UNIVERSITY OF TORONTO

FRONTIERS IN PHYSIOLOGY

43RD ANNUAL CONFERENCE
BAHEN, UNIVERSITY OF TORONTO

2023

PROGRAM

FRONTIERS IN PHYSIOLOGY SCHEDULE

43RD ANNUAL CONFERENCE
BAHEN, UNIVERSITY OF TORONTO

UNIVERSITY OF TORONTO

MORNING

8:00 8:40	REGISTRATION AND BREAKFAST Welcome Gifts, Lanyards	Atrium
8:40 8:50	WELCOME ADDRESS Chair's and VPs Welcome	BA1160
8:50 10:00	ORAL SESSION 1 5 Presentations	BA1160
10:00 10:10	BREAK Coffee & Tea Refreshments	
10:10 11:00	POSTER SESSION 1 25 Presentations	Atrium
11:00 11:10	BREAK Coffee & Tea Refreshments	Atrium
11:10 12:20	ORAL SESSION 2 5 Presentations	BA1160
12:20 12:50	3 MINUTE THESIS 5 Presentations	BA1160

LUNCH 12:50 1:30

AFTERNOON

1:30 2:50	KEYNOTE PRESENTATION Karin Bornfeldt, PhD	BA1160
2:50 3:00	DEPARTMENT PHOTO Department of Physiology	Courtyard
3:00 3:10	BREAK Coffee & Tea Refreshments	
3:10 4:00	POSTER SESSION 2 26 Poster Presentations	Atrium
4:00 4:10	BREAK Coffee & Tea Refreshments	
4:10 5:30	ORAL SESSION 3 6 Presentations	BA1160
5:30 6:30	RECEPTION Presentation & TA Awards Presented Raffle Winners Platform Updates RECEPTION AT MYHAL ATRIUM	BA1160 & MYHAL

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WELCOME ADDRESS

Dear esteemed colleagues, students, and faculty,

We are delighted to welcome you to the University of Toronto for the Frontiers in Physiology conference. We extend our warmest greetings and offer a heartfelt welcome to each participant who has chosen to join us in advancing the boundaries of our understanding in science.

Our gathering epitomizes the shared interest and collective will to engage, learn, and further deepen our knowledge in the field of physiology. This conference is not just a meeting, but a place to grow, innovate and pave the path for future scientific breakthroughs.

This year, we are privileged to include a variety of features in our program: student poster presentations that highlight the work of our upcoming scientists, oral presentations by researchers, and a special keynote address by none other than the distinguished Professor Karin Bornfeldt. Professor Bornfeldt's transformative work in the field of cardiovascular physiology and metabolism is a testament to the power of innovation and relentless inquiry.

These presentations and discussions embody the spirit of our conference: that by sharing our insights and discoveries, we can collectively push the frontiers of physiology, leading to new understandings, treatments, and therapies. As we navigate through these sessions, let us appreciate the value of diverse perspectives, respect the essence of scientific inquiry, and embody the spirit of collaboration and unity.

The Frontiers in Physiology conference is a forum that believes in the value of every contribution. We are certain that the experiences, knowledge, and discussions shared today will inspire new insights and fruitful collaborations.

We hope that this conference will not only provide you with an opportunity to share your research but also help you build a network with other enthusiasts in the field. In the spirit of the conference, we also encourage you to explore our great city of Toronto, a place that embodies diversity, creativity, and innovation.

We thank you for your participation and wish you a fulfilling and stimulating conference experience. Thank you to our Chair, Dr. Scott Heximer, the Graduate Coordinators, Drs. Zhong-Ping Feng, Anthony Gramolini, and Helen Miliotis, and administrative staff Paula Smellie, Jenny Katsoulakos, Rosalie Pang, Yeonkyung Namkoong, Julia Tausch, Justin Kim, and their colleagues. Thank you to all of those who generously volunteered their time to judge abstracts and oral and poster presentations. We appreciate all of the trainees involved in FIP, from attendees to presenters, thank you for sharing your work and contributing to the success of FIP.

Welcome to the Frontiers in Physiology conference, and welcome to the University of Toronto.

Alicia Gibbs, Radu Gugustea, Delphine Ji
FIP Coordinators
University of Toronto

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KARIN E. BORNFELDT, PHD

Dr. Karin Bornfeldt, a highly esteemed figure in the scientific community, is renowned for her groundbreaking contributions to the fields of vascular biology, diabetes, and its complications. She is a recognized thought-leader whose tireless pursuit of understanding the intricate mechanisms behind accelerated vascular disease in diabetes has been a cornerstone in the field.

Currently serving as a Professor of Medicine and Biochemistry at the University of Washington, Dr. Bornfeldt oversees research endeavors that focus on the nexus between glucose, lipids, and inflammation and how they interact to affect blood vessels in people with diabetes and metabolic syndrome. Her work has not only advanced our understanding of these complex interactions but has also paved the way for potential therapeutic strategies that could benefit millions of people worldwide.

Prior to her tenure at the University of Washington, Dr. Bornfeldt's academic journey was marked with significant milestones. Earning her Ph.D. in Experimental Medicine from Lund University in Sweden, she has spent years building upon her expertise and advancing our understanding of diabetes-related vascular complications.

Dr. Bornfeldt's professional dedication is reflected in her impressive list of published works, with numerous peer-reviewed articles to her name. Her research has been met with international acclaim, earning her a place amongst the elected members of the prestigious Association of American Physicians.

We are truly honored to welcome Dr. Karin Bornfeldt to our conference. We look forward to her presentation, where her deep well of knowledge, combined with her knack for effective communication, is certain to provide not only valuable insights but also stimulate thought-provoking discussions among our attendees.

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SCOTT HEXIMER, PHD

As Chair of the Department of Physiology, it brings me immense joy to extend a warm welcome to all participants at our 43rd annual Frontiers in Physiology (FIP) Conference. This event, driven by the commendable leadership and dedication of our graduate trainees for the past 43 years, serves to spotlight their remarkable research endeavors.

Our day ahead is brimming with presentations that illustrate the innovative work undertaken by our trainees, who are shaping the future of Physiology in Canada and worldwide. Their unwavering commitment to science and research is a testament to their achievements, of which we are immensely proud.

We are also highly honored to welcome Dr. Karin Bornfeldt to the Department of Physiology. A distinguished biochemist and researcher, Dr. Bornfeldt has made significant strides in understanding the complexities of diabetes and its vascular complications. Her pioneering work at the University of Washington is transforming our understanding of these diseases and has won her international acclaim.

A noteworthy mention must be made of Alicia Gibbs, Radu Gugustea, and Delphine Ji, the diligent FIP co-Chairs and Vice-Presidents of the Graduate Association of Students in Physiology (GASP). Their exceptional efforts have resulted in the stellar program we have lined up today. A special note of thanks to Ms. Raina Ladha, GASP President, and everyone else who has tirelessly worked behind the scenes to bring this year's research day to fruition.

As we embark on a day filled with insightful presentations and productive discussions, I urge you to seize this unique opportunity for cross-platform engagement and collaboration within our extraordinary scientific community.

Thank you for contributing to the success of this event. We hope you enjoy the day and look forward to the stimulating exchange of ideas that lies ahead.

Best regards,

Scot P. Heximer, PhD
Ernest B. and Leonard B. Smith Chair
Department of Physiology
Temerty Faculty of Medicine
University of Toronto

ORAL SESSION 1

8:50 - 10:00 AM BA1160

O1-1: Contribution of White Matter Disruption to Behavioural Problems After Concussion in Children

Eman Nishat

O1-2: Modelling Murine Kidney Fibrosis Utilizing an Ex-vivo Organ Perfusion System

Jorge Castillo

O1-3: Proximate effects of infant lower respiratory tract infections on lung and immune dysfunction: findings from the CHILD Study

Maria Medeleanu

O1-4: Postpartum maladaptations in wound healing and metabolism implicated in development of type 2 diabetes after a gestational diabetes pregnancy

Julie Van

O1-5: Finding the neural basis of memory integration

Ying Wang

P1-1: Argon Inhalation: A Novel Treatment for Neonatal Sepsis

Felicia Balsamo

P1-2: A novel contrast method for cerebral imaging: Transient hypoxia-induced deoxyhemoglobin

Ecesu Sayin

P1-3: Establishing a novel microRNA signature to mitigate Uremic Cardiomyopathy in patients with End Stage Renal Disease.

Carmine Alberga

P1-4: Astrocytic α4GABAA receptors are critical for the anesthetic-induced persistent increase in tonic inhibition in hippocampal neurons

Li Ju

P1-5: Upper small intestinal protein sensing mechanism in feeding and glucose regulation

Daniel Barros

P1-6: Upper small intestinal protein sensing mechanism in feeding and glucose regulation

Joseph Park

P1-7: Effects of ketamine enantiomers and their HNK metabolites on hippocampal synaptic transmission and plasticity
Muchun Han

P1-8: Nephrectomy impacts physiological mechanisms that support rat brain oxygenation following acute hemodilution
Kyle Chin

P1-9: Breakdown of the apical spectrin network leads to endothelial dysfunction and vascular stiffening in pulmonary arterial hypertension
Sonja Sulstarova

P1-10: Top-down control of human motor thalamic neuronal activity during the auditory oddball task
Frhan Alanazi

P1-11: Targeting NINJ1-mediated plasma membrane rupture to prevent tumour lysis syndrome
Keane Fuerte

P1-12: Developmental changes in the brain networks supporting continuous speech processing
Kristen Li

P1-13: Effect of dexamethasone on expression and activity of P-glycoprotein and breast cancer resistance protein in human fetal brain endothelial cells
Nikola Ivanovski

P1-14: Regulation of TGF-β Signalling and its Impact on Energy Homeostasis in Hypothalamic Neuro

Aws Mustafa

P1-15: Elucidating NINJ1 protein-protein interactions

Suhel Patel

P1-16: Identifying the expression of CEPT- 1 enzyme/gene in pancreatic islets and its role in the development of type 2 diabetes

Yasmin Rabiee

P1-17: The Effect of Prenatal THC and CBD Exposure on Neuroanatomy and Behaviour in the Adult Rodent

Megan Drupais

P1-18: Investigating the role of key sarco(ends)plasmic reticulum shaping proteins on cardiomyocyte structure and development

Kateleen Jia

P1-19: Ex vivo perfusion of the native and humanized murine pancreas to model Type 1 Diabetes.

Marwa Sadat

P1-20: Conserved early upregulation of prefrontal cholinergic signalling across different species and models of Alzheimer's disease

Saige Power

P1-21: Associating ion channel alternative splicing with neuronal intrinsic electrophysiological properties using patch-seq
Nuo Xu

P1-22: Identifying novel intracellular interactions driving membrane-bound tumour necrosis factor reverse signalling
Yasaman Mostafaie

P1-23: Upper Small Intestinal Lipid Sensing in Feeding Regulation
Rachel Kuah

P1-24 The Effect of Prenatal THC and CBD Exposure on Neuroanatomy and Behaviour in the Adult Rodent
Ameer Sarwar

P1-25: Characterization of a common CERS2 polymorphism and links to type 2 diabetes development
Wenyuan He

P1-26: Static Uterine Stretch Polarizes Peripheral Human Monocytes into M1 Macrophage in Preparation for Labor
Adam Boros

ORAL SESSION 2

11:10 AM - 12:20 PM BA1160

O2-1: NINJ1 forms large pore-like structures within the plasma membrane during pyroptosis

Jazlyn Borges

O2-2: Macro-molecular Protein Complex of Voltage dependent Calcium Channel Gating Neurotransmitter Release in Central Synapses

Rayan Saghian

O2-3: Optimizing a pumped artificial placenta circuit in a swine model of the extremely preterm human fetus

Alex Charest-Pekeski

O2-4: Large-scale Proteomic Analysis Of Caltubin-dependent Pathways Promoting Long-term Memory Formation

Julia Bandura

O2-5: Mapping human placental cell lineages via transcriptome analysis

Young June Kim

3 MINUTE THESIS

12:20 - 12:50 PM BA1160

3MT-1: How Our Brain Cells Help Us Move: The Painful Truth

Nikhil Asapu

3MT-2: Comparison of diffusion MRI techniques in detecting microscopic brain damage

Peter Liu

3MT-3: What's poppin: molecular determinants of synapse diversity in the mammalian brain

Raphael Chan

3MT-4: Developmental changes in the brain networks supporting continuous speech processing

Kristen Li

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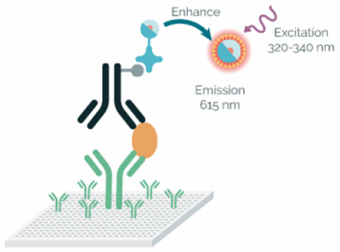
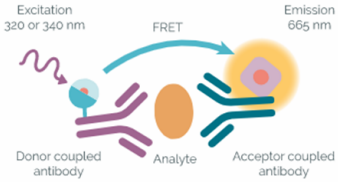
TRF



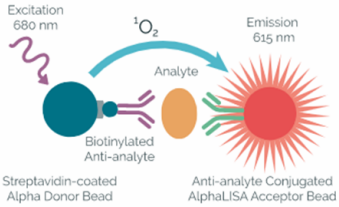
Time Resolved Fluorescence

TR-FRET



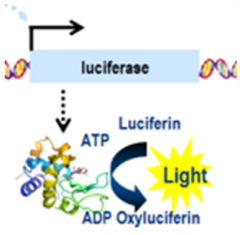
Time Resolved
Fluorescence Resonance Energy Transfer

ALPHA



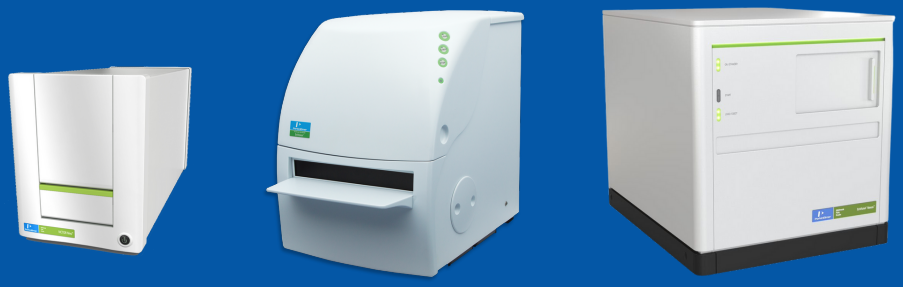
Amplified Luminescent
Proximity Homogeneous Assay

Luminescence



Luciferin-Luciferase
"Lites"

Wash ← → No Wash



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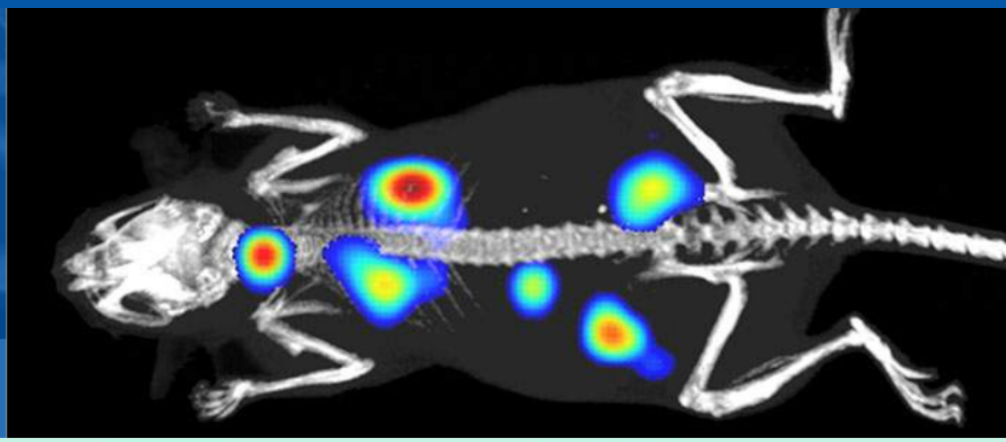

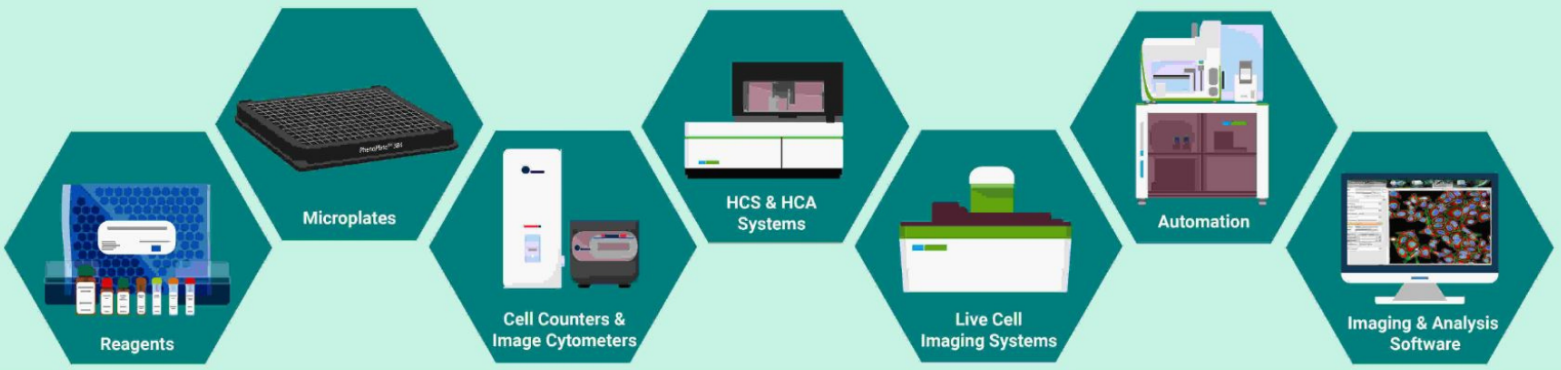

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P2-1: An ex-vivo perfusion method for the mouse uterus
Yvonne Ping

P2-2: A novel peptide to prevent excessive cell-surface expression of α5GABAA receptors in mouse hippocampal neurons
MeiFeng Yu

P2-3: Investigating the potential role of purine signalling in the modulation of NPY by a plant phytohormone.
Calvin Lieu

P2-4: ACELLULAR ex vivo lung perfusion silences pro-inflammatory pathways in human lung endothelial and epithelial cells
Jamie Jeon

P2-5: The role of GABAA receptor overactivity in cognitive impairment after surgery
Joycelyn Ba

P2-6: Using whole decellularized mouse kidney scaffolds to direct human endothelial progenitor cells towards a mature and functional vasculature in an ex vivo culture system
Anupama Bhadwal

P2-7: Role of AMPA glutamate receptors in hippocampal synaptic plasticity and neuronal activation during learning and memory.
Radu Gugustea

P2-8: Nasal microbiome in infancy is differentially abundant in children who later develop respiratory disease
Yu Chen Qian

P2-9: Comparison of different sample preparation methods for plasma peptide profiling
Yihan (Lucia) Luo

P2-10: GLP-1R is required for resveratrol in exerting its metabolic beneficial effect in HFD challenged male mice
Jia Nuo Feng

P2-11: Mechanism of Rac1 in Contributing to LTP and Social Memory Impairments in Alzheimer's Disease
Haorui Zhang

P2-12: Keeping a native pig kidney alive as long as possible in the ex vivo perfusion system by optimizing oxygenation
Chun Tat Lui

P2-13: Temporal dynamics of neuronal excitability in the lateral amygdala mediates allocation to an engram supporting conditioned fear memory
Annelies Hoorn

P2-14: Investigating aT-Catenin Phosphorylation and Evaluating its Cardioprotective Effects in Heart Failure

