



42ND ANNUAL
Frontiers in
Physiology



MAY 20, 2022

Conference Program

Hart House

MORNING

8:00 - 8:40	Registration & Breakfast	Great Hall
8:40 - 8:50	Welcome Address <i>Dr. Scott Heximer, FIP Co-Chairs (Eman Nishat, Kelvin Lee, Joseph Lee)</i>	Great Hall
8:50 - 10:00	Oral Session 1	Great Hall
10:10 - 11:00	Poster Session 1	Music Room
11:10 - 12:20	Oral Session 2	Great Hall

AFTERNOON

12:20 - 12:50	3-Minute Thesis Competition	Great Hall
12:50 - 13:30	Lunch	Great Hall
13:30 - 14:50	Keynote Presentation <i>Dr. Polly Matzinger</i>	Great Hall
14:50 - 15:00	Departmental Photo	Quad
15:10 - 16:00	Poster Session 2	Music Room
16:10 - 17:20	Oral Session 3	Great Hall

EVENING

17:20 - 19:00	Reception <i>Presentation and TA Awards Presented</i> <i>Platform Updates</i> <i>Closing Remarks (FIP Co-Chairs, Dr. Scott Heximer)</i> <i>Cake Cutting Ceremony</i>	Great Hall
----------------------	-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	-------------------





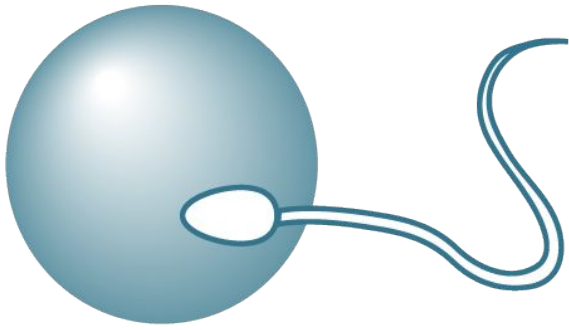
Looking for ways to launch your career?

We've got tips for that!

Visit RBC On Campus on
the 1st Floor, UofT Student
Commons Building



on campus



CReATe

BioBank

A biobank is a collection of biological samples linked with anonymized patient information, which is organized in a structured, readily analyzable format and made available to researchers.

The **CReATe Biobank** is an investigator initiated biobanking program founded in 2015 as an adjunct to the CReATe Fertility Centre (CFC) in Toronto. Under the leadership of Dr. Clifford Librach, we are entirely focused on human reproductive biology-related samples.

It is our mission to facilitate research in fertility and to support the development of novel diagnostic tests and treatments. Our samples along with de-identified clinical data are obtained from consenting patients who are going under fertility treatments. The collected samples can then be used by researchers in REB-approved studies to improve the diagnosis and treatment of infertility and increase scientific knowledge.

Our biobank contains more than 25,000 samples and is continuously growing each day

