypothalamus act on upstream targets to increase feeding, and decrease energy expenditure. Neuronal inflammation can disrupt the energy regulating abilities of NPY/AgRP neurons by impairing insulin sensitivity, glucose sensing, and secretion of feeding neuropeptides. Genetic ablation of the canonical inflammation pathway can lead to improvements in metabolic illness such as a decrease in food intake and weight loss. We would like to explore the ability of anti-inflammatory reagents in reducing neuronal inflammation in an immortalized NPY/AgRP-expressing cell-line. We hypothesize that the pro-inflammatory cytokine tumour necrosis factor alpha (TNFα), which is induced by high-fat exposure, will activate the canonical inhibitor of IkappaB kinase beta/nuclear factor kappa B (IKK-β/NFκB) pathway in the rHypoE-7 cell-line. Furthermore, pre-treatment with anti-inflammatory reagent sodium salicylate (NaSal) and anti-diabetes drug metformin will alleviate the inflammatory response induced by TNFα exposure. Cells were exposed to 10 ng/mL of TNFα for 4, and 24 h, with or without 1 h pre-treatment of 1 mM NaSal and/or 20 μM metformin. In the rHypoE-7 cell-line, 10 ng/mL of TNFα significantly up-regulated IκBα ($P<0.0001$), interleukin-6 ($P<0.05$), and TNFα ($P<0.05$) mRNA expression at 4 h. 1 h pre-treatment with 1 mM NaSal and/or 20 μM metformin alone did not alleviate the TNFα-induced up-regulation of IκBα mRNA expression. However, co-pretreatment with 1 mM NaSal and 20 μM metformin significantly inhibited the TNFα-induced up-regulation of TNFα mRNA expression at 24 h ($P<0.05$). These results show the activation of the canonical IKK-β/NFκB inflammatory pathway with the pro-inflammatory cytokine TNFα in an NPY/AgRP-expressing cell-line. These data indicate that a combined co-pretreatment with anti-inflammatory drug NaSal and anti-diabetes drug metformin can directly affect TNFα-induced inflammatory at the level of the TNFα gene in NPY/AgRP neurons. Together, these results demonstrate the positive anti-inflammatory effects of combined NaSal and metformin treatment.