Daniel Davoudpour

P2-15: Neural entrainment of a naturalistic conversation in varying working memory loads

Priyanka Prince

P2-16: Molecular mechanisms driving maternal cardiac hypertrophy

Zi Qi (Jessica) Lin

P2-17: Developmental defects in nanoscale reorganization of AMPARs and quantal transmission in a mouse model of fragile X syndrome

Maria Gurma

P2-18: Metabolic Characterization of a Common CERS2 Polymorphism and its Risks for Type 2 Diabetes Development

Wenyue Ye

P2-19: Inflammasome Activation in Pulmonary Arterial Hypertension: The Role of Gasdermin D

Anna Foley

P2-20: Regulation of neuronal development by a two-pore potassium channel

Neeraja Ramakrishnan

P2-21: Biopsychosocial subtypes of paediatric concussion predict behavioural impairments
Prashanth Velayudhan

P2-22: Confocal and Mass Spectrometry-based Investigation of REEP5 Depletion by AAV9 in the Mouse Heart
Michelle Di Paola

P2-23: Delineating the potent appetite suppressant effects of a novel phytohormone and its mechanism of action in defined hypothalamic neurons
Cindy Zhang

P2-24 Automatic synthesis of astrocytic trees in silico through generative modeling
Zhenyang Sun

P2-25: Optical interrogation of cholinergic and glutamatergic pre-motor inputs to a medullary circuit essential to rhythmic breathing
Raina Ladha

ORAL SESSION 3

4:10 - 5:30 PM BA1160

O3-1: Elucidating heterogeneity between left and right ventricle-derived cardiac fibroblasts

Michael Dewar

O3-2: Activation of Arcuate nucleus Glucagon-like Peptide-1 receptor-expressing neurons regulates energy homeostasis

Ishnoor Singh

O3-3: Breaking Through the Defense: Biofilm Disruption Using Alveolar-Like Macrophages Expressing a Psl-Glycoside Hydrolase

Sajad Sadat

O3-4: Latent transforming growth factor binding protein - 2 deficiency improves cardiac function post myocardial infarction

Fahad Ehsan

O3-5: Faceted impact of Ω3-polyunsaturated fatty acids on Kv1.2 channels and inhibitory neurotransmission

Tian Kong

O3-6: Engram specific synaptic potentiation is important for fear memory formation and expression in vivo

Matteo Saderi

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2023

ABSTRACTS

O1-1: Contribution of White Matter Disruption to Behavioural Problems After Concussion in Children

Eman Nishat 1, 2; Shannon Scratch 3, 4, 5; Stephanie H Ameis 6,7; Anne L Wheeler 1, 2
1 Department of Physiology, Temerty Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada
2 Neurosciences and Mental Health, The Hospital for Sick Children, Toronto, Ontario, Canada
3 Department of Paediatrics, Temerty Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada
4 Rehabilitation Sciences Institute, Temerty Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada
5 Bloorview Research Institute, Holland Bloorview Kids Rehabilitation Hospital, Toronto, Ontario, Canada
6 Department of Psychiatry, Temerty Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada
7 Cundill Centre for Child and Youth Depression, Margaret and Wallace McCain Centre for Child, Youth and Family Mental Health, Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, Toronto, Ontario, Canada

Background: Many children with concussion exhibit long-lasting externalizing (e.g., aggression, inattention) and internalizing behaviors (e.g., anxiety, depression), with greater rates of persistent problems in females. Establishing the contribution of (1) pre-existing behavioral problems and (2) injury to the brain's vulnerable white matter to long-lasting behavioral problems has been a challenge due to a lack of pre-injury behavioral and imaging data.
Methods: From the Adolescent Brain Cognitive Development Study, we examined 206 11-12- year-old children who experienced a concussion within two years after baseline data collection. Behavioral problems were assessed with the Child Behavior Checklist. In 100 of these children, white matter microstructure was characterized by neurite density (ND) from restriction spectrum modeling of diffusion MRI. Linear regression modeled the contribution of (1) pre-injury behavior and (2) pre- to post-injury change in ND to post-injury behavior.
Results: When controlling for pre-injury scores, post-injury internalizing scores were higher in female but not male children with concussion compared to children with no concussion history (interaction: t=-1.9, p=.061; F: t=-2.8, p=.005; M: t=-0.52, p=.601). Externalizing scores were higher post-injury in both sexes with concussion (t=2.2, p=.025). Younger children with concussion had less maturational change in ND pre- to post-injury in superficial white matter (t=2.3, p=.021). Less change in ND was associated with higher internalizing scores in females only (interaction: t=2.4, p=.021; F: t=-2.1, p=.044; M: t=0.98, p=.332).
Discussion: Concussions elicit behavior problems beyond those that exist pre-injury. Injury to the brain's vulnerable white matter may be a biological substrate for long-lasting internalizing behaviors in females.

O1-2: Modelling Murine Kidney Fibrosis Utilizing an Ex-vivo Organ Perfusion System

Jorge Castillo-Prado 1,2 , Anupama Bhadwal 1,2 , Ian Rogers 1,2,3,4
1 Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto ON M5G 1X5, Canada
2 Department of Physiology, University of Toronto, Toronto ON M5S 1A8, Canada
3 Department of Obstetrics and Gynecology, Mount Sinai Hospital and the University of Toronto, Toronto ON M5G 1E2, Canada
4 Soham & Shaila Ajmera Family Transplant Centre, University Health Network, Toronto, ON, M5G 2C4, Canada

Chronic kidney disease (CKD) affects 11-13% of the population worldwide. A hallmark of CKD is fibrosis which exacerbates kidney dysfunction and is linked to increased mortality. Animal models of fibrosis involve invasive procedures that can affect the animal's health and experiment results. Also, disease stages are missed since the organs of interest cannot be analyzed without invasive techniques. Ex-vivo whole-organ perfusion (EVOP) provides a solution by enabling real-time, longitudinal analysis at the whole-organ level. Also, controlled culture conditions lead to reduced alterations between experiments. We hypothesize that a mouse kidney can be maintained using EVOP for 14 days and that renal fibrosis can be reproduced in an EVOP system through ureteric obstruction (UO) and cisplatin-induced nephrotoxicity.

Following kidney isolation and renal artery canulation, kidneys were cultured using EVOP for 4, 7, and 14 days. Kidneys were also subjected to UO and cisplatin administration to induce fibrosis and were then cultured ex-vivo for 4 and 7 days. We determined that tissue viability and proper marker expression (nephrin, LTL, AQP2) were sustained for up to 7 days. We determined that our system meets the oxygen demand of a mouse kidney. Urinalysis demonstrated proper urine protein and glucose content. Preliminary results revealed higher amounts of collagen deposits present in UO and cisplatin kidneys at days 4 and 7, indicating fibrosis development. Future steps include optimizing the culture medium to extend EVOP times to 14 days. We will also analyze the gene expression of control and fibrotic kidneys to help improve the EVOP
model.

O1-3: Developmental defects in nanoscale reorganization of AMPARs and quantal transmission in a mouse model of fragile X syndrome

Maria Gurma 1,2 , Ankur Bodalia 2 , Adam Fekete 2 , Lu-Yang Wang 1,2
1 Department of Physiology, University of Toronto
2 Program in Neurosciences and Mental Health, SickKids Research Institute

Excitatory synapses undergo rapid remodeling during early sensory development by changing the abundance, composition, and nano-organization of postsynaptic glutamate receptors to enable neurotransmission. Dysregulation of synaptic remodeling can lead to neurodevelopmental disorders such as fragile X syndrome (FXS), caused by a mutation in the gene encoding fragile x mental retardation protein (FMRP). It is unknown how FMRP deletion impacts the nano-organization of AMPARs and quantal transmission during early synaptic development. Using the calyx of Held synapse in the auditory brainstem, where AMPARs undergo a developmental subunit switch from slow-gating GluA1- to fast-gating GluA4-dominant, we applied expansion (ExM) and STED microscopy to map nanoscale differences in subsynaptic localization of GluA1- and GluA4-AMPARs between wild-type (WT) and Fmr1-/- mice at pre- (P8-10) and post-hearing (P16-19) stages. We see partially mismatched GluA1- and GluA4-AMPAR nanodomains, supporting the bimodal distribution of fast and slow mEPSCs in WT mice, which is altered in Fmr1-/- synapses. The bimodal distribution of fast and slow mEPSCs in immature Fmr1-/- synapses phenocopies that of mature WT synapses. Basal mEPSC frequency was significantly higher, and less sensitive to an elevation of extracellular Ca2+ in Fmr1-/- synapses, indicating altered presynaptic remodeling. Our study suggests that the loss of FMRP accelerates developmental remodeling of both pre- and postsynaptic elements underlying quantal transmission, implicating the critical role of FMRP in controlling the pace of activity-dependent synaptic maturation.

O1-4: Postpartum maladaptations in wound healing and metabolism implicated in development of type 2 diabetes after a gestational diabetes pregnancy

Julie Van1,2, Yihan Luo1,2, Feihan Dai1, Erica Gunderson3,4, Hannes Rost5,6, Michael Wheeler1,2
1 Department of Physiology, Faculty of Medicine, University of Toronto; Toronto, Ontario, Canada.
2 Metabolism Research Group, Division of Advanced Diagnostics, Toronto General Research Institute; Toronto, Ontario, Canada.
3 Division of Research, Kaiser Permanente Northern California, Oakland, CA
4 Department of Health Systems Science, Kaiser Permanente Bernard J. Tyson School of Medicine, Pasadena, CA.
5 Department of Molecular Genetics, University of Toronto; Toronto, Ontario M5S 1A8, Canada.
6 Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto; Ontario M5S 3E1, Canada.

Excitatory synapses undergo rapid remodeling during early sensory development by changing the abundance, composition, and nano-organization of postsynaptic glutamate receptors to enable neurotransmission. Dysregulation of synaptic remodeling can lead to neurodevelopmental disorders such as fragile X syndrome (FXS), caused by a mutation in the gene encoding fragile x mental retardation protein (FMRP). It is unknown how FMRP deletion impacts the nano-organization of AMPARs and quantal transmission during early synaptic development. Using the calyx of Held synapse

in the auditory brainstem, where AMPARs undergo a developmental subunit switch from slow-gating GluA1- to fast-gating GluA4-dominant, we applied expansion (ExM) and STED microscopy to map nanoscale differences in subsynaptic localization of GluA1- and GluA4-AMPARs between wild-type (WT) and Fmr1-/- mice at pre- (P8-10) and post-hearing (P16-19) stages. We see partially mismatched GluA1- and GluA4-AMPAR nanodomains, supporting the bimodal distribution of fast and slow mEPSCs in WT mice, which is altered in Fmr1-/- synapses. The bimodal distribution of fast and slow mEPSCs in immature Fmr1-/- synapses phenocopies that of mature WT synapses. Basal mEPSC frequency was significantly higher, and less sensitive to an elevation of extracellular Ca2+ in Fmr1-/- synapses, indicating altered presynaptic remodeling. Our study suggests that the loss of FMRP accelerates developmental remodeling of both pre- and postsynaptic elements underlying quantal transmission, implicating the critical role of FMRP in controlling the pace of activity-dependent synaptic maturation.

O1-5: Identifying and manipulating a hippocampal engram supporting memory integration

Ying Wang 1,2 , Jung Hoon Jung 1 , Chen Yan 1,4 , Margaret L. Schlichting 3 , Katherine D. Duncan 3 , Paul W. Frankland 1,2,3,4 and Sheena A. Josselyn 1,2,3,4
1 Neurosciences & Mental Health, Hospital for Sick Children, Toronto, Ontario, Canada
2 Department of Physiology, University of Toronto, Toronto, Ontario, Canada
3 Department of Psychology, University of Toronto, Toronto, Ontario, Canada
4 Institute of Medical Sciences, University of Toronto, Toronto, Ontario, Canada

Previously acquired information is thought to be stored in ensembles of neurons (or engrams). Most rodent engram studies have focused on conditioned fear memory, which suggests that ensembles of two memory events occurring closely together are more likely to be integrated. Whether these findings generalize to other types of memory are unknown. Here we investigated how CA1 pyramidal hippocampal neurons are allocated to an engram supporting a specific spatial rewarding memory and whether spatial events can be linked through engram co-allocation. Mice were trained to navigate towards two different un-marked predetermined regions in two distinct arenas to receive rewarding brain stimulation using spatial cues. We found that mice tended to integrate two memories by searching both target regions of trained environments when they occurred with a 3h delay, but not in the 27h delay group. To further examine the flexibility of engram ensembles, we artificially forced memory co-allocation and dis-allocation by manipulating the excitability of allocated neurons with optogenetics. Interestingly, mice with a 27h delay showed integrated memories of trained environments after manipulation, while mice a 3h delay showed separated searching behavior for different target regions of trained environments. Together, these results indicate that neurons with higher excitability in the CA1 of dorsal hippocampus are preferentially allocated to an engram supporting a rewarding spatial memory, and that the process of memory linking of two spatial events through engram co-allocation is affected by time.

O2-1: NINJ1 forms large pore-like structures within the plasma membrane during pyroptosis

Jazlyn P. Borges1,2, Allen Volchuk3, Isabelle Jansen4, Neil M. Goldenberg3,5,6, Benjamin E. Steinberg1,2,5,6
1 Program in Neuroscience and Mental Health, The Hospital for Sick Children, Toronto, Ontario, Canada;
2 Department of Physiology, Temerty Faculty Medicine, University of Toronto, Toronto, Ontario, Canada;
3 Program in Cell Biology, The Hospital for Sick Children, Toronto, Ontario, Canada;
4Abberior Instruments, Göttingen, Germany;
5Department of Anesthesia and Pain Medicine, The Hospital for Sick Children, Toronto, Ontario, Canada;
6Department of Anesthesiology and Pain Medicine, University of Toronto, Toronto, Ontario, Canada

Plasma membrane rupture is the common terminal event of multiple cell death pathways, including the pro-inflammatory programmed cell death pathway pyroptosis. Cell rupture was recently found to be an active process mediated by NINJ1. NINJ1 is postulated to cluster within the plasma membrane to mediate rupture; however, the mechanism by which NINJ1 clusters mediate membrane rupture remains unknown. We aimed to determine the membrane organization of

NINJ1 clusters in primary mouse macrophages using super-resolution microscopy. Using stimulated emission depletion microscopy (STED), we first show that during pyroptosis, NINJ1 clusters to form large pore-like structures ~150 nm in diameter. We next used MINFLUX nanoscopy to resolve NINJ1 clusters with dimensions below the resolving power of STED and delineate the three-dimensional (3D) spatial organization of basal and activated NINJ1 clusters at the single-digit nanometer scale. By MINFLUX, we confirmed the presence of > 100 nm NINJ1 pore-like structures and resolved smaller NINJ1 clusters in both 2D and 3D. We show that basal NINJ1 is organized only in small puncta. During pyroptosis, 13.3% of NINJ1 structures were pore-like with a largest inner diameter ranging from ~10 to 175 nm, mean ~39.1 nm ± 25.9 nm. Our work is the first to report NINJ1 forms pore-like structures of sizes that would permit the release of large DAMPs. Our work reveals structural insight into the mechanisms of NINJ1-mediated plasma membrane rupture, which will inform the development of tissue preservation strategies and therapeutics for the numerous pathologies in which lytic cell death has an important role.

O2-2: Macro-molecular Protein Complex of Voltage dependent Calcium Channel Gating Neurotransmitter Release in Central Synapses

Rayan Saghian, 1 2; Jason Arsenault, 2; Bassam Tawfik, 2; Lu-Yang Wang, 1 2
1 Dept. of Physiology, University of Toronto;
2 Neurosciences & Mental Health, Hospital for Sick Children

Neuronal communication depends highly on integrating the action potentials with calcium dependent neurotransmitter release through physical couplings between voltage-gated calcium channels (VGCCs) and synaptic vesicles (SVs) at nerve terminals. An evolutionarily conserved set of proteins that form active zones at the synaptic terminals bridges the activity of VGCCs to the fusion of vesicles. Whereas significant progress has been made in uncovering fusion machinery, much remain to be discovered about the release and refilling mechanisms of SVs. In this study, we hypothesize that intracellular domains of P/Q type voltage-gated calcium channels (Cav2.1) may serve as the core scaffold for organizing macro-molecular complex for the release sites. We have developed an experimental approach focusing on Cav2.1 in the cerebellum where this channel is most abundantly found. By combining Mass Spec, FLIM-FRET and super-resolution microscopy and patch-clamp electrophysiology, we identified a series of new interacting proteins with Cav2.1, among which “Protein Y” was found dynamically regulate the loading of glutamate into SVs via novel, direct interactions with Cav2.1 and vesicular glutamate transporter (VGLUT), significantly impacting synaptic strength and short-term plasticity. Our long-term goal of this project is to shed light on the modular composition of the active release machinery with “Protein Y” as a key building block to better understand the molecular organization of neurotransmission in healthy and diseased brain.

O2-3: Optimizing a pumped artificial placenta circuit in a swine model of the extremely preterm human fetus

Alex J. Charest-Pekeski1,2, Steven K.S. Cho1,2, Liqun Sun3, Tanroop Aujla1,2, Alejandro Floh3,4, Ayman Sheta5, Mark J. McVey6,7, Lynn Crawford-Lean8, Celeste Foramen8, Dariusz Mroczek3, Brahmdeep Saini2,9, Jessie Mei Lim1,2, Megan Quinn10, Jack R.T. Darby10, Janna L. Morrison10, Mike Seed3, & Christoph Haller8
1 Department of Physiology, University of Toronto, Toronto, Ontario, Canada
2 Translational Medicine, The Hospital for Sick Children, Toronto, Ontario, Canada
3 Division of Cardiology, The Labatt Family Heart Centre, The Hospital for Sick Children, Toronto, Ontario, Canada
4 Department of Critical Care Medicine, The Hospital for Sick Children, Toronto, Ontario, Canada
5 Department of Pediatrics, Division of Neonatology, The Hospital for Sick Children, Toronto, Ontario, Canada
6 Department of Pain and Anesthesia, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada
7 Department of Physics, Ryerson University, Toronto, Ontario, Canada
8 Division of Cardiovascular Surgery, The Labatt Family Heart Centre, The Hospital for Sick Children, University of Toronto, Toronto, Canada
9 Institute of Medical Science, University of Toronto, Toronto, Ontario, Canada
10 Early Origins of Adult Health Research Group, Health and Biomedical Innovation, UniSA: Clinical and Health Sciences, University of South Australia, Adelaide, South Australia, Australia

Introduction
An artificial placenta aims to maintain the fetal circulation while supporting the physiologic development of vital organs. In the current study, we compared the hemodynamics of a pumpless and pumped artificial placenta (AP) circuit in a fetal pig model.
Methods
In utero umbilical vein (UV) flow was measured in fetal pigs (n=16; 107±3 gestational age (GA); term=115 days) using phase contrast MRI and, fetal heart rate (HR) data was acquired by carotid artery cannulation (n=6; 106GA). Fetal pigs were delivered via caesarian section and cannulated via the umbilical vessels and supported on a pumpless (n=12, 98±4 days) and a pumped AP circuit (n=13, 102±4 days).
Results
Fetal pigs were supported on a pumpless AP for 11±3hours and a pumped AP for 46.4±46.8 hours (range: 3.4-177.8 hours). In utero UV flow and HR data were 173±45mL/min/kg, and 130±10bpm. Compared to controls, UV flow on the pumpless circuit was subphysiologic (97±39mL/min/kg; P=0.007); whereas there was no significant difference in UV flow between in utero animals and those supported on the pumped AP (143±47ml/min/kg; P=0.202). Fetal pigs were tachycardiac on both pumpless (206±38bpm; P<0.0001) and pumped (205±28bpm; P<0.0001) AP circuits compared to controls, and fetuses were hypertensive on the pumped AP (P=0.013).
Conclusions
The addition of a pump greatly improved the survival of fetuses on the AP. However, we continued to observe hemodynamic decompensation following hours of AP support. This raises the need for further modifications to the circuit to improve hemodynamic stability in this model of the extremely preterm human fetus.