OUR Services

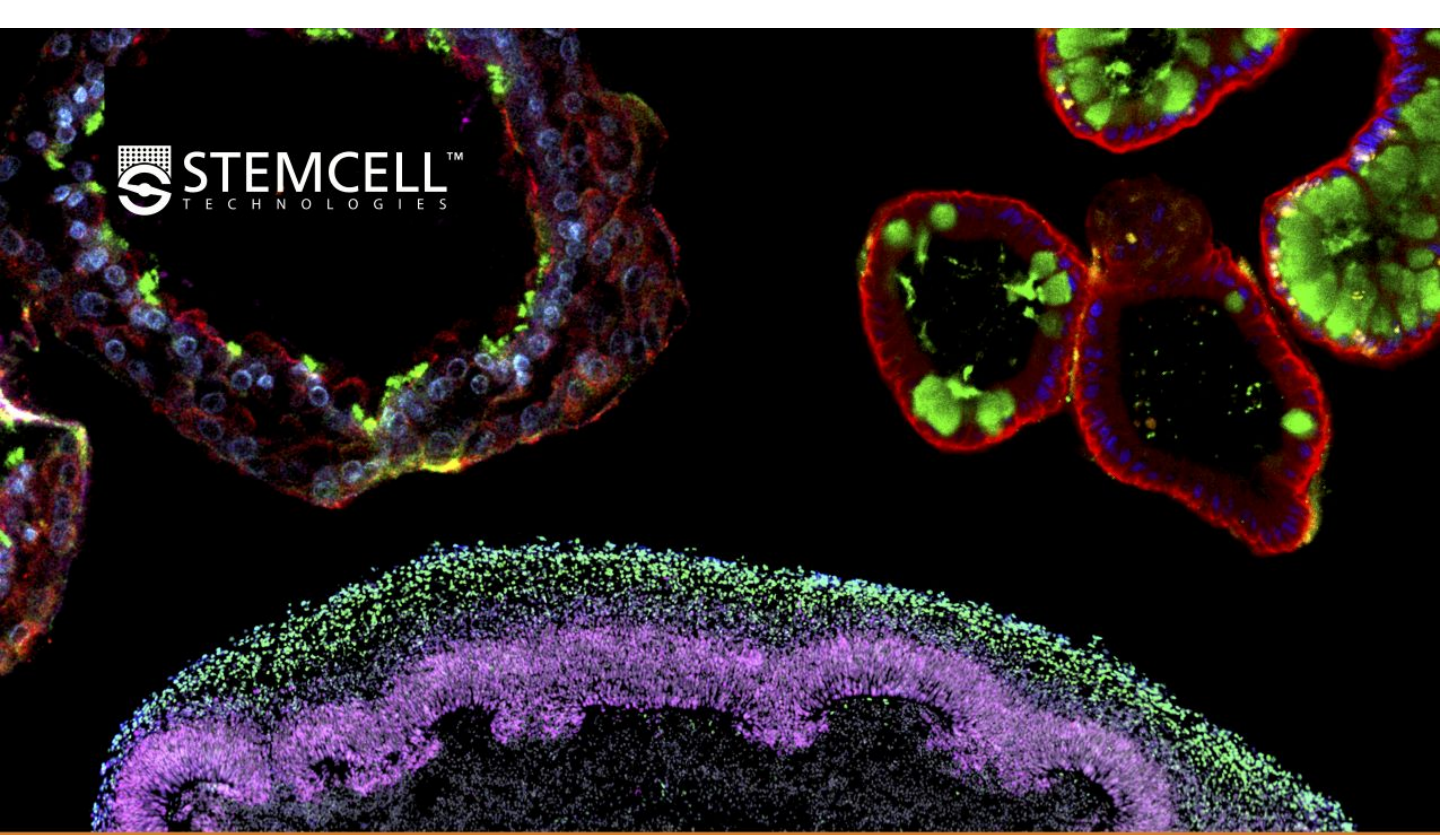
The CReATe Biobank has developed a comprehensive library of annotated patient samples and offers the following services

- Access to retrospectively collected biospecimens
- Prospective collection of samples tailored to your requirements
- Banking services according to your study directions

Access

Our internal researchers formally started utilizing banked biological samples for internal research projects in 2016 and are currently relying on the biobank for the majority of their research projects, but we also collaborate with external parties. External researchers gain access via collaboration with the CReATe Research Program and receive a waiver of payment to use our biobanking services.

For any inquiries, please contact our Clinical Research Manager at Sahar@createivf.com.



MORE EXPERIMENTING. LESS TROUBLESHOOTING.

The inherent variability of culturing human tissues as well as the complexity of do-it-yourself media present a challenge in generating reproducible cultures from one experiment to the next. STEMCELL's organoid media and kits have been designed to standardize your organoid workflows, enabling you to spend more time experimenting and less time troubleshooting.