ILEAL FATTY ACID SENSING MECHANISMS REGULATE GLUCOSE HOMEOSTASIS *IN VIVO*

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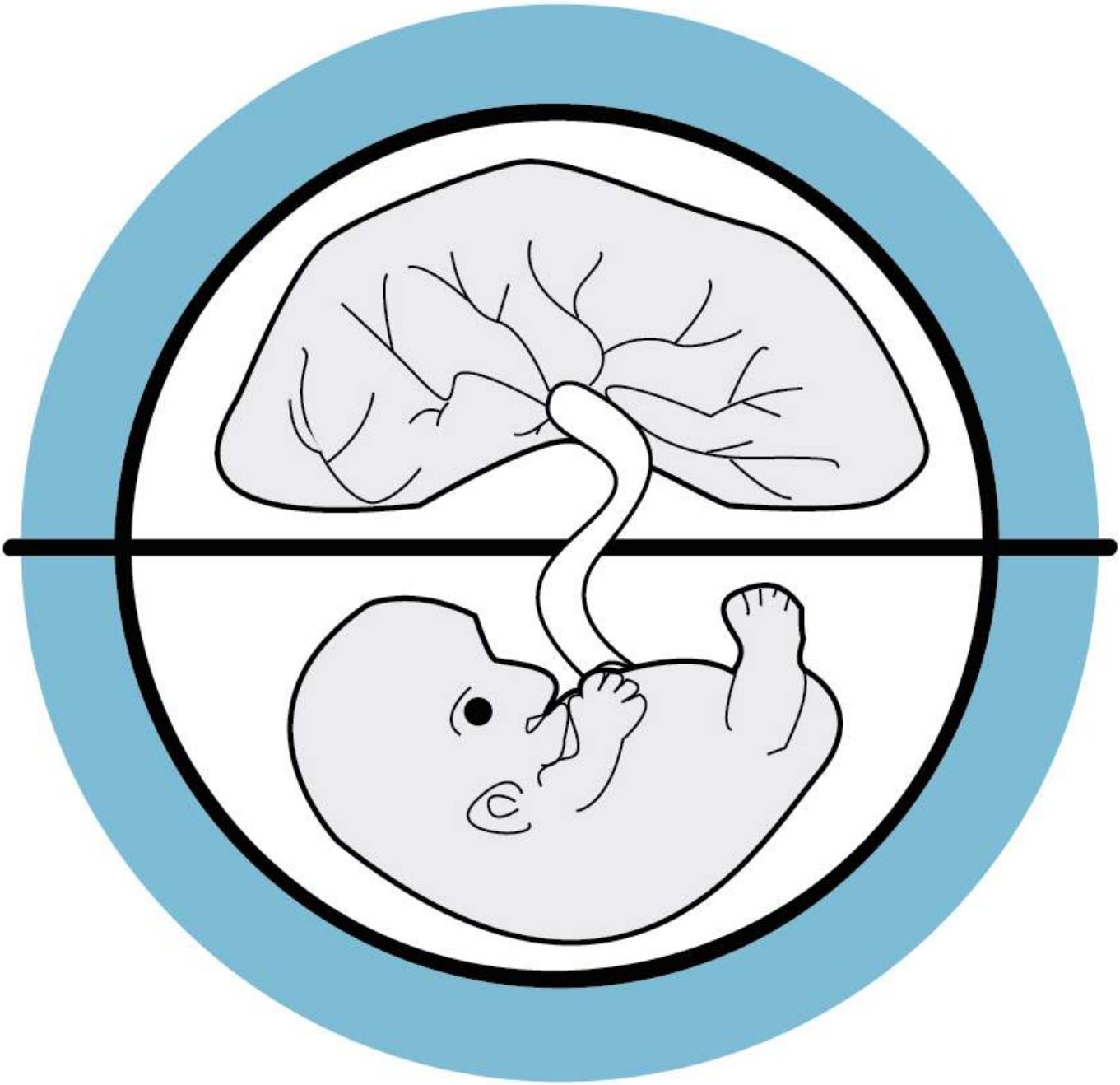
Long-chain-fatty acids (LCFAs) are readily found in the diet, whereas short-chain fatty acids (SCFAs) are byproducts of dietary fibre that are only fermented in the distal intestine. Given that nutrients reach the ileum rapidly within minutes of feeding and lipids in the duodenum lower hepatic glucose production (GP) via a duodenal LCFA-CoA-dependent neuronal network, it remains to be determined if the ileum regulates glucose homeostasis via a conserved sensing mechanism on GP regulation. Further, since the GLP-1 receptor (GLP-1R) is found on the nodose ganglion, which is in close proximity to the site of intestinal GLP-1 secretion (ie induced by LCFA and SCFA), receptor activation and vagal firing may mediate the effects of FAs. Thus, we hypothesize that FAs activate ileal-sensing mechanisms to lower GP via a GLP-1R dependent neuronal network. We first report that intraileal LCFAs: oleic acid (OA), linoleic acid (LA) or SCFA: propionate infusions decreased GP with no changes in glucose uptake during a basal insulin euglycemic clamp. We demonstrated that intestinal LCFA sensing mechanisms are conserved along the small intestine given that the effect of ileal OA and LA was abolished with triacsin-c, an acyl-CoA synthase inhibitor coinfusion, suggesting that LCFA-CoA formation in the ileum, like the duodenum, is required. In contrast, the GP lowering effect of propionate was dependent on ileal GPR43 receptor signaling (as demonstrated by an ileal GPR43 targeted knockdown approach), but not an intracellular mechanism. However, the effects of both LCFA and SCFA infusions were diminished with intraileal coinfusion of either exendin-9, a GLP-1R antagonist, or tetracaine, a local anesthetic. In summary, LCFAs and SCFAs activate an ileal GLP-1R dependent neuronal network to lower GP via ileal LCFA-CoA formation or GPR43 activation, respectively.



REPRODUCTION & DEVELOPMENT POSTERS

JUMONJI C DOMAIN CONTAINING PROTEIN 6 (JMJD6) – A NOVEL REGULATOR OF VHL IN THE HUMAN PLACENTA

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Persistent hypoxia is implicated in the pathogenesis of placental-associated pathologies such as preeclampsia, a disorder complicating 5-7% of all pregnancies and responsible for fetal and maternal morbidity. We previously reported that placentae from severe preeclamptic pregnancies have elevated Hypoxia Inducible Factor 1A (HIF1A), a transcription factor and master regulator of oxygen homeostasis. Emerging evidence implicates Jumonji C domain containing histone demethylases and lysyl hydroxylases as oxygen sensors and regulators of hypoxic gene expression. Our work revealed that JMJD6 is a transcriptional target of *HIF1A* and an indirect regulator of HIF1A stability via its actions on von Hippel-Lindau protein (VHL), a key executor of HIF1A degradation in normoxia. However, the precise mechanism by which JMJD6 regulates VHL remains to be established; hence, in the current study, we investigated this. Recent work has uncovered a growing list of proteins targeted by JMJD6-mediated hydroxylation of lysine residues, impacting on their stability. To understand whether JMJD6 stabilized VHL via lysyl hydroxylation, we exposed JEG3 cells to the lysyl hydroxylase inhibitor, minoxidil. Minoxidil treatment destabilized VHL, which failed to be restored upon JMJD6 overexpression. We next examined whether JMJD6 stabilized VHL by interfering with its degradation. Paradoxically, proteasomal inhibition by Mg132 destabilized VHL. Since protein degradation can alternatively occur via the lysosome, we exposed JEG3 cells to the lysosomal inhibitor, NH_4Cl, and found that inhibition of lysosomal degradation increased VHL. Furthermore, treatment of JEG3 cells with NH_4Cl along with JMJD6 overexpression promoted its nuclear translocation and SUMO1-mediated VHL SUMOylation, a process known to stabilize VHL by maintaining its nuclear expression. Accordingly, minoxidil treatment decreased VHL SUMOylation. In conclusion, our data suggests that JMJD6-mediated lysyl hydroxylation stabilizes VHL by inducing its nuclear SUMOylation, where it is free to degrade HIF1A. Altered JMJD6 function in preeclampsia may indirectly contribute to increased HIF1A stability by decreasing VHL expression. (Supported by CIHR)