O2-4: Large-scale Proteomic Analysis Of Caltubin-dependent Pathways Promoting Long-term Memory Formation

Bandura J1, Chan C2, Sun, H-S3, Wheeler AR2,4,5, Feng Z-P1
1 Departments of Physiology
2 Chemistry
3 Surgery
4 Donnelly Centre for Cellular and Biomedical Research
5 Institute of Biomedical Engineering University of Toronto

Formation of long-term memory (LTM) is essential for survival. Though many proteins implicated in LTM formation have already been identified, the biological complexity of learning behaviours necessitates the involvement of a diverse complement of signaling pathways. Previously, our lab has leveraged a well-established LTM model, aversive operant conditioning of aerial respiratory behaviour in the pond snail Lymnaea stagnalis, and reported cellular mechanisms underlying aversive operant conditioning-related LTM. The current study focuses on identifying novel pathways mediated by caltubin, a novel calcium-binding protein, in LTM formation, combining this advantageous animal model with a powerful large-scale, high-throughput proteomics approach. We identified 308 differentially-expressed CNS proteins between snails exhibiting LTM or no LTM, using label-free quantification of protein abundance via LC-MS/MS. We then identified 431 6xHis-caltubin-interacting protein complexes using affinity purification-LC-MS/MS. 66 proteins intersected between sets, representing LTM-relevant candidate proteins for regulation by caltubin. BLASTp annotation against the human proteome showed a network of highly-interconnected proteins (enrichment p-value < 1e-16), while enriched GO terms included “proteasome-activating activity” and “clathrin heavy chain binding”. We further validated these findings and identified for the first time critical proteins which may contribute to caltubin-dependent pathways promoting LTM formation. Taken together, these findings suggest caltubin may regulate proteasome activity and clathrin-mediated exocytosis in LTM. This is the first bottom-up protein-protein interaction screen in L. stagnalis and provides an exciting opportunity to identify novel and potentially evolutionarily conserved protein pathways of LTM formation. Future work will characterize the physiological relevance of novel caltubin interactors in this model and beyond.

O2-5: Mapping Human Placental Cell Lineages via Transcriptome Analysis

Young June Kim 1 , Pascale Robineau-Charette 1 , William Tony Parks 2 , Brian Cox 1 , Jesse Gillis 1
1 Department of Physiology, University of Toronto
2 Department of Laboratory Medicine and Pathobiology, University of Toronto

A healthy pregnancy requires proper placentation, as the placenta plays a crucial role in maternal-fetal interactions during

gestation. However, the precise regulatory and molecular mechanisms underlying placental development are poorly understood, and the transcriptional programs that define trophoblast cells in early embryos remain unknown. Here, we have identified gene expression patterns crucial for cell-type specification and development at the transcriptional level by tracking the placental cell lineages to a few progenitor trophoblasts. An integrative meta-analysis of placental single-cell RNA sequencing (scRNA-seq) data was performed, where the replicability score of the cell types in four first-trimester scRNA-seq datasets was high (AUROC > 0.9) for the three trophoblast-lineage cell types: Cytotrophoblasts, syncytiotrophoblasts, and extravillous trophoblasts. A cell type-specific marker gene set (meta-markers) was derived across the four datasets defining the three trophoblast-lineage cell types. The top 30 meta-markers for the three cell types were highly connected in the human gene regulatory network (AUROC > 0.8). Furthermore, bulk RNA sequencing (bulk RNA-seq) was performed on seven lobules from two placentas for cell lineage tracing. Genetic bottlenecks occur during early embryonic development, where trophoblasts are clonally expanded in different placental lobes. Distinct placental lineages were traced by measuring the variance in random allelic expression over different lobules, also supported by pseudo-bulk analysis of the scRNA-seq data from our initial meta-analysis. This study elucidates the initial steps of human placentation by identifying gene markers and cell types crucial for proper placental development, suggesting further complex analyses, such as an interspecies comparison of the gene markers.

O3-1: Elucidating Heterogeneity Between Left and Right Ventricle-derived Cardiac Fibroblasts

Dewar, Michael Bradley.(1,2), Shah, Haisam.(1,2), Langburt, Dylan.(1,2), Ehsan, Fahad.(1,2), Hacker, Alison(2), Heximer, Scott.(2,3).

1 Department of Physiology, University of Toronto, Toronto, Ontario, Canada

2 Translational Biology and Engineering Program, Ted Rogers Centre for Heart Research, Toronto, Ontario, Canada

3 Heart & Stroke/Richard Lewar Centre of Excellence for Cardiovascular Research, University of Toronto, Toronto, Ontario, Canada.

Cardiac fibrosis is a major risk factor for cardiovascular disease, leading to impaired electrical conduction and reduced ventricular compliance in the heart. Despite numerous reports on the heterogeneity of cardiac fibroblasts (CFs), there are no direct comparisons between those in the left ventricle (LV) and right ventricle (RV). Due to their environmental and developmental differences, we hypothesize that LV and RV-derived CFs will display transcriptomic differences that influence their response to injury. Bulk RNA-seq of heart tissue from uninjured male mice revealed 442 differentially expressed genes ($p<0.05$, $n=4$) with numerous fibrosis-related genes such as IGFBP3, COL8A1, CTGF, ASPN, and POSTN being the most significantly different. Single-cell RNA-seq analysis identified nine sub-populations, two of which displayed LV vs RV differences. CFs marked by high expression of POSTN, COL8A1, and CTGF were more abundant in the LV, whereas CFs marked by high expression of IGFBP3, FGL2, and SFRP2 were more abundant in the RV. Comparisons with published datasets suggest that POSTN-high CFs are primed for differentiation into injury-induced CFs, while IGFBP3-high CFs have an unknown function. Single-cell RNA-seq of pressure-overloaded ventricles 14 days post-injury showed that the LV developed a larger population of THBS4+ injury-induced CFs. Furthermore, the RV uniquely displayed expansion of INMT-high CFs, which are present in the uninjured heart. These findings demonstrate that LV and RV-derived CFs display baseline transcriptomic differences that may cause their diverging responses to pressure-overload injury. Understanding these differences is critical for determining whether distinct therapies are required for treating LV and RV fibrosis.

O3-2: Activation of Arcuate nucleus Glucagon-like Peptide-1 receptor-expressing neurons regulates energy homeostasis

Ishnoor Singh1, Le Wang2, Wenyue Ye1, Zhiping P. Pang2,5,6, Denise D. Belsham1,7, Michael B. Wheeler1,4

1 Department of Physiology, University of Toronto, 1 King's College Circle, Toronto, ON, M5S 1A8, Canada.

2 The Child Health Institute of New Jersey, Robert Wood Johnson Medical School, Rutgers, The State University of New Jersey, New Brunswick, NJ, 08901, USA.

3 Department of Medicine, Division of Endocrinology, Metabolism and Nutrition, Robert Wood Johnson Medical School, Rutgers, The State University of New Jersey, New Brunswick, NJ, 08901, USA.

4 Division of Advanced Diagnostics, Metabolism, Toronto General Research Institute, ON, Toronto, Canada.
5 Department of Neuroscience and Cell Biology, Rutgers, The State University of New Jersey, New Brunswick, NJ, 08901, USA.
6 Department of Pediatrics, Robert Wood Johnson Medical School, Rutgers, The State University of New Jersey, New Brunswick, NJ 08901, USA.
7 Departments of Obstetrics/Gynecology and Medicine, University of Toronto, Toronto, ON M5S 1A8, Canada

Central nervous system (CNS) control of metabolism plays a pivotal role in maintaining energy balance. In the brain, Glucagon-like peptide 1 (GLP-1), encoded by the proglucagon 'Gcg' gene, produced in a distinct population of neurons in the nucleus tractus solitarius (NTS), has been shown to regulate feeding behavior leading to the suppression of appetite. However, neuronal networks that mediate endogenous GLP-1 action in the CNS on feeding and energy balance are not well understood. This is mainly due to the presence of diverse neuronal subtypes and complex central neuronal connectivity.
We systematically analyzed the distribution of GLP-1R-expressing neurons and axonal projections of NTSGcg proglucagon expressing neurons in the mouse brain. GLP-1R neurons were found to be broadly distributed in the brain and specific forebrain regions, particularly the hypothalamus, including the arcuate nucleus of the hypothalamus (ARC), received dense NTSGcg neuronal projections. For this reason, the impact of GLP-1 signaling in the ARC, a brain region known to regulate energy homeostasis and feeding behavior was examined. Application of GLP-1R specific agonist Exendin-4 enhanced the ARC pro-opiomelanocortin (POMC) neuronal population's action potential firing frequency and miniature excitatory postsynaptic spontaneous currents amplitude. Using a chemogenetic approach to activate the ARC GLP-1R neurons by using Cre-dependent hM3Dq AAV in the GLP-1R-ires-Cre mice, we established that acute activation of the ARC GLP-1R neurons significantly suppressed food intake with mild effects on blood glucose.
These results highlight the importance of central GLP-1 signaling within the ARC that express GLP-1R which upon activation, regulates energy homeostasis.

O3-3: Breaking Through the Defense: Biofilm Disruption Using Alveolar-Like Macrophages Expressing a Psl-Glycoside Hydrolase

Sajad Sadat 1, 2; Michael L. Litvack 1; Deepa Raju 3; Vanessa Hsing 1; P. Lynne Howell 3, 4; Martin Post 1, 2
1 Translational Medicine Program at The Hospital for Sick Children, Toronto, Canada;
2 Department of Physiology, University of Toronto, Toronto, Canada;
3 Molecular Medicine Program at The Hospital for Sick Children, Toronto, Canada;
4 Department of Biochemistry, University of Toronto, Toronto, Canada

In Cystic Fibrosis (CF), high morbidity and mortality arise from biofilm accumulation due to colonization of the bacterium Pseudomonas aeruginosa (PA) in the lungs. These biofilms are impenetrable to the immune system and antibiotics. I hypothesized that stem cell-derived Alveolar-Like Macrophages (ALMs) that have been genetically modified to secrete a glycoside hydrolase enzyme (PslG) that disrupts PA biofilms could represent a novel CF therapy. I have shown the effectiveness of these cells by displaying that the secretions produced by these cells significantly disrupt mature biofilms in an in-vitro model with both lab and patient-isolated PA strains. Furthermore, these PslG-ALMs survive and secrete PslG in the airways of healthy mice for up to 14 days. This pulmonary genetically modified macrophage transplantation does not result in a host-immune response, as no anti-PslG antibodies were detected in the mouse serum. Moreover, I have determined that quality of the in-vivo secretions of these PslG-ALMs is sufficient and effective to disrupt the in-vitro biofilms. In the future, I will apply these PslG-ALMs to an established in-vivo wound-infection biofilm model (as no pulmonary biofilm model exists). I hypothesize that the cells will potentiate the effects of antibiotics in the wound infection model. In summary, my research shows the in-vitro efficacy to disrupt biofilms and the in-vivo utility of PslG-ALMs. This new technology may help expand the therapeutic options or compliment standard antibiotic therapy for CF patients. It also demonstrates the potential of ALMs as a drug delivery mechanism that can constituently express a protein of interest.

O3-4: Latent transforming growth factor binding protein - 2 deficiency improves cardiac function post myocardial infarction

Fahad Ehsan

Cardiac fibrosis and myocardium remodeling are key factors contributing to heart failure, especially after myocardial infarction (MI). Fibroblasts are the main cell type involved in extracellular matrix (ECM) protein deposition, leading to pathological remodeling of the heart. LTBP2 is a protein released by fibroblasts as part of the fibrotic response and is consistently upregulated in heart failure, with a suggested role in the progression of fibrosis. In this study, we hypothesize that in the absence of LTBP2, the heart would have improved cardiac function following MI. Methods: We used LTBP2 knockout mice and modeled MI by ligating the left anterior descending artery. Echocardiography was performed to analyze cardiac function in KO and Wt infarcted mice at 7 and 28 days post-MI. Results: LTBP2 KO mice showed significantly better ejection fractions (KO = 24.1±4.9% vs. WT = 9.2±1.4%, p-value < 0.01) and fractional shortening 28 days post-MI compared to WT mice. Furthermore, the KO mice had improved fractional shortening of the walls at the apical and middle sections of the left ventricle 28 days post-MI. Additionally, KO infarcted hearts had improved wall strain and a greater extent of wall contraction at the infarcted apical region post MI. Conclusion: Our findings suggest that LTBP2 plays a key role in the progression of deteriorating cardiac function in heart failure. Future studies will investigate the fibrotic profile of WT and KO-infarcted hearts. In conclusion, our study suggests that targeting LTBP2 may have therapeutic potential for improving cardiac function in heart failure.

O3-5: Multifaceted impact of Ω3-polyunsaturated fatty acids on Kv1.2 channels and inhibitory neurotransmission

Tian Kong 2 , Jason Arsenault 1 , Bassam Tawfik 1 , Lu-Yang Wang 1,2*
1- Program in Neurosciences & Mental Health, Sick Kids Research Institute, Toronto, ON, M5G 1X8, Canada
2-Department of Physiology, Faculty of Medicine, University of Toronto, Toronto, Canada

Principal neurons encode information by varying their firing rates and patterns fine-tuned through GABAergic interneurons. We have shown voltage-gated potassium channels (Kv1.2) are enriched in GABAergic interneuron nerve terminals where its downregulation leads to excessive GABA release and over-inhibition of Purkinje neurons in the cerebellum of Fmr1-KO mice, a Fragile X Syndrome (FXS) model. Docosahexaenoic acid (DHA), a Kv1.2 positive allosteric modulator, normalizes this inhibitory overtone in vitro and rescues behavioral phenotypes in vivo, indicating Kv1.2 as a target for FXS. In the stable Kv1.2-GFP CHO cell-line, we investigated the effect of three Ω3-polyunsaturated fatty acids, i.e. DHA, eicosapentaenoic acid (EPA) and alpha-linolenic acid (ALA), on Kv1.2 activity, expression, and localization. Electrophysiological recordings and in silico docking revealed that DHA has the highest potency among three. It acutely accelerates Kv1.2 activation and decelerates deactivation. Through in silico simulation and site-directed mutagenesis, we demonstrated that DHA directly binds to a deep cavity that shifts the voltage sensor (S4) of Kv1.2. Chronically, DHA promotes Kv1.2 trafficking to cytoplasmic membranes from the intracellular pool. In vitro immunohistochemistry revealed that Kv1.2 level is positively correlated with presynaptic PSD95 expression in cerebellum. The DHA-induced upregulation of Kv1.2 expression was likely by promoting a redistribution of presynaptic PSD95 to interact with and stabilize cytoplasmic Kv1.2 channels.
These mechanistic insights facilitate in silico and high-throughput drug screening and validation of efficacy in cerebellar slices. Ultimately, this enables us to search for novel compounds that upregulate Kv1.2 functions as therapeutic candidates to treat FXS and other disorders with loss-of-function mutations in Kv1.2 or over-inhibition.
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O3-5: Engram specific synaptic potentiation is important for fear memory formation and expression in vivo

Mattei Saderi

Memories are thought to be encoded in the brain via synaptic strengthening among memory engram cells. It has not been possible to observe if learning potentiates synaptic connections (i.e., measure synaptic strength before and after learning) and simultaneously show that these changes are causally related to memory expression (i.e., they occur in engram neurons). Here, we used a novel engram allocation-based approach to observe synaptic changes in engram neurons upon learning in-vivo. We assigned selected Medial Entorhinal Cortex (MEC) neurons to a context dependent fear memory in mice. To do this, we expressed an adeno-associated virus with both excitatory (blue light) and inhibitory (red light) opsins in MEC and implanted an optrode above MEC terminals in the Dentate Gyrus (DG). Briefly increasing the excitability of infected MEC terminals in mice that underwent fear conditioning (by shining blue light immediately before training) allocated the fear memory to virus-infected neurons. 24 hours after conditioning, during red light exposure, mice showed a reduced memory expression demonstrated by decreased freezing behaviour. In a separate cohort of animals, we used in-vivo electrophysiology to record pre- and post-learning strength of MEC to DG allocated neurons' synapses. Remarkably, we found that synapses originating from allocated MEC neurons were potentiated 24 hours after learning. Finally, to assess a causal relationship between engram-specific synaptic potentiation and memory, in the same animals, we used optogenetics to induce synaptic depression 24 hours after learning in the terminals of allocated neurons. We observed that synaptic strength reversed to pre-learning levels.

P1-1: Argon Inhalation: A Novel Treatment For Neonatal Sepsis

Felicia Balsamo 1,2 , Carol Lee 2 , Peter Li 2 , Agostino Pierro 1,2
1 University of Toronto, Department of Physiology, Toronto, ON, Canada
2 Division of General and Thoracic Surgery, Translational Medicine Program, The Hospital for Sick Children, Toronto, ON, Canada

Background/Aim Sepsis is a leading cause of mortality in neonates. Argon is an emerging interest in the field of noble gas therapy. Neonates with severe sepsis are commonly mechanically ventilated creating an opportunity for introducing a new inhalation therapy. We aimed to investigate argon inhalation as a novel experimental therapy in neonatal sepsis.
Methods Sepsis was established in pup mice by LPS intraperitoneal injection [20mg/kg] on postnatal day 9.
Study 1: Argon was administered by inhalation (70% argon, 30% oxygen) into a chamber housing the pup mice (n=12) for 30 hours. Sepsis controls were housed in the chamber containing room air (n=11).
Study 2: To avoid hypothermia, septic mice were receiving argon (n=13) and septic controls (n=14) were maintained in the chamber positioned in an incubator at 35°C for 6 hours. Breastfed pups served as normal controls (n=6). Small intestine and colon, target organs in neonatal sepsis, were harvested from survivors.
Results
Study 1: Argon inhalation significantly reduced sepsis mortality.
Study 2: At 35°C temperature, there was 50% survival in argon sepsis compared to 20% in control sepsis. In the intestine, morphology was improved and inflammatory cytokines IL-1B and IL-6, were significantly decreased in argon sepsis compared to septic controls (level similar to non-septic mice).
Conclusions Argon inhalation is a novel treatment for neonatal sepsis, reducing mortality and counteracting the acute systemic inflammatory response in the small intestine and colon. Argon inhalation can be translated into humans as septic neonates are commonly mechanically ventilated.

P1-2: Dynamic susceptibility contrast perfusion imaging using a novel contrast agent: Transient hypoxia-induced deoxyhemoglobin

Ece Su Sayin1,2, Olivia Sobczyk2,3, James Duffin1,3, Harrison Levine1,2, Julien Poublanc2, Joseph A. Fisher1,3

& David J. Mikulis2
1 Department of Physiology, University of Toronto, Toronto, Ontario, Canada.
2 Joint Department of Medical Imaging and the Functional Neuroimaging Lab, University Health Network, Toronto, ON, Canada
3 Department of Anesthesia and Pain Management, University Health Network, University of Toronto, Toronto, ON, Canada.