LEARN MORE

www.stemcell.com/OrganoidMedia

Copyright © 2022 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design, Scientists Helping Scientists, IntestiCult, PneumaCult, and STEMdiff are trademarks of STEMCELL Technologies Canada Inc. All other trademarks are the property of their respective holders. While STEMCELL has made all reasonable efforts to ensure that the information provided by STEMCELL and its suppliers is correct, it makes no warranties or representations as to the accuracy or completeness of such information.

QIAcuity[®] Digital PCR System

Fast. Scalable. Reliable.



DON'T BE LIMITED BY TRADITIONAL FLUORESCENCE

Multiplexed Westerns without Compromises

Meet the ChemiDoc MP Imaging System

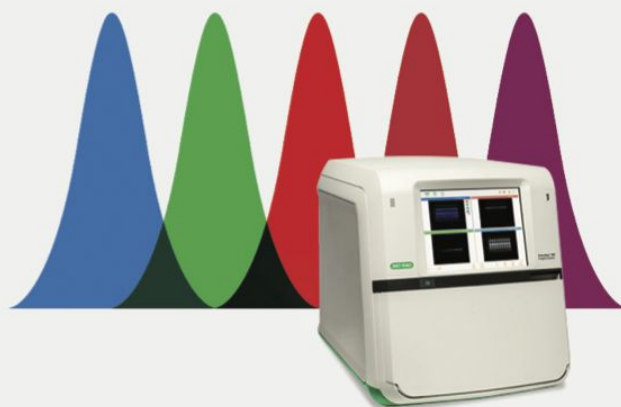
The performance of film meets the quantitation capabilities of digital.

Get the fluorescence performance you expect with higher multiplexing (3 targets vs. 2) and more fluorophore options.

For more information:

Peter Seto

peter_seto@bio-rad.com



Discover the power of Droplet Digital PCR



Bio-Rad's Droplet Digital PCR (ddPCR) Systems

For more information:

Angela Watt-Shirley

angela_watt-shirley@bio-rad.com

- Achieve greater sensitivity, accuracy and precision
- Applications include: Cancer biomarker studies and copy number variation, pathogen detection, NGS library quantification, gene expression analysis, environmental monitoring
- Quantify difficult samples, low abundant targets; FFPE, single cell, cfDNA
- Use existing primers/probe assays or order from our extensive catalogue of validated assays; www.bio-rad.com/digital-assays

Streamline Your Sample Storage and Management with these Solutions from ^{MJS} BioLynx

TubeWriter™ 360

High-speed Labware Labeling

TubeWriter™ 360 is a high-speed inkjet printer that prints directly on a wide variety of labware such as tubes, vials, slides, cassettes and microplates.



Watch the TubeWriter™ 360 in action.

Micronic 2D Coded Sample Storage Tubes

Standardize your Sample Storage

Micronic offers a complete line of sample storage tubes with volumes ranging from 0.30 mL to 6.00 mL that fit in the 96-well, 48-well or 24-well racks. The tubes are available non-coded, alphanumeric coded or with a unique 2D Data-Matrix code.



Find the right tubes for your samples.

Hamilton Storage LabElite I.D. Capper

Automated Screw Cap Decapper and 2D Barcode Tube Reader

The all-in-one I.D. Capper enables labs to combine decapping/capping and high-speed barcode reading within one device. Easily swap decapping heads to decap tubes in 24-, 48-, and 96-format tube racks, all on a single device.

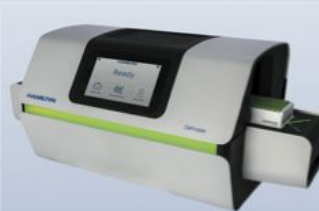


Watch the LabElite I.D. Capper in action.

Hamilton Storage LabElite® DeFroster

Automated Rack Frost Remover

The LabElite® DeFroster quickly removes frost that may obscure 2D barcodes on sample racks. It cleans sample racks using mechanical brushes and defrosting/de-icing solvent.



Learn more about the LabElite® DeFroster.