PRENATAL GLUCOCORTICOID TREATMENT MATURES THE DEVELOPING BLOOD-BRAIN BARRIER (BBB) AND ALTERS RESPONSIVENESS TO TGF-β1

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Pregnant women at risk of preterm birth are treated with synthetic glucocorticoids(sGC) to reduce risk of respiratory distress syndrome. This treatment coincides with astrocyte differentiation. Transforming growth factor-β1(TGF-β1) is released from differentiating astrocytes and we have shown it to increase activity of the bbb efflux transporter P-glycoprotein(P-gp; abcb1) in fetal brain endothelial cells(BEC). P-gp is important for fetal brain protection against xenobiotics. However, little is known about the impact of prenatal sGC exposure on fetal bbb. Evidence suggests that prenatal sGC exposure matures the bbb by increasing expression tight junction(TJ) proteins. In this study, we hypothesized that in vivo exposure to sGC would mature the fetal bbb and alter responsiveness to TGF-β1. Pregnant guinea pigs were treated with dexamethasone or saline on gestational day(GD) 48 and 49 and euthanized on GD50. BEC from fetal brains were treated with TGF-β1(1-10 ng/ml) for 8h. P-gp activity was assessed using fluorescent substrate assay. TJ function was measured by FITC-dextran tracer. Levels of genes that encode TJ proteins, P-gp and TGF-β1 receptors were quantified by qRT-PCR. Levels of abcb1 and genes that encode TJ proteins were increased in BEC from sGC fetuses compared to those from control fetuses($p<0.05$). TGF-β1 treatment increased P-gp function and abcb1 in BEC from control fetuses($p<0.05$). These BEC also displayed decreased permeability to FITC-dextran and increased expression of genes that encode TJ proteins($p<0.001$). In contrast, these effects were not observed in BEC from sGC fetuses following TGF-β1 stimulation. Further analysis revealed that sGC exposure upregulated TGF-β1 receptors alk1 and tgfbr2 mRNA expression compared to control($p<0.05$). In vivo sGC treatment matures the bbb as it increases P-gp and TJ function. These BEC also display decreased responsiveness to TGF-β1, an effect similar to that seen in postnatal BECs from normal pregnancies. Upregulation of TGF-β1 receptors in BEC from sGC fetuses may be indicative of this decreased sensitivity to TGF-β1. This study has identified novel mechanisms by which antenatal sGC exposure matures the fetal bbb, altering brain protection to xenobiotics, including maternally administered therapeutic drugs.

TRANS-DIFFERENTIATING ADULT SOMATIC CELLS AND DIFFERENTIATING INDUCED PLURIPOTENT STEM CELLS TO SIX2+ RENAL PROGENITORS FOR KIDNEY CELL THERAPY

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Kidney transplantation remains to be the only effective treatment for end-stage kidney disease. Cell therapies are promising treatments but there are currently no effective kidney cell therapies. The challenges that impede the development of effective kidney cell therapies include identification and production of patient-matched renal progenitors that can functionally integrate into the damaged areas. It is known that Six2+ renal progenitors have the ability to self-renew and differentiate into almost all cells of the nephron, and these characteristics make them ideal candidates for kidney cell therapy. Since it is unethical and not sustainable to harvest renal progenitors from developing human fetuses, we are developing protocols to trans-differentiate adult somatic cells and differentiate induced pluripotent stem cells (iPSCs) to Six2+ renal progenitors. Our protocols will allow for the generation of patient-matched progenitors with reduced immunogenicity and improved cell engraftment. Trans-differentiation: we identified and generated doxycycline-inducible expression vectors for three transcription factors and three homeobox genes involved in inducing and maintaining Six2 expression. Overexpression of the six genes in adult tubule cells induced Six2 expression in up to 19% of the cells. Trans-differentiation efficiency was highly dependent on the post-transfection culture conditions as transfected cells plated on collagen IV-coated plates and cultured in the presence of trichostatin A exhibited the highest efficiency. Differentiation: we developed a protocol consisting of three defined media to differentiate embryonic stem cells to Six2+ progenitors. When reseeded onto decellularized kidney scaffolds, these Six2+ progenitors self-organized into tubular structures, which strongly suggest that our protocol generates robust renal progenitors. This differentiation protocol is being applied to blood, urine and fibroblast iPSCs to determine the adaptability of our protocol and whether epigenetic memory influences differentiation efficiency. Finally, we will assess the functionality of the trans-differentiated and differentiated Six2+ progenitors by transplanting them into mouse models of renal ischemia/reperfusion injury.