Background: Dynamic susceptibility contrast (DSC) magnetic resonance imaging (MRI) requires a susceptibility contrast agent to allow for the calculation of perfusion measures at rest. Currently this requires the intravascular injection of gadolinium-based contrast agents (GBCA), engendering medical risks, cost and environmental drawbacks. Here we use hypoxia-induced dOHb (THx-dOHb) as a suitable contrast agent for DSC perfusion imaging and validate against a clinical standard, GBCA.
Methods: We studied 8 healthy controls, 8 patients with steno-occlusive disease and 8 patients with low-grade glioma in a 3-Tesla MRI scanner running T2* acquisition sequences. THx-dOHb was induced via an automated gas blender running feed-forward gas algorithm targeting 2 consecutive reductions of pulmonary PO2 from 95 mmHg to 40 mmHg followed by full reoxygenation within a single inhalation. A second T2* sequence was acquired following an intravenous injection of 5 ml of GBCA. All images were analyzed, and resting perfusion measures were calculated using a standard tracer kinetic model.
Results: The calculated resting perfusion measures showed similar voxel-wise proportional changes in T2* signal throughout the brain. Bland-Altman analysis indicated little bias or difference in hemodynamic measures between methods. Both techniques allow for the distinction between the healthy and affected hemispheres consistent with the clinical notes of the patient population.
Conclusions: The resting perfusion measures obtained from THx-dOHb are spatially and quantitatively comparable to those obtained using a bolus of GBCA in the same healthy controls and patients. The advantages of THx-dOHb as a contrast agent include it being non-invasive, no risk of allergy, no tissue injury in patients with pre-existing renal failure and no environmental damage.

P1-3: Establishing a novel microRNA signature to mitigate Uremic Cardiomyopathy in patients with End Stage Renal Disease.

Carmine Alberga1, Dr.Filio Billia2, Dr.Christopher Chan3.
1 Department of Physiology, University of Toronto, Toronto, Ontario, Canada.
2 Joint Department of Medical Imaging and the Functional Neuroimaging Lab, University Health Network, Toronto, ON, Canada
3 Department of Anesthesia and Pain Management, University Health Network, University of Toronto, Toronto, ON, Canada.

Left ventricular hypertrophy (LVH), often observed in patients with end-stage renal disease (ESRD), is not only associated with increased risk of developing heart failure but also associated with increased mortality. The interplay between the heart and the kidney is coined as cardiorenal syndrome, wherein the diseased state of one organ influences the state of the other. Of particular interest, there are two known biomarkers, namely: (i) fibroblast growth factor 23 (FGF23) and (ii) Klotho that have been shown to have a reciprocal and potential role in in patients with renal disease displaying LVH; however, their exact mechanism of action has not been clearly delineated. MicroRNAs (miRs) are small non-coding RNA fragments that are 22 nucleotides in length that bind and hinder the expression of their desired target mRNA at the post-transcriptional level. While there are a few specific miRs that have been identified in patients with LVH and CKD, the role of these specific miRs remains unclear. Therefore, we are assessing the differential expression of circulating miRs in patients of two specific groups of dialysis: (i) conventional hemodialysis, and (ii) frequent hemodialysis. We will focus the specific miRs that may regulate the FGF23/Klotho axis, aiming to develop potential therapeutic targets to mitigate the development of LVH in patients in ESRD.

P1-4: Astrocytic α4GABAA receptors are critical for the anesthetic-induced persistent increase in tonic current in neurons

Li Ju(1), Arsène Pinguelo(1), Anthony Ariza(1), Himaben Gohil(1), Dian-Shi Wang(1), Michael B Wheeler(1),

Beverley A Orser(1,2,3)
1 Department of Physiology, University of Toronto;
2 Department of Anesthesiology and Pain Medicine, University of Toronto, Toronto, Ontario, Canada;
3 Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada.

Background: Exposure to anesthetic drugs including etomidate triggers a sustained increase in tonic current in neurons that is associated with cognitive deficits. Astrocytes are necessary for this effect, as etomidate triggers the sustained increase in tonic current in astrocyte-neuron cocultures but not in neurons alone. Anesthetic-sensitive GABAA receptors (GABAARs) are expressed in astrocytes, so we hypothesized that activation of GABAARs in astrocytes is necessary to trigger the sustained increase in tonic current in neurons. The first goal of this study was to identify the subtypes of GABAARs in astrocytes. Next, we knocked out a major anesthetic-sensitive population of GABAARs on astrocytes, and investigated whether this approach abolishes the etomidate-induced increase in tonic current in neurons.
Methods: Expression of GABAAR subunits in cultured astrocytes and neurons was profiled using ddPCR. For the major anesthetic-sensitive receptor subtype, knockout(KO) and wildtype(WT) cortical astrocytes were cocultured with CD1 hippocampal neurons. Cocultures were treated with etomidate for 1h followed by complete media change which tonic current was measured using whole-cell patch clamp 24h later.
Results: We found that astrocytes predominantly expressed α4 and α2, as well as β1, β3 and γ3 subunits. We created cocultures using α4KO astrocytes, since α2KO mice are non-viable. Etomidate increased tonic current in neurons cocultured with α4WT astrocytes but not in neurons cocultured with KO astrocytes.
Conclusion: These results suggest that α4GABAARs in astrocytes are necessary for the etomidate-induced increase in tonic current in neurons. These receptors may represent a novel target to mitigate cognitive deficits after general anesthesia.

P1-5: Small intestinal protein sensing mechanism in feeding and glucose regulation

Daniel R. Barros(1,2), Rosa J.W. Li(1,2), Yu-Mi Lim(2,3), Song-Yang Zhang(2), Rachel Kuah(1,2), Anna Gao(5), Tony K.T. Lam(1,2,4,5)
1 Department of Physiology, University of Toronto, Toronto, Canada
2 Toronto General Hospital Research Institute, UHN, Toronto, Canada
3 Medical Research Institute, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea
4 Institute of Medical Science, University of Toronto, Toronto, Canada
5 Banting and Best Diabetes Centre, University of Toronto, Toronto, Canada

High-protein feeding stimulates the secretion of GLP-1 and lowers body weight and blood glucose; however, the site of action(s) remains uncertain. Interestingly, direct small intestinal protein infusion activates calcium sensing receptor (CaSR) and peptide transporter 1 (PepT1) to increase GLP-1 secretion, but the role of gut protein sensing on energy and glucose homeostasis in vivo remain under debate and their underlying small intestinal mechanism requires investigation. We performed intravenous glucose tolerance test and refeeding studies and infused 8% (w/v) casein hydrolysate into the upper small intestine (USI) of male rats. We found that USI casein vs. saline infusion increased glucose tolerance in chow and HF-fed rats and suppressed feeding in chow but not HF rats. We inhibited CaSR by injecting lentivirus expressing shRNA of CaSR into the USI (reduced expression by ~40%) and CaSR knockdown negated casein to increase glucose tolerance but not to lower feeding. Instead, viral-mediated PepT1 knockdown by ~50% negated USI casein infusion to lower feeding as similar to previously reported for glucose control. Thus, CaSR and PepT1 are required for casein in the USI to increase glucose tolerance, while PepT1 but not CaSR mediates casein to lower feeding, in chow rats. The differential role of PepT1 vs CaSR may explain why HF diet disrupts casein to regulate feeding but not glucose tolerance as HF inhibited PepT1 expression but increased CaSR expression in the USI. In sum, we discover differential role of USI CaSR vs. PepT1 in the feeding and glucose regulatory abilities of protein sensing.

P1-6: Effects of ephedrine, phenylephrine, and norepinephrine on myometrial contractility

Joseph Park1,2 HBSc, Oksana Shynlova1,2,3 PhD, John Kingdom2,3 MD FRCSC, Binu Jacob4 MSc PhD, Tess

Engel4 BSc, Stephen Lye2,3 PhD, Mrinalini Balki1,2,3,4 MD
1 Department of Physiology, Temerty Faculty of Medicine, University of Toronto, Toronto, ON, Canada
2 The Centre for Women's and Infant's Health, Lunenfeld-Tanenbaum Research Institute Sinai Health System, Toronto, ON, Canada.
3 Department of Obstetrics and Gynecology, Temerty Faculty of Medicine, University of Toronto, Toronto, ON, Canada
4 Department of Anesthesiology and Pain Medicine, Temerty Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada

Introduction: Maternal hypotension during caesarean deliveries (CD) is seen in about 80% of cases. To combat this, vasopressors are administered to constrict blood vessels and increase blood pressure, however the adrenergic receptors specific for these drugs are also expressed on the smooth muscle layer of the uterus (myometrium).
The objectives of this study are: 1) Assess the effects of ephedrine, phenylephrine, and norepinephrine on the contractility of human myometrium with and without oxytocin. 2) Determine the biomolecular signalling pathways of vasopressors on myometrial contractility through protein and gene analysis.
Methods: 1) An ex-vivo study was conducted using myometrial biopsies obtained from elective CD. Tissues were dissected into 8 strips and suspended individually in an organ bath apparatus containing physiological salt solution. Strips were equilibrated in solution for 2hrs or until spontaneous contractions developed, then cumulative dose-response of vasopressor from 10-10 to 10-6 M was conducted every 10 minutes in the presence or absence of oxytocin. The primary outcome was the motility index (amplitude * frequency). 2) An in-vitro study using isolated human smooth muscle cells and whole tissue biopsies is currently being conducted through western blot, immunocytochemistry, and RT-qPCR.
Resslts: Results show that administration of vasopressors did not significantly increase motility index. However, co-administration of ephedrine and oxytocin significantly increased motility index ($p<0.05$), whereas co-administration of phenylephrine or norepinephrine did not significantly increase motility index.
Conclusion: Co-administration of ephedrine and oxytocin after delivery has the potential to increase myometrial contractility and reduce the risk of uterine atony during cesarean delivery.

P1-7: Effects of ketamine enantiomers and their HNK metabolites on hippocampal synaptic transmission and plasticity

Muchun Han1,2 Patrick J. Tidball2, John Georgiou2,3, Graham L. Collingridge1,2,3
1 Department of Physiology, University of Toronto, Toronto, Ontario, M5S 1A8, Canada
2 Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Sinai Health System, Toronto, Ontario, M5G 1X5, Canada
3 TANZ Centre for Research in Neurodegenerative Diseases, University of Toronto, Ontario, M5S 1A8, Canada

Ketamine, an anesthetic and recreational psychedelic, at lower doses acts as a rapid and persistent antidepressant after a single dose in patients with treatment-resistant major depressive disorder. While several hypotheses have been proposed to explain its acute action, the mechanism underlying its persistent effect is unknown and there is controversy about whether ketamine acts directly or via its metabolism to (2R,6R)-hydroxynorketamine (HNK). Extending our previous work (Kang et al., 2020; PMID: 33088919), we explored whether ketamine itself, as an NMDA receptor (NMDAR) antagonist, has synaptic effects that could explain its long-lasting antidepressant action independent of (2R,6R)-HNK. Using CA3-CA1 field potential recordings in stratum radiatum of mouse hippocampal slices, we show that (S)- and (R)-ketamine substantially inhibited synaptic NMDAR-mediated responses and LTP, without affecting basal AMPA receptor (AMPAR)-mediated transmission. Surprisingly, we found that a short (20 min) application of (S)-ketamine leads to a sustained depression of NMDAR-mediated transmission that persists after drug washout. Conversely, the ketamine metabolites (2S,6S)- and (2R,6R)-HNK had only minor effects on NMDAR transmission and plasticity and, in contrast to previous reports, did not modulate basal AMPAR transmission or paired-pulse facilitation. Together, our findings suggest that a long-lasting modulation of synaptic NMDAR function may contribute to ketamine's sustained actions, independently of its metabolism to HNK.

P1-8: Nephrectomy Impacts Physiological Mechanisms that Support Rat Brain Oxygenation Following Acute Hemodilution.

Chin, Kyle 1,2; Jiang, Helen 2; Joo, Sarah 2; Steinberg, Benjamin E 1,3; Goldenberg, Neil M 1,3; Baker, Andrew J 2,4,5; Mazer, C David 1,2,5; and Hare, Gregory MT1,2,4.
1 Department of Physiology, University of Toronto, Toronto, ON, Canada
2 Department of Anesthesia, St Michael's Hospital, Toronto, ON, Canada
3 Department of Anesthesia and Pain Medicine, The Hospital for Sick Children, The University of Toronto, Toronto, ON, Canada
4 Keenan Research Centre for Biomedical Science in the Li Ka Shing Knowledge Institute, St. Michael's Hospital, Toronto, ON, Canada.
5 Institute of Medical Science, University of Toronto, Toronto, ON, Canada

Anemia is associated with acute kidney injury (AKI) and stroke in perioperative patients [1-3]. There is also evidence to support that the kidneys are a critical sensor of changes in blood oxygen content and may initiate cardiovascular responses to preserve brain oxygenation during anemia. We performed acute nephrectomy to test the hypothesis that the kidney is essential in maintaining brain tissue oxygen tension during acute hemodilutional anemia.
We performed bilateral nephrectomy or sham surgery followed by acute isovolemic hemodilution of 50% of the estimated blood volume on otherwise healthy Sprague-Dawley rats. We measured heart rate, mean arterial pressure, brain microvascular pO2, arterial blood gases and cooximetry. Heart rate and brain microvascular pO2 were lower at baseline (after nephrectomy and prior to hemodilution) in nephrectomised rats. Following hemodilution, brain microvascular pO2 decreased further in nephrectomised rats compared to baseline and sham. The decrease resolved 40 minutes after hemodilution.
Bilateral nephrectomy resulted in changes in baseline physiology and resulted in an acute reduction in brain microvascular pO2 after acute hemodilution supporting the hypothesis that the kidney contributes to maintain brain oxygenation during acute anemia. The kidney's capacity as an oxygen sensor [4,5] may contribute to this function. Further assessment of physiological parameters, including cardiac output and cerebral blood flow and systemic biomarkers of tissue hypoxia, may help elucidate the mechanism(s).

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P1-9: Breakdown of the apical spectrin network leads to endothelial dysfunction and vascular stiffening in pulmonary arterial hypertension

Sonja Sulstarova1,2, Anna Foley1,2, Sivakami Mylvaganam1,3, Benjamin E Steinberg1,2,4,5, Spencer Freeman1,3 Neil M Goldenberg1,2,4,5
1 Program in Cell Biology, Hospital for Sick Children;
2 Department of Physiology, The University of Toronto;
3 Department of Biochemistry, University of Toronto
4 Department of Anesthesia and Pain Medicine; 5Department of Anesthesiology, The University of Toronto

Pulmonary arterial hypertension (PAH) is a devastating disease, characterized by endothelial dysfunction and lung

vascular remodeling. In PAH, endothelial cells hyperproliferate, have imbalanced production of vasoactive molecules, and exhibit abnormal responses to shear stress. Recently, a critical role for a spectrin cytoskeletal network in sensing of shear and regulation of vascular stiffness has been described in systemic endothelial cells. The loss of spectrin resulted in the disappearance of caveolae, dysregulated eNOS activation, and a lack of calcium response to shear. Since spectrin can be broken down in vivo by calpain, which is activated in PAH, we hypothesized that spectrin breakdown may represent a key mechanism underlying endothelial dysfunction in PAH patients and animal models. The presence of an apical spectrin network in primary human pulmonary artery endothelial cells, as well as rat pulmonary arterioles, was confirmed by immunostaining. The lungs of monocrotaline (MCT) treated rats, a model for PAH, displayed elevated activity of calpain and caspase-3, both of which function to degrade spectrin. Furthermore, there was a reduction in full-length spectrin and increase in spectrin breakdown products in the lungs of MCT rats as compared to controls. In MCT rats, immunostaining revealed a decrease in spectrin expression, along with decreased levels of caveolin-1 compared to controls. Furthermore, spectrin knockdown caused internalization of caveolin-1, loss of shear-stimulated eNOS activation, and increased cellular stiffness. Our studies detail a critical role for the spectrin cytoskeleton in the development of endothelial dysfunction and highlight the therapeutic potential of preventing its breakdown via calpain inhibition.with increased risk of developing heart failure but also associated with increased mortality. The interplay between the heart and the kidney is coined as cardiorenal syndrome, wherein the diseased state of one organ influences the state of the other. Of particular interest, there are two known biomarkers, namely: (i) fibroblast growth factor 23 (FGF23) and (ii) Klotho that have been shown to have a reciprocal and potential role in in patients with renal disease displaying LVH; however, their exact mechanism of action has not been clearly delineated. MicroRNAs (miRs) are small non-coding RNA fragments that are 22 nucleotides in length that bind and hinder the expression of their desired target mRNA at the post-transcriptional level. While there are a few specific miRs that have been identified in patients with LVH and CKD, the role of these specific miRs remains unclear. Therefore, we are assessing the differential expression of circulating miRs in patients of two specific groups of dialysis: (i) conventional hemodialysis, and (ii) frequent hemodialysis. We will focus the specific miRs that may regulate the FGF23/Klotho axis, aiming to develop potential therapeutic targets to mitigate the development of LVH in patients in ESRD.

P1-10: Top-down control of human motor thalamic neuronal activity during the auditory oddball task

Frhan I. Alanazi 1,2 Suneil K. Kalia 2, Mojgan Hodaie 2 , Adriana Lopez Rios 3, Andrés M. Lozano2, Luka Milosevic2, William D. Hutchison1,2.

1 Department of Physiology, University of Toronto, Canada

2 Krembil Brain Institute, Leonard St, Toronto Ontario, Canada

3 Hospital Universitario San Vicente Fundación, Medellin (Rionegro), Colombia

The neurophysiology of selective attention in visual and auditory systems has been studied in animal models but not with single unit recordings in human. Here, we recorded neuronal activity in the ventral intermediate nucleus as well as the ventral oral anterior, and posterior nuclei of the motor thalamus in 25 patients with parkinsonian (n = 6) and non-parkinsonian tremors (n = 19) prior to insertion of deep brain stimulation electrodes while they performed an auditory oddball task. In this task, patients were requested to attend and count the randomly occurring odd or "deviant" tones, ignore the frequent standard tones and report the number of deviant tones at trial completion. The neuronal firing rate decreased compared to baseline during the oddball task. Inhibition was specific to auditory attention as incorrect counting or wrist flicking to the deviant tones did not produce such inhibition. Local field potential analysis showed beta (13–35 Hz) desynchronization in response to deviant tones. Parkinson's disease patients off medications had more beta power than the essential tremor group but less neuronal modulation of beta power to the attended tones, suggesting that dopamine modulates thalamic beta oscillations for selective attention. The current study demonstrated that ascending information to the motor thalamus can be suppressed during auditory attending tasks, providing indirect evidence for the searchlight hypothesis in humans. These results taken together implicate the ventral intermediate nucleus in non-motor cognitive functions, which has implications for the brain circuitry for attention and the pathophysiology of Parkinson's disease.

P1-11: NINJ1-mediated Plasma Membrane Rupture leads to Tumor Lysis Syndrome

K.P.B Fuerte1,2, A. Volchuk2, J.P. Borges3, N.M. Goldenberg1,2,4,5
1 Department of Physiology, University of Toronto, Ontario, Canada.
2 Program in Cell Biology, SickKids Research Institute, Toronto, Ontario, Canada.
3 Program in Neuroscience and Mental Health, Hospital for Sick Children, Toronto, Ontario, Canada.
4 Department of Anesthesia and Pain Medicine, The Hospital for Sick Children, Toronto, Ontario, Canada.
5 Department of Anesthesiology and Pain Medicine, University of Toronto, Ontario, Canada

Tumor lysis syndrome (TLS) is commonly seen in patients with hematological or solid organ cancers, and occurs when tumor cells rapidly lyse and release their intracellular contents. This can occur either spontaneously or in response to chemotherapy. TLS causes potentially fatal complications, including hyperkalemia, hypocalcemia, hyperphosphatemia, and hyperuricemia, leading to arrhythmias, seizures, and organ failure. Currently, preventative or therapeutic measures against TLS are limited, and establishing such a treatment is needed. NINJ1, a cell-surface transmembrane protein, has recently been shown to be necessary for plasma membrane rupture (PMR) following a variety of injurious stimuli, including chemotherapeutic agents. Using a variety of tumor cell lines, we now demonstrate that NINJ1 is expressed across many human tumors. Knockout of NINJ1 in mouse bone marrow-derived macrophages inhibits chemotherapy-induced cell rupture. In response to cytotoxic drugs, substantial cell lysis occurs in these tumor cells, as indicated by lactate dehydrogenase (LDH) release. Taken together, our data suggest a role for NINJ1 in tumor cell rupture, which we will explore in animal models of TLS in the future, in order to design NINJ1-targeted therapies to prevent or treat TLS.