MULTIGENERATIONAL PROGRAMMING OF ANXIETY RELATED GENE EXPRESSION IN THE PREFRONTAL CORTEX AFTER ANTENATAL GLUCOCORTICOID

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Glucocorticoids are necessary regulators of fetal neural development. Prenatal exposure to excess glucocorticoids is implicated in the development of psychiatric disorders through altered expression of anxiety- and stress-regulatory genes in the prefrontal cortex. Here, we investigated whether prenatal exposure to synthetic glucocorticoids programs transcription of anxiety and stress genes in the prefrontal cortex across two generations following paternal transmission. Pregnant guinea pigs received 3 courses of either betamethasone (Beta; 1mg/kg; n=12) or saline (C; n=11) at 75% of gestation (term ~69 days). Male offspring were mated with naïve females to generate F2 animals. RNA was extracted from the prefrontal cortex of F1 (C n=6, Beta n=4) and F2 females (C n=7, Beta n=5) at PND 40. mRNA levels of stress and anxiety related genes, *Gnb1*, *Fkbp5*, *Gr* and *Mr*, were measured by q-RTPCR. *Mr* expression was significantly reduced in F2 Beta females ($p<0.05$) compared to C; there was a strong trend towards reduced *Mr* in F1 Beta females ($p=0.057$). *Gnb1* expression was significantly reduced in F1 Beta females only ($p<0.05$). There were no differences in the expression of *Fkbp5* and *Gr*. Prenatal exposure to synthetic glucocorticoids resulted in reduced expression of genes associated with anxiety (*Gnb1*) and stress response (*Mr*) in first and second generation juvenile female offspring. These data suggest that antenatal glucocorticoid exposure leads to lasting changes in gene expression in female offspring which can be inherited via paternal transmission.

THE ROLE OF BRCA1 IN MODULATION OF GLUCOCORTICOID RECEPTOR ACTIVITY IN HIGH-GRADE SEROUS OVARIAN CARCINOGENESIS

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A *BRCA1* germline mutation increases a woman's lifetime risk of developing high-grade serous ovarian cancer (HGSOC) up to 43-fold. Increased lifetime ovulatory events also associate with increased risk. Studies in our lab suggest the post-ovulatory pro-inflammatory milieu persists to a greater extent in *BRCA1* mutation carriers compared to control women. Slowed remediation of pro-inflammatory signaling may potentiate accumulation of DNA damage and ultimately promote malignant transformation. Preliminary evidence suggests that glucocorticoid receptor (GR) activity, a major anti-inflammatory mediator, may be modulated by BRCA1. Impairment of GR signaling by *BRCA1* mutation may contribute to increased HGSOC risk. Our objective is to determine if BRCA1 deficiency affects GR signaling in HGSOC. Our results indicate

that GR levels in several ovarian cancer cells examined correlate with BRCA1 levels. GR transcriptional activity was greater in UWB1.289 + *BRCA1* (BRCA1-restored) compared to parental UWB1.289 (BRCA1-null) cells. A similar effect on signaling was also observed with ectopic *GR* expression, indicating a facilitating role of BRCA1 that is independent of an effect of GR protein levels. Our preliminary results indicate that this effect may not involve a BRCA1-GR interaction. Further, dexamethasone treatment decreased IL-1b-induced COX2 expression, an NFkB gene target, in nonmalignant fallopian tube cells but did not affect COX2 levels in UWB1.289 +/- BRCA1 cells. Microarray analysis is currently underway for patient-derived FTE cells and UWB1.289 +/- *BRCA1* to determine the global impact of BRCA1 expression on GR response. Overall, BRCA1 expression correlates with GR levels and enhances transcriptional activity independent of its impact on GR levels. However, preliminary data suggest that BRCA1 may not impact GR transrepression of NFkB in malignant HGSOC cells.