P1-12: Age-related increases in right hemisphere dominance during prosodic processing in children

Kristen E. Li1,2, Andrew Dimitrijevic1,3,4, Karen A. Gordon2,4, Elizabeth W. Pang2,5, Hansel M. Greiner6,7, Darren S. Kadis1,2
1 Department of Physiology, University of Toronto, Toronto, ON, Canada
2 Neurosciences and Mental Health, Hospital for Sick Children, Toronto, ON, Canada
3 Department of Otolaryngology, Sunnybrook Health Sciences Centre, Toronto, ON, Canada
4 Department of Otolaryngology, University of Toronto, Toronto, ON, Canada
5 Division of Neurology, Hospital for Sick Children, Toronto, ON, Canada
6 Division of Neurology, Cincinnati Children Hospital Medical Center, OH, United States
7 Department of Pediatrics, College of Medicine, University of Cincinnati, OH, United States

Language comprehension is a complex process involving an extensive brain network. Brain regions responsible for prosodic processing have been studied in adults; however, much less is known about the neural bases of prosodic processing in children. Using magnetoencephalography (MEG), we mapped regions supporting speech envelope tracking (a marker of prosodic processing) in 80 typically developing children, ages 4 to 18 years, completing a stories listening paradigm. Neuromagnetic signals coherent with the speech envelope were localized using dynamic imaging of coherent sources (DICS). Across the group, we observed coherence in bilateral perisylvian cortex. We found age-related increases in coherence to the speech envelope in the right superior temporal gyrus ($r = 0.31$, $df = 78$, $p < 0.05$) and primary auditory cortex ($r = 0.27$, $df = 78$, $p < 0.05$); age-related decreases in coherence to the speech envelope were observed in the left superior temporal gyrus ($r = -0.25$, $df = 78$, $p < 0.05$). This pattern may indicate a refinement of the networks responsible for prosodic processing during development, where language areas in the right hemisphere become increasingly specialized for prosodic processing. Altogether, these results reveal a distinct neurodevelopmental trajectory for the processing of prosodic cues, highlighting the presence of supportive language functions in the right hemisphere. Findings from this dataset of typically developing children may serve as a potential reference timeline for assessing children with neurodevelopmental hearing and speech disorders.

P1-13: The effect of synthetic glucocorticoid exposure on expression and activity of P-glycoprotein and breast cancer resistance protein in human fetal brain endothelial cells

Nikola Ivanovski, 1,2 Phetcharawan Lye, 1,2 Hafsah Mughis,1,2 Stephen Matthews. 1,2
1 Dept. of Physiology, University of Toronto, Toronto, ON, Canada;
2 Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada

Introduction: P-glycoprotein (P-gp, ABCB1) and breast cancer resistance protein (BCRP, ABCG2) are two primary multidrug transporters involved in neuroprotection at the developing blood-brain barrier (BBB). These proteins efflux a variety of substrates, including synthetic glucocorticoids (sGCs), a class of drugs routinely administered to women at risk of preterm labour. While sGCs have been shown to regulate P-gp and BCRP in other tissues, there is limited information about the effect of sGCs on these proteins at the human fetal BBB. We hypothesized that treatment with the sGC dexamethasone (DEX) will alter P-gp and BCRP expression and activity in human fetal brain endothelial cells (hfBECs) isolated from 2nd trimester fetuses.
Methods: hfBECs (N=6) were cultured and treated with DEX (10-9M-10-7M). P-gp and BCRP activity were assessed at 4h, 24h, and 48h after treatment through intracellular accumulation of fluorescent substrates Calcein-AM and Ce6, respectively. Expression of ABCB1 and ABCG2 were assessed at 24h and 48h after treatment by qPCR (10-8M-10-7M).
Results and Discussion: DEX treatment down-regulated expression of ABCG2 at both doses and timepoints and up-regulated P-gp activity at 4h (10-7M) compared to vehicle. This is the first study to investigate the effects of DEX on P-gp and BCRP in hfBECs. Future experiments will assess the effect of DEX on P-gp and BCRP protein levels as well as glucocorticoid-responsive genes in hfBECs. A better understanding of P-gp and BCRP regulation by sGCs at the developing BBB may lead to strategies for preserving neuroprotection and improving neurodevelopmental outcomes.

P1-14: Regulation of TGF-β Signalling and its Impact on Energy Homeostasis in Hypothalamic Neurons

Aws Mustafa 1 , Wenyuan He 1 , Denise D. Belsham 1,2,3 .
1 Physiology,
2 Obstetrics and Gynaecology,
3 Medicine, University of Toronto, Toronto, ON, Canada

Obesity represents a growing epidemic that arises from disruptions to whole-body energy homeostasis and altered circadian rhythms. Central to its etiology is the overexposure of the hypothalamus, the chief regulator of body weight and food intake, to free saturated fatty acids, notably palmitate. Palmitate-induced neuroinflammation damages neurons by triggering the release of pro-inflammatory cytokines and overactivation of immune cells. Recent studies suggest a potential involvement of transforming growth factor beta (TGFβ) in the development of hypothalamic neuroinflammation. To understand the regulation and function of TGFβ signalling in the hypothalamus, we first examined the mRNA expression of various TGFβ- related genes in immortalized hypothalamic neurons. TGFβ1, TGFβ2, TGFβ3, TGFβ1R, and TGFβ2R were robustly expressed in mHypoA-BMAL1-WT/F, mHypoA-BMAL1-KO/F, and mHypoE-46 hypothalamic neurons. 24 hours treatment of 50 μM palmitate significantly altered, TGFβ2, and TGFβ1R expression. Notably, palmitate robustly altered TGFβ2, with an apparent phase-shifting effect. To determine the rhythmicity of TGFβ2 expression, a synchronized time- course was conducted in mHypoA-BMAL1-WT/F and mHypoA-BMAL1-KO/F cells. TGFβ2 is rhythmically expressed in both cell lines, according to circadian-detection algorithms Cosinor, Lomb-Scargle, and JTK Cycle. These results suggest the existence of a BMAL1-independent rhythmic system that regulates TGFβ2. Experiments are ongoing to assess the effects of TGFβ2 treatment on gene expression involved in energy homeostasis. Overall, this study discovered rhythmic expression of TGFβ2 and demonstrated that palmitate disrupts TGFβ signaling in hypothalamic neurons. A better understanding of the underlying mechanisms that link nutrient excess to hypothalamic dysfunction is critical for the development of effective prevention and treatment strategies.

P1-15: Elucidating NINJ1 Protein-Protein Interactions

Suhel Patel1,2, Allen Volchuk1,2, Benjamin Steinberg1,2,3,4, Neil Goldenberg1,2,3,4
1 Department of Physiology, University of Toronto, 1 King's College Circle, Medical Sciences Building, Toronto, ON, M5S 1A8, Canada.

2 Program in Cell Biology, Hospital for Sick Children, 686 Bay St, Toronto, ON, M5G 0A4, Canada.
3 Department of Anaesthesia and Pain Medicine, Hospital for Sick Children
4 Department of Anaesthesiology and Pain Medicine, University of Toronto

The cell surface protein, NINJ1, was recently identified as having a critical role in mediating plasma membrane rupture (PMR) during lytic cell death. PMR results in the release of inflammatory cytokines and large damage associated molecules such as HMGB1 into the extracellular environment which enhance the pro-inflammatory response and damage host tissue. Furthermore, a more recent study has demonstrated that during lytic cell death NINJ1 forms a high molecular weight multimer. However, despite intense interest in NINJ1 mediated PMR, the molecular mechanism by which NINJ1 clusters, and the components comprising these clusters remain unknown. Biotin antibody recognition (BAR) is a proximity based labelling approach which is both a sensitive and specific method available to detect putative interactors and proximal proteins in the vicinity of a specific protein of interest. Here, we show using the BAR approach, that NINJ1 clustering on the plasma membrane is associated with a previously unidentified protein intermediate which interacts with NINJ1 and modulates its clustering upon encounter with a lytic cell death stimulus. Identification of NINJ1-associated proteins will provide insight into the molecular mechanism of NINJ1-clustering mediated PMR and will present new targets for the development of novel therapeutics used for cell and tissue preservation.

P1-16: Identifying the expression of CEPT- 1 enzyme/gene in pancreatic islets and its role in the development of type 2 diabetes

Yasmin Rabiee 1 , Ishnoor Singh 1 , Julie Van 1 , Alpana Bhattacharjee 1 , Yihan Luo 1 , Wenyue Ye 1 , Feihan Dai 1 , Michael B. Wheeler 1,2
1Department of Physiology, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada
2 Metabolism Research Group, Division of Advanced Diagnostics, Toronto General Research Institute, Toronto, Ontario Canada

Located on ER and nuclear membranes, CEPT-1 is the final enzyme in phospholipid biosynthesis pathway that converts 1,2-Diacylglycerol (DAG) to Phosphatidylcholines (PC) and Phosphatidylethanolamines (PE) which are the two main phospholipids in eukaryotic cells. CEPT-1 was identified as being a dysregulated protein in a cohort of females who went through gestational diabetes mellitus (GDM) and progressed to type 2 diabetes (T2D). CEPT-1 has been found to play a role in regulating insulin secretion, but its role in insulin sensitivity and its mechanism of action in T2D is not well understood. In this study, we first investigated the expression levels of CEPT-1 in mouse and human pancreatic islets. Its gene expression was confirmed by performing PCR and qPCR in mouse and human islets. To determine the protein expression, we performed western blots on the same set of samples. To localize CEPT-1 in pancreatic islets, we conducted immunocytochemistry on our samples. After analyzing the images quantitatively, the results showed that CEPT-1 is found in more than 90% of both α-cells and β-cells. Based on our findings and previous studies we proposed that β-cell specific knockout of the CEPT-1 gene could help understand the role of CEPT-1 in T2D. After acquiring the mice, we will test them and their control littermates for glucose tolerance and insulin sensitivity using glucose and insulin tolerance tests. We will measure serum insulin levels and perform histological analysis of pancreatic tissue to assess changes in β-cell function and mass.

P1-17: The Effect of Prenatal THC and CBD Exposure on Neuroanatomy and Behaviour in the Adult Rodent

Megan Drupals 1,2 , Janice Hicks 1 , Shoshana Spring 3 , John G. Sled 3,4 , Brian J. Nieman 3,4 , Benjamin Steinberg 1,2 , Michael Salter 1,2
1 Neuroscience and Mental Health, The Hospital for Sick Children, Toronto, ON, Canada;
2 Department of Physiology, University of Toronto, Toronto, ON, Canada
3 Department of Medical Biophysics, University of Toronto, ON, Canada
4 Translational Medicine, The Hospital for Sick Children, Toronto, ON, Canada

Introduction: Existing human and rodent studies have found some convergent and some contradictory findings on the impact of prenatal cannabinoid exposure on offspring. Using a mouse model developed to reflect pre- and peri-gestational THC and CBD exposure, we examined the effects on neuroanatomy and a range of behaviours in young adult offspring. Methods: Beginning 7 days prior to mating and continuing throughout pregnancy, female mice were subcutaneously injected once daily with either 5mg/kg THC, 60mg/kg CBD, or vehicle. At 9 weeks of age, offspring brains were imaged using MRI. A separate cohort of mice underwent a battery of behavioural tests which included accelerod, elevated plus maze (EPM), open field, and novel object recognition (NOR). Results: No differences in brain structure volumes were detected when comparing drug-exposed adult offspring brains to vehicle controls in neither males nor females. However, we found sex- specific behavioural differences in EPM and NOR which point to alterations in anxiety and novelty-seeking behaviour. In EPM, male, but not female CBD- and THC-exposed animals spent significantly more time in the open arms compared to vehicle controls, indicating a lower anxiety response (ANOVA with post-hoc, p<0.05). In NOR female CBD-exposed mice displayed an altered object interaction time course favouring exploration of the novel object (ANCOVA, p<0.05). Conclusion: Gestational exposure to cannabinoids affects behaviour in adult rodents. We found a reduction in anxiety-like behaviour in THC and CBD-exposed male mice, and an increase in novelty-seeking behaviour in CBD-exposed females. These behavioural changes occurred without alterations in the volume of brain regions, none of which were significantly different across groups when measured using MRI.

P1-18: Investigating the role of key sarco(ends)plasmic reticulum shaping proteins on cardiomyocyte structure and development

Kateleen Jia 1,2 , Michelle Di Paola 1,2 , Cristine J. Reitz 1,2 , Allen C.T. Teng 1,2 , Anthony O. Gramolini 1,2
1 Department of Physiology, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada M5S 1M8
2 Translational Biology and Engineering Program, Ted Rogers Centre for Heart Research, Toronto, Ontario, Canada M5G 1M1

The sarco(endo)plasmic reticulum (SR/ER) is an essential organelle in cardiac muscle cells that regulates many vital functions, such as Ca 2+ cycling, muscle contractility, organelle communication, protein synthesis and trafficking. The depletion of the key structural proteins, REEP5, RTN4, ATL3, and CKAP4, cause structural disorganization of the adult SR/ER, however the development of the SR/ER structure in neonatal cardiomyocytes is poorly understood. We assessed the hypothesis that the depletion of structural proteins would prevent the proper formation of the structure of the SR/ER in developing cultured mouse neonatal cardiomyocytes (CMNC). Through confocal microscopy of wild type CMNCs at days 2, 4, and 7 in culture, the developmental maturation of the SR/ER structural development was evaluated. Further investigations will assess the effect of single gene knockdowns of each structural protein of interest (REEP5, RTN4, ATL3, and CKAP4) via lentiviral shRNA constructs, to assess their individual effects on SR/ER structural development. The single gene knockdown models will be examined for cell viability as well as ER stress activation, at each point of development. The findings will provide a detailed timeline of the structural development of the SR/ER and may reveal the effects of critical structural proteins during the developmental time period.

P1-19: Ex vivo perfusion of the native and humanized murine pancreas to model Type 1 Diabetes.

Marwa Sadat 1,2 , Amanda Fantin 1,2 , Jorge Castillo-Prado 1,2 , Amatullah Fatehi 1,2 , Yvonne Ping 1 , Ian M. Rogers 1,2,3,4
1 Department of Physiology, University of Toronto, Toronto, ON, M5S 1A8, Canada
2 Lunenfeld-Tanenbaum Research Institute, Toronto, ON, M5G 1X5, Canada
3 Soham & Shaila Ajmera Family Transplant Centre, University Health Network, Toronto, ON, M5G 2C4, Canada
4 Department of Obstetrics and Gynecology, University of Toronto, Toronto, ON, M5G 1E2, Canada

Pancreatic diseases present a significant global health challenge, with a high unmet therapeutic need due to a lack of proper disease modeling and the inefficacy of current treatments. The bioengineering of a pancreas through the combination of decellularized donor organs and patient-derived stem cells presents a promising therapeutic approach. An acellular

pancreas that incorporates the extracellular matrix (ECM) has the potential to facilitate organ-specific differentiation without the need for additional growth factors. While pluripotent stem cells do not differentiate to pancreas cells when cultured in the ECM, recellularization of lung ECM with definitive endoderm (DE) cells has been successful. Thus, we hypothesize that the integration of human iPSC-derived DE cells into the acellular mouse pancreas will result in mature pancreatic tissue. In this study, we will decellularize the pancreas using our optimized protocol, and we will repopulate the acellular mouse pancreas both through the vasculature and the ductal system. The recellularized organs will grow while kept in our laboratory's ex vivo organ perfusion system and their function will be tested through a glucose-stimulated insulin secretion test. We will also use both the native and humanized pancreas to generate a type 1 diabetes mellitus (T1DM) disease model. Thus far, we have successfully created a T1DM model in the native pancreas via treatment with the beta cell cytotoxin, streptozotocin, as evident by β cell-specific death. Together, these studies will not only allow for successful generation of ex vivo models of pancreatic disease but will also aid in efforts for generation of patient-specific organs for transplant.

P1-20: Conserved early upregulation of prefrontal cholinergic signalling across different species and models of Alzheimer's disease

Saige Power , Dr. Sridevi Venkatesan , Madeleine Ridgway , Sarah Qu , Janice McNabb , Prof. JoAnne McLaurin , Prof. Evelyn Lambe

Deficits in attention and executive function occur relatively early in Alzheimer's disease (AD). To interrogate the system most relevant for this cognitive decline, we examine the impact of early AD pathology on the cholinergic excitation of the prefrontal cortex. We take advantage of models of AD in two different species to examine early-disease changes: the TgF344-AD rat model that recapitulates the human trajectory of AD pathology, and a compound transgenic mouse that permits optogenetically-triggered release of endogenous acetylcholine (opto-ACh) in the presence of TgCRND8-AD pathology. In the AD rat, we find a significant, unexpected enhancement of deep-layer pyramidal neuron responses to exogenous acetylcholine. This change is specific to the early-disease state, as it is not observed in younger or older rats. We then use the opto-ACh AD mouse to investigate disease impact on cholinergic synaptic function. Paralleling the AD rat results, the opto-ACh AD mouse shows a significant upregulation of the prefrontal cholinergic response in early-to-mid-disease that is lost in older animals. Since upregulation of cholinergic signalling in AD models of both species is observed before cognitive deficits become overwhelming, we hypothesize that a form of molecular compensation may be at work. We are therefore probing the underlying mechanisms to identify new treatment targets to ameliorate the disruption of attention and executive function in AD.

P1-21: Associating Ion Channel Alternative Splicing With Neuronal Intrinsic Electrophysiological Properties Using Patch-seq

Nuo Xu, Melanie Davie, Derek Howard, Nayeon Hyun, Shreejoy Tripathy. Centre for Mental Health and Addiction.

The small intestine detects nutrients such as lipids to regulate feeding and glucose homeostasis by activating a gut-brain axis. Although the specific mechanisms are not fully elucidated, small intestinal lipid sensing pathways involve vagal afferent signalling to the hindbrain to lower food intake and glucose production in chow but not high-fat (HF) fed conditions. We have previously shown that metformin, a first-line therapy for obesity-associated diabetes that also lowers food intake, enhances glucose-sensing in the upper small intestine (USI) to lower glucose production in HF-fed rats. However, whether metformin enhances USI lipid sensing to regulate feeding in HF-fed rats is unknown. We infused 10% Intralipid into the USI of HF rats and performed refeeding studies. First, we found that USI Intralipid vs. saline infusion suppressed food intake by ~30% in chow-fed rats during refeeding. In 3d HF-fed rats exhibiting hyperphagia prior to fasting, USI Intralipid vs. saline failed to suppress food intake, indicating that short-term HF feeding disrupts USI lipid

sensing and dysregulates feeding. Importantly, metformin (50 mg/kg) administered 1d prior rescued the USI Intralipid effect by suppressing feeding by ~22%. Of note, the 1d metformin pretreatment per se did not alter food intake in rats that received a USI saline infusion the next day. Thus, we have found that metformin enhances USI lipid sensing to lower food intake in short-term HF feeding. Our future studies will elucidate the underlying mechanism of this interaction, potentially revealing novel targets for lowering food intake and weight in obesity-associated type 2 diabetes.

P1-22: Identifying novel intracellular interactions driving membrane-bound tumour necrosis factor reverse signalling

Yasaman Mostafaie1,2, Julia Hanchard1,3,4, Darcy Lidington1-4, Steffen-Sebastian Bolz*1-4
1 Department of Physiology, Faculty of Medicine, University of Toronto;
2 Ted Rogers Centre for Heart Research;
3 Toronto Centre for Microvascular Medicine at TBEP, University of Toronto;
4 Qanatpharma Canada Ltd.

Elevated total peripheral resistance (TPR) is a hallmark of many cardiovascular diseases and furthers disease progression. We previously demonstrated that myogenic tone, mediated by membrane bound tumour necrosis factor (mTNF) reverse signalling, is a primary modulator of TPR. Thus, inhibiting mTNF reverse signalling should reduce TPR with the potential to improve disease outcome. However, since mTNF is a ubiquitously expressed protein with critical biological functions, its indiscriminate inhibition is prone to cause widespread adverse effects. Therefore, therapeutics aiming to reduce myogenic tone and hence, TPR must target other elements of mTNF reverse signalling. To identify these unknown molecular contributors to mTNF reverse signalling, we employed proximity-dependent biotinylation coupled to mass spectrometry (BioID). Our search for proteins interacting with mTNF's cytoplasmic domain identified 42 high confidence hits. Gene ontology analysis revealed that molecularly, these proteins are structural constituents of the cytoskeleton involved in actin and cell adhesion molecule binding. This aligns well with our underlying hypothesis that smooth muscle mTNF functions as a mechanosensor that tethers to its receptors and initiates myogenic vasoconstriction in response to changes in transmural pressure. GO analysis also identified proteins involved in MAPK and RhoA signalling pathways, which are critically involved in myogenic vasoconstriction. The most abundant hit within proximity of mTNF is a filamentous cytoskeletal protein that plays a crucial role in maintaining the structural integrity and stability of cells. This protein has also been shown to interact with essential proteins involved in different cell signalling pathways, namely, RhoA signalling and Calcium-dependent signalling pathways. Thus, we hypothesize that this protein is essential for formation of signalling complexes with mTNF and interacts with other proteins to activate reverse signalling. Other notable hits include phosphatases and regulatory proteins known for modulating G-protein, MAPK, and Rho signaling. Future Directions: We will employ a bioluminescence-based approach to quantify the strength of interactions detected by BioID, and identify small molecule inhibitors disrupting these protein-protein interactions. These newly discovered inhibitors could potentially be developed into novel therapeutics targeting augmented myogenic tone and hence, elevated TPR to improve disease management and clinical outcome of cardiovascular diseases.

P1-23: Metformin Action and Upper Small Intestinal Lipid Sensing in Feeding Regulation

Rachel Kuah1,2, Daniel Barros1,2, Song-Yang Zhang1, Tony K.T. Lam1,2, 3, 4, 5
1 Department of Physiology, University of Toronto, Toronto, Canada
2 Toronto General Hospital Research Institute, UHN, Toronto, Canada
3 Department of Medicine, University of Toronto, Toronto, Canada
4 Institute of Medical Science, University of Toronto, Toronto, Canada
5 Banting and Best Diabetes Centre, University of Toronto, Toronto, Canada

The small intestine detects nutrients such as lipids to regulate feeding and glucose homeostasis by activating a gut-brain axis. Although the specific mechanisms are not fully elucidated, small intestinal lipid sensing pathways involve vagal afferent signalling to the hindbrain to lower food intake and glucose production in chow but not high-fat (HF) fed conditions. We have previously shown that metformin, a first-line therapy for obesity-associated diabetes that also lowers

food intake, enhances glucose-sensing in the upper small intestine (USI) to lower glucose production in HF-fed rats. However, whether metformin enhances USI lipid sensing to regulate feeding in HF-fed rats is unknown. We infused 10% Intralipid into the USI of HF rats and performed refeeding studies. First, we found that USI Intralipid vs. saline infusion suppressed food intake by ~30% in chow-fed rats during refeeding. In 3d HF-fed rats exhibiting hyperphagia prior to fasting, USI Intralipid vs. saline failed to suppress food intake, indicating that short-term HF feeding disrupts USI lipid sensing and dysregulates feeding. Importantly, metformin (50 mg/kg) administered 1d prior rescued the USI Intralipid effect by suppressing feeding by ~22%. Of note, the 1d metformin pretreatment per se did not alter food intake in rats that received a USI saline infusion the next day. Thus, we have found that metformin enhances USI lipid sensing to lower food intake in short-term HF feeding. Our future studies will elucidate the underlying mechanism of this interaction, potentially revealing novel targets for lowering food intake and weight in obesity-associated type 2 diabetes.

P1-24: Gene co-expression profiles characterize neuronal cell-cell spatial relationships

Ameer Sarwar ,2, Xiaoyin Chen3, Stephan Fisher4,5, Tony Zador4, & Jesse Gillis1,2,4,6,7 *;
1 Department of Cell and Systems Biology, University of Toronto,
2 Terrence Donnelly Center for Cellular & Biomolecular Research, University of Toronto,
3 Allen Institute for Brain Science,
4 Cold Spring Harbor Laboratory,
5 Institut Pasteur,
6 Department of Physiology, University of Toronto, 7 Department of Molecular Genetics, University of Toronto
* Correspondence: jesse.gillis@utoronto.ca

Neurons within a tissue communicate with each other and form functional networks. However, proximal cells are more likely to form networks given spatial constraints on signaling1. Using mouse whole-brain single-cell gene expression data2, we explore co-localization of gene pairs to probe neuronal cell-cell spatial relationships. Since spatial co-localization between genes is equivalent to their co-expression across cells, we compute pairwise correlations between genes at the single-cell resolution. Next, we group physically proximal or transcriptionally similar cells and compute pairwise correlations using group-averaged values. We find that correlations using groups of proximal cells become progressively positive or negative with larger group sizes. However, no trend is observed for groups of transcriptionally similar cells. To assess the robustness of our results, we permute cell labels and find no relationship, indicating that gene co-expression within local cell groups exhibits bona fide signals.

We next find gene pairs with progressively positive or negative correlations at larger group sizes and explore their co-expression profiles at single-cell resolution. Compared to those of random gene pairs, we observe highly stratified and boundary co-expression profiles for the positive and negative pairs, respectively, showing that our method uncovers spatially well-delineated patterns. Indeed, we find similar profiles using cells that express one or both genes in a pair, indicating that our approach is robust to zero-inflated data. After registering and annotating our data with the Allen Institute's Common Coordinate Framework version 3 (CCFv3) mouse brain atlas3, we are investigating in 3D brain region-specific or global co-expression patterns, a study that will be complete by the time of the poster presentation.

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P1-25: Regulation of Insulin Signaling by the miR-16 Family in Hypothalamic Neuronal Models.

Wenyuan He1, Neruja Loganathan1, Kimberly W.Y. Mak1, Emma K. McIlwraith1, and Denise D. Belsham1,2,3
1 Physiology,
2 Obstetrics and Gynaecology,
3 Medicine, University of Toronto, Toronto, ON, Canada

Insulin has a critical role in regulating food intake and peripheral metabolism through signaling via INSR and IGF1R in hypothalamic neurons. The development of cellular insulin resistance in hypothalamic neurons is a significant contributor to obesity and its associated comorbidities, but the molecular changes to neuronal insulin signaling are not fully understood. This study aimed to understand the involvement of microRNA (miRNAs), which inhibit specific protein expression, in the regulation of insulin signaling and resistance in hypothalamic neurons.
To profile miRNAs in the whole hypothalamus and immortalized hypothalamic neuronal cell lines, the Affymetrix-GeneChip-miRNA-4.0 Array was used. Notably, the miR-16 family, including miR-15b-5p and miR-322-5p, was highly expressed miRNAs in each case. To study the involvement of miRNAs in hyperinsulinemia-induced neuronal insulin resistance, the mHypoE-46 neurons were treated with 100 nM insulin for 24 hours induce cellular insulin resistance, as characterized by decreased INSR-beta protein and a resulting decrease in insulin-induced phosphorylation of AKT. Insulin overexposure upregulated miR-15b-5p, which induced insulin resistance when overexpressed in mHypoE-46 neurons. Additionally, overexpression of miR-322-5p in mHypoA-59 neurons decreased the Igf1r mRNA and the INSR-beta protein. The results of this study suggest that the miR-16 family, particularly miR-15b-5p and miR-322-5p, inhibit insulin signaling, and changes in miRNA expression are associated hypothalamic cellular insulin resistance. Additionally, the identification of additional miRNAs associated with neuronal insulin resistance and ongoing functional validations will also be presented. The knowledge derived from these studies will provide insight into miRNA-based diagnostics and therapeutics for early central insulin resistance in humans.

P1-26: Static Uterine Stretch Polarizes Peripheral Human Monocytes into M1 Macrophage in Preparation for Labor

Adam Boros-Rausch1,2, Lubna Nadeem1, Oksana Shynlova*1,2,3 & Stephen Lye*1,2,3.
1 LTRI at SHS, ON, Canada;
2 Depts of Physiology and
3 Ob/Gyn, U of T, Toronto, ON, Canada

Our previous data has shown that a crosstalk between macrophages and uterine myocytes (MYO) enhances myometrial contractility. Macrophages could be polarized to M1 (pro-inflammatory) and M2 (anti-inflammatory) phenotypes depending on tissue environment. We hypothesised that stretch- induced myometrial cytokines contribute to monocytes differentiation into macrophages and polarization to M1 or M2. Myometrial biopsies were collected from elective caesarean section (term not in labor, TNL). Myocytes were plated on the Collagen I-coated Flexcell plates (200,000/well) and subjected to static mechanical stretch (23% elongation) for 24 hours using Flexcell system FX-5000. Conditioned media from non-stretched (NS-CM) and stretched (SCM) MYO were collected. Maternal peripheral blood was collected from pregnant TNL women, mononuclear cells (PBMCs) were isolated and cultured with NS-CM, SCM, MCM or control serum-free media (SFM) for 7 days. Flow Cytometry was used to characterize cell differentiation and polarization. Monocytes treated for 7 days with MCM show a dramatic 156.8-fold increase in CD68 macrophage marker as compared to negative control SFM; M1 marker HLA-DR show 344.5-fold increase, and 19.5-fold increase in CD80. M2 marker CD206 increased 33-fold, and CD163 - 2.9-fold. Treatment of monocytes with SCM/NS-CM for 7 days both caused 2.7-fold increase in CD68 as compared to SFM. SCM treatment induced macrophage polarization manifested by 525.5-fold increase in M1 marker HLA-DR, and 53.9-fold increase of CD80 as compared to NS-CM treated monocytes. M2 markers CD206 and CD163 did not change significantly by SCM. This work points to a putative mechanism leading to macrophage polarization in the uterus and myometrial activation in preparation for labor onset.

P2-1: An ex-vivo perfusion method for the mouse uterus

Yvonne Ping, HBSc Student [1,2], Jorge Castillo Prado, MSc Student [1,2], Ian Rogers, PhD [1,2,3]
1 Department of Physiology, University of Toronto, Toronto, Ontario, Canada M5S 1A1
2 Sinai Hospital, LTRI, Toronto, Ontario, Canada M5G 1X5
3 Department of Obstetrics and Gynecology, University of Toronto, Toronto, Ontario, Canada M5S 1A1

Studying embryonic implantation and developmental events can be challenging due to the inaccessible nature of embryos. Current imaging methods only provide snapshots of development and require removing the uterus from the mouse, potentially impeding growth. Isolation and growth of post-implantation embryos ex-utero have proven to be difficult and negate important fetal-placenta-uterus interactions. An ex-vivo organ perfusion (EVOP) that can support the murine uterus for studying embryo development in real-time is needed. A uterus EVOP system would provide easier access to the embryos for imaging and real-time analysis whilst maintaining the embryos in a physiologic environment for growth. This culture system will also enable the study of blastoid implantation and the role of the uterus in embryonic development. For this, the uterus is isolated, and the abdominal aorta (AA) is cannulated and connected to a bioreactor previously developed by the Rogers Lab. The ideal culture medium for the mouse uterus is also being determined through the testing of different culture media to meet its metabolic needs; essential amino acids, hemoglobin, and nutritional supplements can be added. To study the performance of the ex-vivo cultured uterus, oxygen consumption is measured using oxygen sensors at the aorta and vena cava. Additionally, H&E and Caspase-3 staining are used for histopathological analysis of organ viability and culture media compatibility. The ex-vivo uterus culture will enable observation of fetal-placenta-uterus interactions and development in real-time, which are currently inaccessible both in situ and in vitro. This system will help us understand fundamental questions of developmental biology.

P2-2: Targeting excessive cell-surface expression of α5GABAA receptors with a novel peptide to mitigate perioperative neurocognitive disorders

MeiFeng Yu1, Anthony Ariza1, Setareh Malekian Naeini1, Li Ju1, Gang Lei1, Dian-Shi Wang1, Lilia Kaustov3, Beverley A. Orser1,2,3
1 Departments of Physiology
2, Anesthesiology & Pain Medicine University of Toronto, Toronto, ON, Canada
3 Department of Anesthesia Sunnybrook Health Sciences Centre, Toronto, ON, Canada

INTRODUCTION: Excessive α5GABAA receptor (α5GABAAR) activity is associated with a variety of cognitive, developmental and mood disorders. Currently, there are limited strategies to treat or prevent these disorders. Cell-surface expression of α5GABAARs is regulated by radixin, a cytosolic protein that anchors α5GABAARs. Disrupting the interaction between radixin and α5GABAARs may reduce cell-surface expression. Our lab designed a peptide (US patent 10,981,954) that mimics the binding site of radixin on α5GABAARs to disrupt this interaction.
METHODS: Cultured hippocampal neurons were prepared from embryonic mice and cultured for 13–15 days. Neurons were treated with ifenprodil, an NMDA receptor antagonist that increases cell-surface expression of α5GABAARs, or vehicle for 24 h. A subset of neurons were cotreated with ifenprodil and TAT-peptide or TAT-scrambled peptide for 24 h. Cell-surface expression of α5GABAARs was assessed using multicolor immunofluorescent staining and biotinylation assays.
RESULTS AND CONCLUSIONS: Ifenprodil increased cell-surface expression of α5GABAARs identified by both immunofluorescence and biotinylation assays. Ongoing experiments will determine whether the peptide prevents the increase in cell-surface expression. We anticipate that our results will elucidate a promising novel strategy to treat disorders associated with excessive α5GABAAR activity.

P2-3: Investigating the potential role of purine signalling in the modulation of NPY by a plant phytohormone.

Calvin V. Lieu 1 , Denise D. Belsham 1, 2, 3
1 Department of Physiology, University of Toronto, Ontario, M5S 1A8, Canada
2 Departments of Medicine, University of Toronto, Ontario, M5S 1A8, Canada
3 Departments of Obstetrics and Gynaecology, University of Toronto, M5S 1A8, Canada

Compound X (CX) is a plant phytohormone that controls plant cell differentiation and division. Recent studies in mammalian tissue have uncovered potential anti-aging, antioxidant, and anti-inflammatory effects. We hypothesized that CX may modulate expression of the orexigenic feeding neuropeptide NPY through purinergic signalling. To assess the effects of CX on Npy expression, we treated multiple Npy-expressing hypothalamic neuronal models, mHypoE-41, mHypoE-44, and mHypoE-46, with increasing concentrations of CX in a 24-hour timecourse. We then measured Npy mRNA expression via RT-qPCR. We found that CX was able to decrease Npy mRNA expression in the mHypoE-46 and mHypoE-44 lines but increased it in the mHypoE-41 line. We investigated the involvement of two P2 purine receptor candidates by cotreating our cell lines with endogenous purine agonists, ATP or UDP, and CX for 16 hours. We found that ATP was able to induce Npy expression in the cell lines treated but was unable to block the effects of CX. Treatment with UDP did not affect the basal expression of Npy but was able to block the suppression of Npy in the mHypoE-44 line. To investigate the involvement of P1 purine signalling, we generated a P1 purine receptor knockout cell line using CRISPR-cas9 and treated the cells with CX in a 24-hour timecourse. Our results demonstrate the ability of CX to modulate Npy expression and contribute to the expanding list of signalling molecules that target hypothalamic neurons and modulate feeding.

P2-4: Acellular EVLP Perfusate Silences Pro-inflammatory Signaling in Human Lung Endothelial and Epithelial Cells

Jamie E. Jeon 1,2 , Lei Huang 1 , Zhiyuan Zhu 1 , Aaron Wong 1 , Shaf Keshavjee 1,2 , & Mingyao Liu 1,2
1 Latner Thoracic Surgery Research Laboratories, University Health Network;
2 Temerty Faculty of Medicine, University of Toronto

Background: Ex vivo lung perfusion (EVLP) with acellular Steen solution is a novel platform that allows evaluation and repair of marginal donor lungs to mitigate lung ischemia-reperfusion (IR) injury. EVLP has shown promising clinical outcomes so far. To reveal cell-type-specific molecular responses to IR and EVLP, we performed transcriptomic analyses on human lung endothelial and epithelial cell cultures simulating the two processes. Methods: We first incubated human pulmonary microvascular endothelial cells (HPMEC) and human lung epithelial cells (BEAS-2B) in DMEM+10%FBS at 37°C until sub-confluent. Next, the cells underwent 18H of cold ischemia in Perfadex solution at 4°C. Then they were perfused for 4H with either DMEM+10%FBS (IR model) or Steen (EVLP model) at 37°C. RNA samples were collected after 18H of cold ischemia and 4H of reperfusion and EVLP. Total bulk RNA sequencing data were analyzed with R, GSEA, and Cytoscape to identify differentially expressed genes and enriched pathways. Results: Endothelial and epithelial cells had significant changes in their gene expressions after IR and EVLP simulations. Pathway enrichment analyses revealed significant upregulation of pro-inflammatory signaling and vascular process and downregulation of various cell metabolism pathways in the IR models. In contrast, the inflammation and vascular process responses were significantly absent in the EVLP models. Conclusion: Human lung endothelial and epithelial cells were activated by IR, but EVLP with acellular Steen solution kept the cells from inducing inflammatory responses. This finding may help explain how acellular EVLP impacts different cell types that result in great clinical outcomes.