LARGE COHORT PLACENTAL MICROARRAY ANALYSIS REVEALS MULTIPLE DISTINCT SUBCLASSES OF HUMAN PREECLAMPSIA

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Preeclampsia (PE) is a complex, heterogeneous disorder of pregnancy, demonstrating a high degree of variability in observed maternal symptoms and fetal outcomes. We hypothesized that this heterogeneity, leading to a lack of robust predictive biomarkers and effective treatments for this disorder, was due to the existence of multiple molecular forms of PE. To address our hypothesis, we created a large (N=330) placental microarray data set consisting of 7 previously published studies and an additional 157 highly annotated samples drawn from the RCWIH BioBank and representing a range of PE presentations. This combined data set was then subjected to unsupervised multivariate model-based clustering, gene-set enrichment analysis, and correlative analysis between cluster membership and over 200 known clinical attributes. Clustering revealed five distinct molecular groups of placental gene expression, with preeclamptic samples falling into four of these clusters, thus supporting our hypothesis. One of these PE groups was a “canonical” subclass with elevated expression of classic PE phenotypes, such as increased secretion and response to hypoxia, and clinical presentation of lower placental weights and early deliveries. Several novel PE clusters were also observed, including: 1) an immunological response group demonstrating a gradient of fetal growth restriction severity; 2) a subclass with chromosomal abnormalities supported by aCGH analysis; and 3) a possible maternal origin of the pathology associated with normal birth weight and term deliveries. Furthermore, the only non-PE-enriched cluster was predominately composed of preterm “control” samples exhibiting signs of infection. Overall, our large cohort analysis of placental gene expression has successfully identified subtypes of PE patients, and represents significant progress towards understanding the underlying molecular pathways and clinical correlations involved in the formation of these groups. Hopefully, this research will eventually lead to the development and implementation of robust biomarkers and personalized treatments for all PE subclasses.

PDSS2 DEFICIENCY IN OOCYTES NEGATIVELY AFFECTS PREIMPLANTATION EMBRYONIC DEVELOPMENT

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Coenzyme Q (coQ) is a lipophilic antioxidant molecule important for various cellular processes, particularly mitochondrial function. During preliminary studies, the protein expression levels of Pdss2, a subunit of a coQ biosynthetic enzyme called prenyl diphosphate synthase, was found to be decreased in oocytes collected from older female mice when compared to levels seen in oocytes collected from younger mice. Further studies showed that oocytes from Pdss2-deficient mice recapitulated many defects seen in oocytes from older mice, and that these females had difficulty reproducing. The objective of this study was to observe any abnormalities in preimplantation embryo development with Pdss2 deficiency, and to understand, if there are abnormalities, why they occur. Female mice with oocyte-specific deletion of Pdss2 were generated using the Cre-lox system; mice homozygous for the floxed Pdss2 gene, $Pdss2^{loxP/loxP}$, were mated with mice expressing oocyte-specific Cre, ZP3 Cre, to ultimately generate $Pdss2^{loxP/loxP,\ ZP3\ Cre}$ mice. Female $Pdss2^{loxP/loxP,\ ZP3\ Cre}$ mice were primed with gonadotropins PMSG and hCG and mated overnight with wildtype males. Zygotes were collected from plugged females on the day of plug, and incubated to track *in vitro* embryo development or flushed from reproductive tract at day 3.5. The progress of the embryos was noted daily.While 76% of the zygotes collected from the control $Pdss2^{loxP/loxP}$ mice were able to progress to the late blastocyst stage, very few zygotes from the $Pdss2^{loxP/loxP,\ ZP3\ Cre}$ females progressed to the same stage (12 %); many zygotes instead arrested at the 2-to 4-cell stage. While delay/arrest of embryogenesis was also observed in in vivo grown embryos, this phenotype was more dramatic in embryos cultured *in vitro*. Pdss2 deficiency in oocytes negatively affects preimplantation development of the ensuing embryos; this may be due to chromosomal abnormalities within the oocytes at the time of ovulation or due to compromised mitochondrial function.

UTF-1 AS A MARKER FOR HUMAN SPERMATOGONIAL STEM CELLS (SSCs) AND ITS APPLICATION IN THE ASSESSMENT OF TELOMERE LENGTH IN AZOOSPERMIC MEN

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Most cases of non-obstructive azoospermia are currently considered idiopathic. Studies on aging have demonstrated that telomere attrition limits cellular replicative capacity, inducing senescence and apoptosis, while elongated telomeres in human sperm are inherited by offspring. We hypothesize that dysregulation of telomere length during spermatogenesis is responsible for the proliferative arrest observed in some cases of NOA men. The objective of this study was to find a suitable marker for the identification of spermatogonial stem cells (SSCs) in human testicular tissue and use this to co-label SSCs for quantification of telomere length using qFISH. REB approval was obtained for this study. Human orchiectomy tissue donated for research was collected at Mount Sinai Hospital following informed consent. Phenotypically normal tissue was processed with standard immunohistochemistry. To identify SSCs, testicular tissue sections were stained for SSEA4, Sall4, GPR125 and UTF-1. Telomere assessment was performed on tissue sections using qFISH with a telomere-repeat containing PNA probe. Images were captured on a fluorescence microscope and analyzed using Imaris 8.0 software. Our results identified UTF-1 as the most reliable marker for human SSCs based on positive staining within a select population of round cells localized along the basement membrane (BM) of the seminiferous tubules. In contrast, staining from other candidate SSC markers did not highlight a specific population of cells with BM localization. Telomere assessment via qFISH successfully hybridized to all telomeres of all cell types within the tissue. Our findings indicate that UTF-1 is a reliable marker for human SSCs and could be used in combination with qFISH to assess telomere length in SSCs. Our future directions are targeted towards co-localization of UTF-1 with telomere qFISH (i.e. immuno-FISH) to compare telomere lengths in SSCs between normozoospermic and NOA men, in addition to identification of markers for other, more differentiated, spermatogenic cell types.