P2-5: The role of GABAA receptor overactivity in cognitive impairment after surgery

Joycelyn Ba,1 Shahin Khodaei,1 Anthony Ariza,1 Setareh Malekian Naeini,1 Dian-Shi Wang,1 Beverley A. Orser1,2,3

1 Department of Physiology, University of Toronto, Toronto, Ontario, M5S 1A8, Canada
2 Department of Anesthesiology & Pain Medicine, University of Toronto, Toronto, Ontario, M5G 1E2, Canada
3 Department of Anesthesia, Sunnybrook Health Sciences Centre, Toronto, Ontario, M4N 3M5, Canada

BACKGROUND: Up to one third of adult surgical patients experience cognitive complications after surgery. Despite its prevalence, the underlying causes are poorly understood. One potential mechanism is an overactivity of extrasynaptic GABAA receptors which manifests as increased tonic currents and cognitive deficits. We previously showed that general anesthetic drugs cause sustained GABAA receptor overactivity that persists long after the drugs have been eliminated from the body. However, the role of surgery is not well understood and there are currently no studies that have measured GABAA receptor activity after surgery. The goal of this study was to characterize extrasynaptic GABAA receptor activity in the hippocampus after anesthesia and surgery.
METHODS: Adult mice underwent laparotomies under isoflurane or sevoflurane anesthesia. Physiological parameters were monitored during surgery. Ex vivo hippocampal slices were prepared 48 to 72 h later. GABAA receptor expression was quantified using cell-surface biotinylation and Western blotting. Tonic inhibitory currents were recorded in hippocampal neurons with whole-cell patch clamp techniques.
RESULTS: Cell-surface expression of extrasynaptic GABAA receptors increased following anesthesia and surgery. GABA antagonist caused a greater decrease in current noise in the slices from surgical mice compared to control, consistent with the hypothesis of greater GABAA receptor function. In addition, there was a trend towards a greater amplitude of tonic inhibitory current in surgical mice.
CONCLUSIONS: These results suggest that a sustained overactivity of GABAA receptors occurs postoperatively and GABAA receptors may be targeted to treat postoperative cognitive deficits.

P2-6: Using whole decellularized mouse kidney scaffolds to direct human endothelial progenitor cells towards a mature and functional vasculature in an ex vivo culture system

Anupama Bhadwal,1,2 Yuchao Niu,1,2 Jorge Castillo-Prado,1,2 Ian Rogers1,2,3,4

1 Department of Physiology, University of Toronto, Toronto, ON, M5S 1A8, Canada
2 Lunenfeld-Tanenbaum Research Institute, Toronto, ON, M5G 1X5, Canada
3 Soham & Shaila Ajmera Family Transplant Centre, University Health Network, Toronto, ON, M5G 2C4, Canada
4 Department of Obstetrics and Gynecology, University of Toronto, Toronto, ON, M5G 1E2, Canada

Over 950 million people globally suffer from chronic kidney disease, however treatment options remain limited. Consequently, there is a high demand for bioartificial kidneys which can be faithful research models. Our aim is to assess the ability of a decellularized mouse renal ECM scaffold to provide human mesoderm cells with critical site-specific cues for adherence, differentiation, and growth, resulting in a mature and functional vasculature. Once a vasculature is established it will allow for future work into recellularizing the epithelium to create a functional organ. Mouse kidney scaffolds are decellularized via perfusion with 0.05% sodium dodecyl sulphate (SDS) at ~0.12mL/min for 24hrs, followed by perfusion with phosphate buffered saline for another 24hrs. Our data shows that the low SDS concentration and flow rate allow for effective cell clearance while maintaining the ECM microarchitecture and glycosaminoglycan (GAG) levels. GAGs sequester site specific growth factors which guide cell differentiation, thus their retention post decellularization is crucial. For revascularization, human endothelial progenitor cells are injected into the renal scaffold via the renal artery. Previous work in the lab has found mesoderm cells derived from human stem cells to be a good starting point for our purposes. The recellularized kidney is cultured for various time points in a bioreactor previously developed in the Rogers lab, specifically designed for mouse kidneys. The tissue is analyzed using immunohistochemistry to determine tissue health (caspase-3), cell proliferation (Ki67), and maturation (CD31, CD144, VEGF). Microarrays/single nucleus RNA sequencing is conducted to identify endothelial populations present within the scaffold.

P2-7: Role of AMPA glutamate receptors in hippocampal synaptic plasticity and neuronal activation during learning and memory

Radu Gugustea

Neurodegenerative disorders with a memory deficit component such as Alzheimer's disease are becoming increasingly common in our aging society. It is believed that memory is physically represented within specialized structures between neurons known as synapses. Through various mechanisms and the involvement of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (AMPARs), synaptic strength can be robustly increased or decreased to produce long-term potentiation (LTP) and depression (LTD), respectively. Learning induces neuronal activation within the brain, but a mechanistic link between synaptic plasticity and learning-induced cell activation remains unknown. This study uses male and female adult age- matched wildtype and transgenic mice featuring genetically altered AMPARs and impaired LTP and LTD to determine the role of AMPAR-mediated changes in synaptic plasticity on learning- induced cell activation. It is hypothesized that AMPARs are altered specifically in learning- induced active cells and that the genetic modification of AMPARs to impair synaptic plasticity will impair the activation of neuronal assemblies following learning. Mice injected with an activity-dependent viral marker into the hippocampus, a critical memory structure, underwent contextual fear conditioning to form a long-term context-dependent memory. Here we report that transgenic mice deficient in long-term contextual fear memory featured reduced learning- induced active cells in the hippocampal dorsal CA1 region relative to wildtype littermates. Follow-up studies are underway to examine potential morphological changes in dendritic spines as well as associated functional impairments using ex vivo whole-cell recordings.

P2-8: Title: Associations of the Infant Nasal Microbiome with Environmental Factors and Asthma Risk Running Title: Infant nasal microbiome, Environmental Factors and Asthma Risk

Authors: Yu Chen Qian1,2, Ruixue Dai2, Anastasia Teterina3, Kelsey Fehr4,5, Myrtha E. Reyna2, Charisse Peterson6, Stuart E Turvey6, Piush Mandhane7 , Elinor Simons8, Wendy Lou3, Meghan Azad8, Marek Smieja8, Jakob Stokholm9 , Jonathan Thorsen9, Michael Surette10, Theo Moraes2 , Padmaja Subbarao1,2

1 Department of Physiology, University of Toronto, Toronto, ON, Canada
2 Division of Respiratory Medicine, Department of Pediatrics, Hospital for Sick Children & Research Institute, Toronto, Ontario, Canada
3 Dalla Lana School of Public Health, University of Toronto, ON, Canada
4 Children's Hospital Research Institute of Manitoba, Winnipeg, MB, Canada
5 Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, MB, Canada
6 Department of Pediatrics, Child & Family Research Institute, BC Children's Hospital, University of British Columbia, Vancouver, British Columbia, Canada
7 Department of Pediatrics, University of Alberta, Edmonton, AB, Canada
8 Department of Pediatrics and Child Health, Children's Hospital Research Institute of Manitoba, University of Manitoba, Winnipeg, Manitoba, Canada
9 COPSAC, Copenhagen Prospective Studies on Asthma in Childhood, Herlev and Gentofte Hospital, University of Copenhagen, Copenhagen, Denmark
10 Faculty of Health Science, McMaster University, Hamilton, Ontario, Canada

BACKGROUND: The nasal microbiome is the collection of microbes that live on the surface of the nose and sinuses. These microbes are shaped by numerous environmental factors and play a role in immune response and disease susceptibility. While it is known that viruses play an important role in asthma; their role in shaping the infant nasal microbiome is unclear. Similarly, the role of the nasal microbiome on respiratory outcomes in childhood are unclear.
AIM: To identify the association between the infant nasal microbiome, viral presence, and asthma risk.
METHODS: The data used in this study were collected from the CHILD Cohort Study, a large Canadian prospective longitudinal cohort. Anterior nares nasal swabs were obtained at a routine visit from infants at 3 & 12 months of age. Bacteria and viruses were identified using 16S rRNA gene sequencing polymerase chain reaction (PCR), respectively. Bacteria were clustered into operational taxonomic units at 99% similarity. Asthma risk at 3 years of age was defined using

a symptom-based risk assessment score.
RESULTS: The infant nasal microbiome was associated with many environmental factors including prenatal smoke exposure and older siblings. Infants with viral presence had decreased alpha diversity and increased Moraxella sp.8 (3 months) and Haemophilus sp.41 (12 months). Finally, infants with high asthma risk had multiple deferentially abundant OTUs at 12 months.
CONCLUSIONS: The infant nasal microbiome is shaped by many infant, maternal and environmental factors including presence of virus. Additionally, dsybiosis of the microbiome is associated with high asthma risk.

P2-9: Comparison of Different Sample Preparation Methods for Plasma Peptide Profiling

Yihan Luo, Julie Van, Michael Wheeler
Endocrine and Diabetes Research Group, Department of Physiology, Faculty of Medicine, University of Toronto

Peptides are often generated as part of the maturation and activation steps of zymogens, prohormones, and other protein precursors by proteolytic activity. These shortened protein fragments therefore reflect the interplay between protein levels and proteolytic machinery of a given tissue. Changes in peptide concentration and profiles in plasma may hold considerable promises in identifying disease-specific biomarkers and therapeutic targets using a non-invasive approach. Despite the technological advances in proteomics, endogenous peptide detection and quantitation from human plasma have been thwarted by the presence of high-abundant proteins, lipids, and other masking compounds. Peptidomics has therefore utilized diverse peptide enrichment methods such as protein precipitation, size exclusion chromatography, solid phase extraction (SPE), and fractionation. To date, the number of plasma peptides identified by label-free quantitation methods varies a lot. It is therefore critical to optimize for peptide coverage for large-scale human plasma peptidomics studies. Here, we compared trichloroacetic acid (TCA) precipitation and sequential precipitation and delipidation (SPD) methods for human plasma peptide enrichment. As a result, TCA method quantified more peptides that SPD (608 vs. 287), and the Pearson correlation of TCA ranges from 0.92 to 0.96 which is higher than the SPD method (0.68 to 0.86). Additionally, 58% of the peptides quantified using TCA had a coefficient variation lower than 20%, whereas all the peptides quantified by SPD had CV greater than the 20% threshold. In conclusion, the TCA method showed higher plasma peptide enrichment with higher reproducibility and quantitative sensitivity. These findings allow subsequent large-scale disease-based peptidomic analysis more robust.

P2-10: GLP-1R is required for resveratrol in exerting its metabolic beneficial effects in HFD challenged male mice

Jia Nuo Feng1,2, Weijuan Shao2, and Tianru Jin1.2
1 Department of Physiology, Temerty Faculty of Medicine, University of Toronto, Toronto, Canada.
2 Division of Advanced Diagnostics, Toronto General Hospital Research Institute, University Health Network, Toronto, Canada.

The beneficial effects of dietary polyphenols including resveratrol have been elucidated during the past 2-3 decades, yet the mechanism underlying their metabolic functions remains elusive or even controversial. Recent investigations revealed that hepatic FGF21 is the common target for both GLP-1RAs and dietary polyphenol intervention. Here we utilized wild type (WT) and GLP-1R-/- mice to access whether GLP-1R is required for resveratrol to exert its beneficial effects. In WT male mice with HFD challenge, concomitant resveratrol intervention (REV-I) for 12 weeks reduced body weight gain and improved glucose tolerance, while in male GLP-1R-/- mice, such metabolic effects were lost. In WT mouse liver and epididymal white adipose tissue (eWAT), REV-I ameliorated HFD-induced FGF21 resistant and stimulated a battery of genes that are involved in lipid homeostasis. In addition, HFD-induced alterations on expressions of a battery of adipose tissue specific genes including those encode for leptin and adiponectin were reversed by concomitant REV-I. REV-I was also shown to exert anti-inflammatory effects in the ileum of WT mice fed with HFD. Specifically, genes that encode for a battery of pro-inflammatory markers including IL-1β and IFN-γ were significantly elevated by HFD challenge, while REV-I attenuated the expression. HFD challenge also reduced IL-10 mRNA level in the WT mouse ileum, while REV-I restored the expression. Together, we bring a novel player GLP-1R to explore the mechanism underlying the metabolic functions of

resveratrol. Collectively, we conclude that the metabolic beneficial effects of resveratrol on reducing body weight gain and improving glucose disposal require GLP-1R.

P2-11: Mechanism of Rac1 in Contributing to LTP and Social Memory Impairments in Alzheimer's Disease

Haorui Zhang 2 ; Haiwang Zhang 1 ; Dongju Lee 1 ; Zhengping Jia 1,2
1 Program in Neuroscience & Mental Health, Sick Kids Research Institute, Toronto, Canada
2 Department of Physiology, University of Toronto, Toronto, Canada

Alzheimer's disease (AD) is a neurodegenerative disease with memory loss, including social memory. The memory loss in AD is believed to associated with synaptic deficit caused by accumulated amyloid-beta (Aβ) peptides. Long-term potentiation (LTP), a long-lasting synaptic mechanism believed to underlie memory, has been widely reported to be impaired in AD. However, how Aβ peptides lead to synaptic impairment and memory loss is unclear. Rac1 is a Rho family small GTPase, serving as a signalling center in regulating actin dynamics and kinase activity, and plays an important role in LTP and memory. Here we show the abnormally elevated Rac1 activity in the hippocampus of APP/PS1 mouse associates with the deficit of LTP in the ventral CA3-CA1 and social memory. Suppressing Rac1 activity via overexpressing its dominant negative mutant in the ventral hippocampus could rescue those deficits. Since our recording experiments suggested unchanged basal transmission and presynaptic function in 3–4-month APP/PS1 mouse, we hypothesized that the elevated Rac1 in APP/PS1 mouse impairs memory and LTP via affecting the activity-dependent surface expression and phosphorylation of AMPA receptors (AMPAR), key mediators of synaptic plasticity. Meanwhile, by creating and overexpressing a novel synthesized protein with Rac1 fused with a biotin ligase, we also aim to screen the dysregulated Rac1 upstream factors by using mass spectrometry. Ultimately, results from this study will reveal new mechanisms by which Rac1 dysfunction contributes to the pathogenic process of AD and may provide new therapeutic strategies for the development of drugs to treat memory impairments in AD.

P2-12: "Keeping a native pig kidney alive as long as possible in the ex vivo perfusion system by optimizing oxygenation"

Chun Tat Lui, Ian Rogers

The goal in our lab is to generate a transplantable human organ using a patient's cells and decellularized pig organs as the substrate. To achieve this goal, we are working on ex-vivo perfusion organ culture as a way to grow and maintain the new organ, which is an essential part of producing artificial organs. My project aims to improve our existing bioreactor system in usability & stability, medium flow, media supplementation, oxygenation and contamination. I redesigned our previous bioreactor to facilitate medium flow. This also included adding flow sensors to investigate the maximum flow rate and to achieve a consistent flow rate to the kidney. Vasodilator, as a supplement, was used to solve the vasoconstriction. Oxygen sensors help me to monitor oxygen consumption during ex vivo organ perfusion. To optimize oxygenation of the medium, I am testing including hemoglobin and altering the length and arrangement of the oxygenator's silicone tubing, for example, parallel or series. Lastly, I will identify the osmolarity of the medium we use and make adjustments to the nutrient content. I successfully maintained a native pig kidney using the ex vivo perfusion system for 24 hours.

P2-13: Temporal dynamics of neuronal excitability in the lateral amygdala mediates allocation to an engram supporting conditioned fear memory

Annelies Hoorn1,2, Asim Rashid1, Paul Frankland1,2,3,4 and Sheena Josselyn1,2,3,4.
1 Program in Neurosciences &Mental Health, The Hospital for Sick Children (SickKids), 555 University Avenue, Toronto, Ontario M5G 1X8, Canada.
2 Department of Physiology, University of Toronto, 1 King's College Circle, Toronto, Ontario M5S 1A8, Canada.
3 Department of Psychology, University of Toronto, 1 King's College Circle, Toronto, Ontario M5S 1A8, Canada.
4 Institute of Medical Science, University of Toronto, 1 King's College Circle, Toronto, Ontario M5S 1A8, Canada.

Memories are encoded by ensembles of neurons (engrams) that are active during learning. Neurons important in an engram are sparsely distributed across the brain. Within a given brain region, eligible neurons compete for allocation to an engram and neurons with increased excitability at the time of training are likely to be allocated to the engram. Neurons with increased excitability during training also have increased excitability for ~6h. Two separate but similar training episodes within a 6h period tend to co-allocate to a similar population of neurons and remembered together. We examined the temporal dynamics of neuronal excitability important for memory allocation. We expressed both an excitatory and inhibitory opsin in the same sparse subset of LA neurons. At different times before fear conditioning, we optically activated this subset of neurons to allocate them to the engram. To examine whether these neurons were critical components of the engram, we tested mice both with and without optical inhibition. We find that stimulation up to 6h, but not 24h before training biases neuronal allocation to the engram. Next, we tested what happens under basal conditions, whereby we labeled intrinsically active neurons at different timepoints prior to learning, using a calcium indicator. We inhibited these neurons during testing and found that intrinsically active neurons 1h, but not 24h prior to learning, indeed encode the memory shown by successful inhibition. Taken together, these findings indicate that excitability in the LA is temporally defined and plays a critical role in neuronal selection to a fear engram.

P2-14: Investigating aT-Catenin Phosphorylation and Evaluating its Cardioprotective Effects in Heart Failure

Daniel Davoudpour, Cristine J. Reitz, Uros Kuzmanov, Anthony O. Gramolini
Department of Physiology, Faculty of Medicine, Translational Biology and Engineering Program, Ted Rogers Centre for Heart Research, University of Toronto, Toronto, Ontario, Canada M5S 1M8

New approaches for understanding the underlying molecular mechanisms of heart failure are crucial for identifying new targets for therapy. Our lab previously conducted a phosphoproteomic screen on myocardial tissue from human heart failure patients and identified aT-catenin as a cardiac-enriched protein that is hyperphosphorylated at 5 sites in dilated cardiomyopathy. aT-catenin has been demonstrated to have cardioprotective effects after heart injury via the Hippo/YAP pathway; however, the role of phosphorylation is unknown. We hypothesize that genetic and pharmacological manipulation of aT-catenin phosphorylation can benefit cardiac structure and function in disease. First, to identify the putative enzymes involved, we performed bioinformatics-based kinase prediction analyses (35 unique kinases predicted). Next, we developed a stable HEK-293T aT-catenin overexpression cell line (2.92±0.14-fold increase vs. control). Thirdly, initial phosphoproteomic screening identified 1/5 phosphosites of interest (S637) by mass spectrometry, with future studies evaluating phosphorylation status following pharmacological targeting of the predicted kinases. Future aims will also assess the cardioprotective effects of aT-catenin phosphorylation in vivo, using AAV9-mediated delivery of wildtype and phospho-null aT-catenin and pharmacologic kinase targeting to evaluate cardiac structure and function post-transverse aortic constriction. Ultimately, understanding how aT- catenin phosphorylation can improve cardiac function will provide insight into new therapies for cardiovascular disease.

P2-15: Neural entrainment of a naturalistic conversation in varying working memory loads

Priyanka Prince 1,2, Joseph Chen 3,4, Trung Le 3,4, Vincent Lin 3,4, and Andrew Dimitrijevic 1,2,3,4
1 Evaluative Clinical Sciences Platform, Sunnybrook Research Institute, Toronto, ON Canada
2 Department of Physiology, University of Toronto, Toronto, ON, Canada
3 Otolaryngology—Head and Neck Surgery, Sunnybrook Health Sciences Centre, Toronto, ON, Canada
4 Faculty of Medicine, Otolaryngology—Head and Neck Surgery, University of Toronto, Toronto, ON, Canada

In a noisy environment with auditory and visual distractions, selective attention to target stimuli can be cognitively demanding, especially in individuals with a hearing impairment or using hearing prostheses such as a cochlear implant (CI). CI users have been shown to rely more on visual input for the understanding of speech stimuli; this can result in an increased listening effort and therefore, more resources utilized from a limited cognitive load. The neural basis of this

relationship between cognition and speech perception and understanding is not fully understood. In this study, using a high-density electroencephalogram (EEG) in CI users, we investigated the neural correlates of speech entrainment to two people having a conversation with background multi-talker noise whilst visual digits appeared on the screen around them. The participant's task was to answer conversation content questions and recall the digits that were presented. Three memory loads were assessed: zero, three, and seven visual digits. Behavioural results showed that as visual load increases, performance on recall for both the conversation and digits decrease. The degree of neural entrainment varied as a function of memory load such that a larger memory load resulted in lower neural tracking. These data provide evidence that natural conversations can be used as a stimulus when probing speech-related cognitive functions in noise listening and working memory.