TGFβ AND IL8 COOPERATE TO POLARIZE DECIDUAL NEUTROPHILS TOWARDS A PRO-ANGIOGENIC PHENOTYPE

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N2 neutrophils are a distinct tumorigenic neutrophil phenotype present in TGFβ-dominant environments which promote progression of cancer via pro-angiogenic mechanisms, among others. Our previous work identified a novel population of neutrophils in 2nd trimester decidua which possess a distinct pro-angiogenic phenotype similar to N2 neutrophils. Together with prior experiments demonstrating that IL-8 can recruit and drive partial differentiation of peripheral blood neutrophils (PBN) to a decidual phenotype, we hypothesized that IL-8 and TGFβ co-operate within the decidua to differentiate infiltrating neutrophils into a phenotype which would aid in the vascular remodelling process. To investigate this hypothesis, PBN isolated from blood of women between 16-18 weeks of gestation were split into four treatment groups: 1) serum-free RPMI; 2) TGFβ + IL8; 3) 2nd trimester decidua-conditioned media (DCM); 4) DCM + IL8 neutralizing antibody + 10uM SB431542 (TGFβ inhibitor). Neutrophils were collected for RNA isolation at 0, 2, 4, and 6 hour timepoints to investigate changes in mRNA expression of angiogenic factors. Changes in functional angiogenic ability were assessed by comparing tube formation of uterine microvascular endothelial cells (UtMVEC) or HTR8 cells following 24h incubation with HL60 cells or PBN having been treated as described above. DCM-treated PBN show significant upregulation of mRNA expression of CXCL2, CXCL3, IL6, IL8, VEGF-A, MMP9, which is partially inhibited by treatment with TGFβ and IL8 inhibitors. Notably, PBN treated with DCM in the presence of TGFβ/IL8 inhibitors demonstrated increased mRNA expression of N1 markers ICAM-1 & TNFα. Functionally, both UtMVEC and HTR8 demonstrated greater tube formation and more advanced networks following incubation with primary neutrophils or HL60 cells pre-treated with DCM or TGFβ/IL8 compared to incubation with SFM-treated or DCM+inhibitor-treated neutrophils. Future experiments will investigate the effect of angiogenic neutrophils on the acquisition of an endothelial phenotype by extravillous trophoblast cells.

THE EFFECT OF PHYSICAL ACTIVITY ON OOCYTE QUALITY AND MITOCHONDRIAL ACTIVITY

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Female fertility is sensitive to maternal energy balance. Maternal energy intake, such as caloric restriction can postpone follicular recruitment for ovulation, while caloric-excess hinders ovulation and oocyte quality. Oocyte quality and

reproductive success are in part regulated by mitochondrial activity, controlling the cellular redox balance and energy availability. This study investigates whether energy expenditure, by moderate-physical activity may improve ovulation, oocyte quality and metabolism. Female ICR mice were exercised (n=9) on a motorized treadmill for 15 weeks (1 hour/3 days/week), and compared to age-matched sedentary controls (n=8). Mice were superovulated, and matured metaphase II oocytes (MII) were retrieved from the oviducts. Moderate-intensity exercised mice had improved oocyte yield when compared to control animals (p=0.0399). MII oocyte quality was microscopically analyzed for fragmentation, chromosomal alignment and markers of DNA damage (γ-H2AX, Cell Signaling). Total mitochondrial content of MII oocytes was greater in exercised animals (p<0.001), with a greater proportion of respiring mitochondria (p=0.028). The oocytes were probed for total and respiring mitochondria (Mitotracker Green/Red, respectively) and outputs of oxidative phosphorylation: ATP, superoxide, and FAD. While there was no difference in ATP and superoxide production; oxides FAD was significantly lower in exercised animals (p=0.0068). There was no difference in proportion of oocytes with chromosomal misalignment or markers of DNA damage. MII oocytes represent the fertilization-competent egg pool, which was increased with physical activity. The increase of total and respiring mitochondria in physically active mice suggest that cell types other than muscle and fat can respond to exercise and could modulate oocyte quality.

MICRORNAs GOVERN TROPHOBLAST STEM CELL FATE VIA REPRESSION OF EMBRYONIC GENE REGULATORY NETWORKS

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The first cell fate specification resulting in the emergence of trophoblast and embryonic stem cells (TSCs, ESCs) occurs as a consequence of an intricate balance between transcription factor regulatory networks. However, cell fate regulation may also occur post transcriptionally. We provide evidence of microRNA involvement in the commitment of TSCs versus the simultaneously specified embryonic lineage. High throughput analyses of ESC transcriptome and TSC microRNAome data was performed to identify TSC-enriched miRNAs targeting the ESC pluripotency gene network. miRNAs were cloned into doxycycline-inducible vectors and introduced to naïve ESCs. Transgenic ESCs were characterized by fluorescence microscopy, flow cytometry and miRNA-Taqman qPCR. miRNAs were induced for 6 days in the presence of an HDAC2 inhibitor and morphology was evaluated. Gene and protein expression was assessed by qPCR, microarray, and western blots respectively. We identified 3 TSC-miRNAs predicted to target 5 mRNAs essential to ESC lineage identity. Ectopic expression of these miRNAs in ESCs caused a downregulation of pluripotency genes and their corresponding proteins, accompanied with an upregulation of TSC markers Cdx2 and Elf5. Upregulated genes were strongly enriched in gene ontology terms associated with placenta/trophoblast development. Moreover, we observed multiple pathways required for sustaining ESC and TSC fate were affected by upregulation of these miRNAs, including signaling and epigenetics. Principle component analysis reveals a unifying trend, as all miRNA-induced lines and a Cdx2-iTSC line more closely resemble naïve TSCs rather then ESCs. Lastly, these lines are stable and self-renewing as they retain geno- and phenotypic resemblance to TSCs even following removal of the transgene-inducing agent and passaging. Using this stem cell conversion model, we show that TSCs contain miRNAs targeting genes essential for sustaining ESC pluripotency, but also that expression of these miRNAs in ESCs is sufficient to drive trans-differentiation to TS-like cells. We are assessing in vivo potential and performing AGO-ChIP and CRISPR-mediated deletions of the miRNAs. Lastly, RNA-seq is being performed on miR15b and Cdx2- lines to elucidate pathways leading to transcription factor- and microRNA- mediated conversion.

PATIENT TO PATIENT CHARACTERIZATION OF HUMAN UMBILICAL CORD MESENCHYMAL STROMAL CELLS.

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Mesenchymal stromal cells (MSCs) were first identified as plastic adherent fibroblast like-cells from the bone marrow. MSCs have gained wide popularity due to their multi-lineage potential and paracrine secretion capabilities. Their therapeutic potential has been shown in various human diseases. However, there is a large variability in MSCs from different sources. It has been suggested that expression of some markers are altered with increasing passage number, while other studies indicate that MSC phenotype does not change. There is also a lack of definitive markers to predict the *in vivo* efficacy of cell cultures. Hence, MSCs from 50 umbilical cord samples were phenotypically characterized by flow cytometry for standard MSC marker expression, at multiple passages. The expression levels of TSG-6 were also examined, as TSG-6 is a protein that is a modulator of inflammation and that as been suggested as a biomarker which may be used to determine the therapeutic ability of the cells. MSCs from umbilical cord samples exhibited a characteristic mesenchymal phenotype, expressing CD44, CD73, CD90, and CD105, but were negative for hematopoietic markers CD34, CD11b, CD19, CD45, HLA-DR. CD105 surface expression was consistently lower when compared with CD73, CD90 and CD44. These findings are in accordance with other studies that have been done on MSCs from other sources, such as umbilical cord blood, umbilical

cord MSCs and BM-MSCs. However, other studies have indicated that CD73, CD90 and CD105 are simultaneously expressed at P1 but decrease slightly until P11. Although there was no significant difference among surface marker expression in our study, TSG-6 analysis with Real-Time PCR indicated that UC-MSCs express distinctively diverse levels of this protein. Since other reports have also shown that BM-MSCs differentially express TSG-6, future experiments will quantitate cytokine secretion differences among samples expressing high vs. low levels of this gene, and determine if there is a wide variation in wound healing ability among these samples in a db/db mouse model.

LUNG TISSUE ENGINEERING: USING NATURE'S PLATFORM TO DRIVE DIFFERENTIATION OF EMBRYONIC STEM CELLS TO FUNCTIONAL LUNG EPITHELIAL CELLS