P2-16: Molecular Mechanisms Driving Maternal Cardiac Hypertrophy in Pregnancy

Zi Qi Lin 1 and Brian Cox 1,2
1 Department of Physiology, University of Toronto
2 Department of Obstetrics and Gynecology, University of Toronto

The maternal cardiovascular system remodels during pregnancy to support the increasing circulatory needs of the growing fetus. In particular, the maternal heart hypertrophies throughout gestation, increasing size, muscle mass, and cardiac output up to 50%, and this change naturally returns to baseline post-partum. Maladaptation of this process is associated with potentially lethal hypertensive disorders of pregnancy, peri- and post-partum cardiomyopathy, and earlier onset of chronic cardiovascular disease, but little is known about the underlying molecular mechanisms. We aim to characterize gene networks driving pregnancy-induced cardiac remodeling by time-series RNA sequencing of the mouse maternal left ventricle on gestational days 8.5 (e8.5), e10.5, and e14.5, along with non-pregnant controls. As e10.5 was found to show the earliest detectable morphological change, these chosen timepoints represent pre-remodeling, early-remodeling, and mid-remodeling stages, respectively. Preliminary gene set enrichment analyses show upregulation of carbohydrate catabolism and mitosis pathways in the pre- remodeling stage. Early-remodeling is characterized by a downregulation in protein synthesis, while mid-remodeling shows decreases in lipid, steroid, and amino acid processing. Interestingly, only the increase in glucose metabolism stays consistent through time. These changes in the transcriptome suggest that pregnancy-induced cardiac remodeling is rapid and dynamic, featuring unique gene signatures at different timepoints. Additional time series echocardiography will help relate these findings to functional changes throughout gestation. This work aims to better understand pregnancy-induced cardiac hypertrophy, which can be translated not only into disease understanding of pregnancy disorders, but also new therapeutic approaches for cardiovascular pathologies with similar phenotypes of cardiac hypertrophy.

P2-17: Developmental defects in nanoscale reorganization of AMPARs and quantal transmission in a mouse model of fragile X syndrome

Megan Drupals 1,2 , Janice Hicks 1 , Shoshana Spring 3 , John G. Sled 3,4 , Brian J. Nieman 3,4 , Benjamin Steinberg 1,2 , Michael Salter 1,2
1 Department of Physiology, University of Toronto
2 Program in Neurosciences and Mental Health, SickKids Research Institute

Excitatory synapses undergo rapid remodeling during early sensory development by changing the abundance, composition, and nano-organization of postsynaptic glutamate receptors to enable neurotransmission. Dysregulation of synaptic remodeling can lead to neurodevelopmental disorders such as fragile X syndrome (FXS), caused by a mutation in the gene encoding fragile x mental retardation protein (FMRP). It is unknown how FMRP deletion impacts the nano-organization of AMPARs and quantal transmission during early synaptic development. Using the calyx of Held synapse in the auditory brainstem, where AMPARs undergo a developmental subunit switch from slow-gating GluA1- to fast-gating GluA4-dominant, we applied expansion microscopy (ExM) to map nanoscale differences in subsynaptic localization of

GluA1- and GluA4-AMPARs between wild-type (WT) and Fmr1-/- mice at pre- (P8-10) and post-hearing (P16-19) stages. We see partially mismatched GluA1- and GluA4-AMPAR nanodomains, supporting the bimodal distribution of fast and slow mEPSCs in WT mice, which is altered in Fmr1-/- synapses. The bimodal distribution of fast and slow mEPSCs in immature Fmr1-/- synapses phenocopies that of mature WT synapses. Basal mEPSC frequency was significantly higher, and less sensitive to an elevation of extracellular Ca2+ in Fmr1-/- synapses, indicating altered presynaptic remodeling. Our study suggests that the loss of FMRP accelerates developmental remodeling of both pre- and postsynaptic elements underlying quantal transmission, implicating the critical role of FMRP in controlling the pace of activity-dependent synaptic maturation.

P2-18: Characterization of a common CERS2 polymorphism and links to type 2 diabetes development

Wenyue Ye1, Alpana Bhattacharjee1, Yasmin Rabiee1, Ishnoor Singh1, Julie A. D. Van1, Michael B Wheeler1,2
1Department of Physiology, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada
2 Metabolism Research Group, Division of Advanced Diagnostics, Toronto General Research Institute, Toronto, Ontario Canada

Ceramide Synthase 2 (CERS2) gene encodes for a dihydroceramide synthase, which is involved in the biosynthesis process of very-long-chain sphingolipids from ceramide, and the downregulation of such sphingolipid metabolism pathway is closely associated with the early-stage type 2 diabetes (T2D) pathogenesis. CERS2 has also been identified as one of the novel biomarkers associated with increased risks of T2D development after a gestational diabetes pregnancy. A common single nucleotide polymorphism (SNP; rs267738) was discovered to be strongly associated with reduced CERS2 enzyme functionalities using humanized knock-in Cers2 polymorphism (Cers2pm) mice model, implying the potential metabolic dysfunction, and increased risk for T2D development. In this study, we conducted both intensive in vivo and ex vivo studies to examine the glycemic impact of the female humanized CERS2 polymorphism mice on a C57BL/6 background at 10-14 weeks on a normal chow diet, and the wildtype C57BL/6 mice were used as controls. No significant difference was found in the insulin tolerance tests, but Cers2pm displayed significant glucose intolerance during glucose tolerance tests. Interestingly, in vitro studies revealed greatly reduced insulin-secretion capacity in Cers2pm by performing glucose-stimulated insulin secretion assay from pancreatic islets, whereas no morphological difference of the isolated islets was found. These data suggest the effect of an SNP in a critical risk factor gene on alternating metabolic functions, especially among the female population. Altogether, this study provides us with a more detailed appreciation of the partial loss of CERS2 functionalities and its associated risk for T2D due to the reduced insulin-secreting ability.

P2-19: Inflammasome Activation in Pulmonary Arterial Hypertension: The Role of Gasdermin D

Anna Foley 1,2; Sonja Sulstarova 1,2; Allen Volchuk 1,2 ; Benjamin E Steinberg 1,2,3,4; Neil Goldenberg 1,2,3,4
1 Program in Cell Biology, Hospital for Sick Children;
2 Department of Physiology, The University of Toronto;
3 Department of Anesthesia and Pain Medicine;
4 Department of Anesthesiology, The University of Toronto

Pulmonary arterial hypertension (PAH) is characterized by substantial pulmonary vascular inflammation, which is a target for novel therapeutic strategies to improve PAH patient survival. A critical component of this response involves the inflammasome, a multi-protein complex found in immune and vascular cells. During activation, a pore-forming protein gasdermin-D (GSDMD) oligomerizes in the plasma membrane, allowing IL-1β to exit and propagate the immune response. The GSDMD pore is also required for subsequent pro-inflammatory cell death, termed pyroptosis. Given the importance of IL-1β and other inflammatory molecules in PAH pathogenesis, we wished to study the importance of GSDMD in PAH. Following 4 weeks of hypoxia, GSDMD-/- mice had a significantly lower right ventricular systolic pressure (RVSP) than littermate controls (32.25 vs 43.9 mmHg, p=0.03). Plasma levels of IL-1 receptor antagonist (IL-1RA; marker of IL-1β production) were higher in WT than GSDMD-/-. In the MCT rat PAH model, GSDMD-/- animals had

reduced RVSP and right ventricle hypertrophy, a physiological change commonly seen in disease as a response to pressure loading. To explore the mechanism of inflammasome activation in the context of hypoxia, we examined hypoxic primary BMDM culture during inflammasome LPS-priming. Triggering of inflammasome activation was increased by hypoxia, with elevated cleaved caspase-1 and secreted IL-1β as compared to normoxic controls. Hypoxia itself stimulated pro-IL-1β mRNA upregulation, providing a possible mechanism for the observed phenomenon. Our studies are the first to show a critical role for GSDMD in a PH mouse model. Together, these data demonstrate that GSDMD may be a therapeutic target in PAH.

P2-20: Regulation of neuronal development by a two-pore potassium channel

Neeraja Ramakrishnan(1,3), Jun Meng(3), Ben Mulcahy(3), Wesley Hung(3), Mei Zhen(1,2,3)
1 Department of Physiology, University of Toronto, Toronto, ON, Canada
2 Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada
3 Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada

The role of neuronal activity in altering structural development of neurons has been extensively studied, but how a key regulator of neuronal activity, membrane excitability, contributes to neural development, is less understood. Previously, we identified TWK-40, a two-pore potassium channel (K2P) in C. elegans, which is expressed in a small subset of neurons, including a pair of premotor interneurons AVA. Using genetic manipulations of twk-40, we showed that changing AVA's RMP alters its motor behaviours: AVA-twk-40(lf) leads to depolarized RMP and increased body motility, whereas AVA-twk-40(gf) leads to hyperpolarized RMP and reduced motility (Meng et al., bioRxiv 2022).
We found that neurons with reduced membrane excitability exhibit morphological deficits, similar to neurons in aged animals. In twk-40(gf) mutants, twk-40 expressing neurons exhibit reduced clustering of a synaptic vesicle marker, swellings along neurites, and fragmented mitochondria. These results suggest that reduced excitability may contribute to neural degeneration. We aim to reveal the effect of excitability on overall circuit morphology and identify specific molecular mechanisms through which excitability regulates synapse and circuit development.

P2-21: Biopsychosocial Subtypes of Paediatric Concussion Predict Behavioural Impairment

Prashanth S Velayudhan1, 2, Shraddha Pai3, 4, Eman Nishat1, 2, Sonja Stojanovski1, 2, Stephanie Ameis1, 5, 6, Keith Yeates7, 8, 9, Anne L Wheeler1, 2
1 Neurosciences and Mental Health, The Hospital for Sick Children, Toronto, Canada
2 The Department of Physiology, University of Toronto, Toronto, Canada
3 The Donnelly Centre, University of Toronto, Toronto, Canada
4 Ontario Institute for Cancer Research, Toronto, Canada
5 Kimel Family Translational Imaging Genetics Research Laboratory, Centre for Addiction and Mental Health, Toronto, Canada
6 Cundill Centre for Child and Youth Depression, Margaret and Wallace McCain Centre for Child, Youth and Family Mental Health, Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, Toronto, Canada.
7 Alberta Children's Hospital Research Institute, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada.
8 Department of Psychology, Faculty of Arts, University of Calgary, Calgary, AB, Canada.
9 Hotchkiss Brain Institute, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada

Background: Neurological outcomes of paediatric concussions are highly heterogeneous. Patient heterogeneity across diverse biological, psychological, and social attributes has made it difficult to know if and why a child will develop a particular impairment. Characterizing patient subtypes is expected to improve our understanding of this heterogeneity, but traditional subtyping methods cannot handle diverse inputs. I hypothesize that similarity network fusion (SNF), a subtyping technique suited for diverse data, will reveal novel subtypes of concussion that are predictive of post-injury impairments.
Methods: 253 children with concussions aged 9-10 years old from the Adolescent Brain Cognitive Development Study were divided into subtypes by SNF using a wide range of biopsychosocial variables. Subtypes were validated in an independent,

held-out sample (N = 67).
Results: Within the original sample, high (n = 114) and low (n = 139) behavioural impairment clusters were found that strongly associated with having any Child Behaviour Checklist impairment (OR = 3.05, 95% CI [1.99, 4.87]). The high impairment cluster had more cases with loss of consciousness (OR = 1.85, 95% CI [1.12, 3.04]) and higher total parent psychopathology scores ($p < 1e-04$; Cohen's $d = 1.04$). These trends persisted in the held-out sample ($p < 0.05$ by permutation testing).
Discussion: Subtyping children with concussion across diverse, non-behavioural inputs can define groups that differ in post-concussive behavioural impairment. These findings suggest loss of consciousness on injury and parent psychopathology may drive post-concussion behavioural impairments. Replication in a second dataset is needed to confirm the clinical significance of these subtypes.

P2-22: Confocal and Mass Spectrometry-based Investigation of REEP5 Depletion by AAV9 in the Mouse Heart

Michelle Di Paola 1,2 , Cristine J. Reitz 1,2 , Uros Kuzmanov 1,2 , Kateleen Jia 1,2 , Allen C.T. Teng 1,2 , and Anthony O. Gramolini 1,2
1 Translational Biology and Engineering Program, Ted Rogers Centre for Heart Research, Toronto, ON M5G 1M1, Canada
2 Department of Physiology, Faculty of Medicine, University of Toronto, Toronto, ON M5S 1M8, Canada

The sarco(endo)plasmic reticulum (SR/ER) is an essential regulator of many key cellular processes, especially those that play a role in the development and progression of cardiac disease. However, many aspects of its structural organization remain poorly defined. REEP5 is a cardiac enriched SR/ER membrane protein, which regulates organization of the highly differentiated SR/ER network and responses to stress. Within cardiomyocytes, Reep5 depletion in vitro results in decreased muscle cell contraction, disrupted Ca 2+ signaling and SR/ER luminal vacuolization. For these studies, in vivo cardiac knock-down of Reep5 in the mouse was achieved using recombinant adeno- associated virus serotype 9 (rAAV9)-mediated gene delivery. Cardiac tissues or isolated cardiomyocytes were harvested at 7 days through to 4 weeks following knock-down, for biochemical and functional assessments. We observed that the largest significant change in REEP5 expression occurred 4 weeks post-rAAV9 injection, correlating to a 78% knock-down, observed by immunoblotting (unpaired t-test, p<0.0001, n=6-8). To assess the biochemical changes induced by Reep5 knock-down, we have established an organelle-specific cardiac proteomic profile, using subcellular fractionation and mass spectrometry (nLC-ESI-MS-HCD-MS). Coupled with high resolution confocal microscopy and 3D mapping, we have examined localization and expression patterns of key SR/ER and mitochondrial proteins, that have altered expression following knock- down of Reep5 at the myocyte-level. These findings provide a detailed understanding of the role that REEP5 plays in maintaining ER homeostasis, SR/ER structure, and general organelle integrity. By identifying the mechanistic significance of REEP5 expression in the heart, we can work to delineate underappreciated pathways in cardiac muscle development.

P2-23: Delineating the potent appetite suppressant effects of a novel phytohormone and its mechanism in defined hypothalamic neurons.

Cindy X. Zhang1, Calvin Lieu1, Denise D. Belsham1,2,3,
1 Physiology,
2 Obstetrics and Gynaecology,
3 Medicine, University of Toronto, Toronto, ON, Canada

Obesity is primarily caused by a positive energy balance where energy intake exceeds expenditure. This balance is tightly regulated by the hypothalamus via the controlled release of feeding-related neuropeptides, like the orexigenic neuropeptide Y (NPY) and agouti-related peptide (AgRP). Our lab recently discovered a novel phytohormone, compound X (CX), that decreases body weight and food intake in mice fed with a 60% high-fat-diet. Moreover, CX robustly reduces Npy expression in mouse immortalized hypothalamic neurons as early as 4 hours after treatment. This study aims to elucidate

the mechanism of CX to promote anorexigenic signals in hypothalamic neurons. RNA- sequencing was performed on NPY/AgRP-expressing cell line, mHypoE-46, upon 4 or 16 hours of 100 μM CX treatment. Candidate pathways were selected via in silico analysis using ErmineR, DAVID, and SwissTargetPrediction. The mTOR pathway was investigated for its high ranking in all algorisms and involvement in energy metabolism. RT-qPCR validation showed that sestrin2 (sesn2), a mTORC1 inhibitor, is significantly enriched over 2-fold in CX-treated neurons. Concurrently, CX upregulated the expression of eukaryotic translation initiation factor 4E (4E- BP1) and unc-51-like autophagy activating kinase (ULK1), which are two direct effectors suppressed by mTORC1. Given the importance of mTOR in feeding regulation, we hypothesized that CX suppresses appetite via inhibiting the central mTOR pathway. Selective activators and inhibitors will be used to confirm the involvement of mTOR in CX signalling. Our study delineates the mechanism of CX in the hypothalamic control of energy balance, providing fundamental insights into a novel, accessible obesity treatment for humans.

P2-24: Automatic synthesis of astrocytic trees in silico through generative modeling

Zhenyang Sun1,2, Maurizio De Pitta1,2
1 Krembil Research Institute, University Health Network
2 Department of Physiology, Temerty Faculty of Medicine, University of Toronto

Astrocytes are prominent glial cells in the brain that shape the anatomy and function of our neural circuits. They do so thanks to their complex branched morphology, which infiltrates the neural tissue and wraps around key neuronal elements, allowing modulation of neural activity by various biochemical pathways through the sites of contact. Characterizing astrocytes branched anatomy is key to understanding their functions in brain circuits. However, there is currently no systematic framework for such a characterization. We present a preliminary automated pipeline to systematically classify astrocyte anatomy based on the extraction of macro- and micro-features from experimental 3D astrocyte tracing. Macro-features are scalar measurements that quantify cell anatomy globally. Micro-features are instead vectorized by the multiple branching compartments of the cell. Deploying techniques from statistics, machine learning, and topological analysis we mine for patterns of macro- and micro-features that completely describe the astrocyte branching architecture. In turn, we use a generative modeling approach to simulate those patterns de novo and synthesize realistic astrocyte membrane scaffolds.

P2-25: Optical interrogation of cholinergic and glutamatergic pre-motor inputs to a medullary circuit essential to rhythmic breathing

Raina Ladha (1), Wenying Liu (1), Hattie Liu (2), Richard Horner (1,2)
1 Department of Physiology, University of Toronto
2 Department of Medicine, University of Toronto

Rationale: Rhythmic motor drive to the upper airway muscles functions to maintain an open airway for effective breathing. This function is compromised by reductions in motor drive during sleep and fails in sleep apnea. The hypoglossal motor nucleus (HMN) is the core of this circuitry but controlling mechanisms have not been identified in-vivo. Hypothesis: We hypothesize that cholinergic and glutamatergic pre-motor inputs to the HMN control endogenous rhythmic motor output during breathing and behavior. Methods: We optically stimulate (10Hz or continuously for 2s, 0-20mW) light-sensitive cation channels on cholinergic and glutamatergic neurons in isoflurane-anesthetized transgenic mice (ChAT-ChR2-EYFP and VGLUT-ChR2-YFP respectively). Stimuli are applied at the HMN and the intermediate reticular nucleus (IRt), the largest source of glutamatergic and cholinergic inputs to the HMN and involved in breathing. Results: We are the first to identify, in-vivo, HMN output responses to optical stimulation of cholinergic neurons at the HMN that are larger than at the IRt (n=15, $P<0.001$; 321% and 216% at 5 and 20mW). HMN output responses to stimulation of glutamatergic terminals at the HMN and IRt neurons were similar (n=4, $P=0.077$) but >6x larger than cholinergic responses at 5mW. Discussion and Future Directions: In-vitro evidence identifies cholinergic inputs to the HMN alter the gain of responses to glutamatergic inputs involved in breathing. We will now test if this interaction occurs in-vivo to explain: (i) modulation

of activity in this medullary circuit essential to rhythmic breathing, and (ii) the marked reductions in activity that occur in behaviors such a rapid-eye-movement sleep.and changes in miRNA expression are associated hypothalamic cellular insulin resistance. Additionally, the identification of additional miRNAs associated with neuronal insulin resistance and ongoing functional validations will also be presented. The knowledge derived from these studies will provide insight into miRNA-based diagnostics and therapeutics for early central insulin resistance in humans.

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