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The interaction of stem cells with the surrounding matrix microenvironment is crucial during embryogenesis and lineage specification. Cellular processes such as proliferation, adherence, spatial arrangement, and differentiation are strongly influenced by cell-matrix signaling. Development of a matrix platform that can recapitulate the *in vivo* environment is a key component to promoting efficient differentiation of pluripotent cells into lung endoderm precursors and ultimately functional, transplantable segments of lung tissue *in vitro*. Herein we demonstrate that isolation of lung extracellular matrix (ECM) by decellularization provides a viable platform for directing the differentiation of seeded embryonic stem cells (ESC) into functional lung epithelial cells. Rat cadaveric lungs were decellularized by sequential tracheal lavages and retrograde pulmonary arterial perfusion using a range of physical, chemical, and enzymatic treatments. Murine ESC were then seeded onto scaffolds following endoderm induction using activin, and cultured at air-liquid interface, in serum free conditions for the duration of three weeks. Cells were cultured with and without supplementation of growth factors. Seeded stem cells express the earliest known lung lineage marker NKX2-1 and adopt an epithelial-like tubular organization. Cells further differentiate to specialized epithelial cell types found in the trachea and bronchioles (club cells, ciliated cells, basal cells) with continued culture on scaffolds. With the addition of specific fibroblast growth factors, retinoic acid, and dexamethasone, at distinct intervals, differentiation to a more distal cell population (alveolar regions) is observed. This work demonstrates that decellularized lung scaffolds effectively recapitulate the lung developmental milieu by supporting the adherence, proliferation, and differentiation of murine ESC into mature populations of lung epithelia. Current work is focused on further maturation of cell-matrix constructs for transplantation purposes.

THE ROLE OF ACID SPHINGOMYELINASE IN MURINE PLACENTATION

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Sphingolipids, a ubiquitous class of membrane lipids, have emerged as important signaling molecules carrying out a myriad of critical cellular functions. Recent findings have implicated sphingolipids as key factors essential for successful pregnancy and embryo development where a disruption in sphingolipid production or metabolism results in early embryonic lethality. Interestingly, the functional role of sphingolipids in placental development has yet to be fully elucidated. Acid sphingomyelinase (aSMase), encoded by the gene *Smpd1,* is an enzyme responsible for the hydrolysis of sphingomyelin into ceramide – the central precursor in sphingolipid metabolism. Best known for its role in the human lysosomal storage disorder Niemann-Pick types A and B, there have been no studies published on aSMase and its role in murine placental differentiation and function. My objective is to investigate how aSMase regulates optimal placental development in the mouse.

When fetal and maternal phenotypes of *Smpd1*-deficient (KO) females during pregnancy were analyzed, we observed a reduction of fetal weights (by ~20%) in KO fetuses carried by KO mothers when compared to WT fetuses carried by WT mothers. This was accompanied by an increased number of resorptions (litter size of ~8 WT pups/~5 KO pups) despite ovulation of comparable number of oocytes. Microscopic inspection of mutant placentas revealed a reduced labyrinthine region (*i.e.,* the site of maternal/fetal exchange) with an increased interhemal distance. Maternal casts also revealed aberrant spiral artery architecture in pregnant KO females. Overall our results reveal an abnormal placental phenotype as well as impaired fetal growth in mice deficient in aSMase.

THE EFFECT OF ORAL *LACTOBACILLUS RHAMNOSUS,* GR-1® AND *LACTOBACILLUS REUTERI,* RC-14® ON THE VAGINAL MICROBIOTA AND CERVICO-VAGINAL CYTOKINES IN LOW RISK PREGANT WOMEN

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Bacterial vaginosis (BV) is characterized by a decrease in lactobacilli and an overgrowth of facultative anaerobic bacteria. Probiotic *L. rhamnosus* GR -1 (GR-1) and *L. reuteri* RC-14 (RC-14) reduces BV recurrence and restores the indigenous lactobacilli in non-pregnant women. We hypothesized that oral GR-1/RC-14 will reverse the abnormal Nugent score of pregnant women to a normal Nugent score, and will modulate the vaginal microbiota and cervicovaginal cytokines across pregnancy. Pregnant women (n=86) with a Nugent score≥ 4 at 13 weeks gestation were randomized to receive 2 capsules orally of either GR-1/RC-14 ($5x10^9$ viable cells) or placebo for 12 weeks. Vaginal swabs were collected at 13, 28 and 35 weeks gestation. Vaginal microbiota were analyzed by sequencing the V6 region of 16S rRNA, and 27 cytokines were measured with a multiplex assay. Significance was assessed with unpaired student's t test, Chi-square test, Two-Way RM ANOVA with Holm Sidak test or Generalized Estimation Equation. *L. iners, L. crispatus, Gardnerella vaginalis* and *Atopobium vaginae* were the most abundant species out of the 93 detected species at 13 weeks. The Nugent score returned to normal in 30% of the women in both the probiotic and placebo groups by 28 weeks. The relative abundance of 26 species including several *Lactobacillus spp*, *G. vaginalis* and *A. vaginae* decreased in both groups at 28 and 35 weeks, when compared to 13 weeks gestation. In contrast, 21 species increased variably with advancing gestation. The anti-inflammatory cytokines IL-4 (placebo group) and IL-10 (probiotic and placebo groups) were increased at 28 weeks, but not at 35 weeks. There was no difference in the vaginal microbiota and cervico-vaginal cytokines between the probiotic and placebo groups. The vaginal microbiota and anti-inflammatory cervico-vaginal cytokines are dynamic across pregnancy. Oral probiotic GR-1/ RC-14 at $5x10^9$ viable cells twice daily for 12 weeks does not alter the gestational age changes.

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