Answer a fun question on our landing page, and we will enter you into our draw for 1 of 20 Adorable Adopted Stuffed Lynx!

Visit www.biolyx.ca/instrumentation_sample_handling_uoftfp

MJS
BioLynx
INC.

Trust • Truth • Help • Love • Listen



1-888-593-5969 • biolyx.ca • tech@biolyx.ca

Selection of some of Diamed's products currently in stock.



GLB150838 - 1250 µl



GEN23-777RL



BPSMCT150-N



DIATEC610-3334



MTCC3150-S



MTCC1020



MTCC2600 Rack
MTCC2601 Bags



MTCC2602 Rack
MTCC2603 Bags

Diamed



MTCC2230



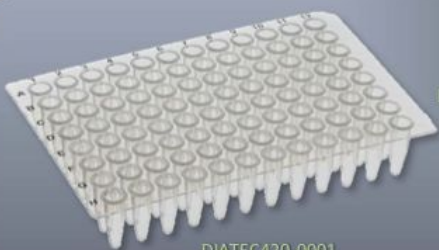
DIAU1002-?



DIATEC610-2740



DIATEC420-1377



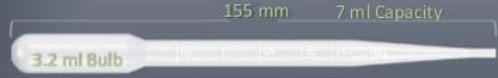
DIATEC420-0001



DIAU1002-4AT

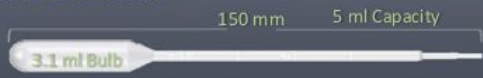


DIAU1002-5AU



DLAQ400-7220S

Non-Sterile & Sterile



DLAQ400-5220S



Roche has a tradition of looking to the past to develop innovative solutions that improve the present, but never at the expense of the future

Come along with us as we celebrate what's always been at the heart of all we do - Life.

We have been partners and leaders in the delivery of diagnostic and pharmaceutical solutions globally for 125 years and here in Canada for 90 years.

We have proven time-and-again that we will push boundaries so the innovation that is to come will be available to impact countless lives in the future.

We are now expanding into Advanced data analytics, Digital Health and AI to create individually tailored treatments. We are working towards integrated personalized healthcare solutions, ensuring that we can fit the right test and the right treatment, to the right patient, at the right time and at the right value.

Our promise

Is to care about this generation, and the ones to come, for the next 125 years and beyond.



Heart & Stroke | Richard Lewar

Centres of Excellence in Cardiovascular Research



**TED ROGERS CENTRE
FOR HEART RESEARCH**

Table of Contents

About our Keynote: Dr. Polly Matzinger.....	12
Message from the Chair..... <i>Dr. Scott Heximer</i>	13
Message from the Vice-Presidents..... <i>Eman Nishat, Kelvin Lee, Joseph Lee</i>	14
Oral Presentation Session 1.....	15
Poster Presentation Session 1.....	16
Oral Presentation Session 2.....	18
3-Minute Thesis Competition.....	19
Poster Presentation Session 2.....	20
Oral Presentation Session 3.....	22
Oral Presentation Abstracts.....	23
Poster Presentation Abstracts.....	31
Organizers and Contributors.....	51



Dr. Polly Matzinger

Ghost Lab, Division of Intramural Research
National Institute of Allergy and Infectious Diseases
National Institutes of Health
USA



Polly Matzinger PhD, Honorary D Phil, is a world-renowned immunologist, who began a new paradigm in Immunology with the publication of her “Danger Model” of immunity and has won several awards, as well as been featured in several films because of her work, including a one-hour BBC documentary on the Danger model titled “Turned on by Danger”. In her pre-scientific life, she worked as a bartender, carpenter, jazz musician, playboy bunny, and dog trainer. She is currently the chief of the Ghost lab, and the section on T cell Tolerance and Memory at the National Institutes of Health. She worried for years that the dominant model of immunity does not explain a wealth of accumulated data and proposed an alternative, the Danger model, which suggests that the immune system is far less concerned with things that are foreign than with those that do damage. This model, whose two major tenets were conceived in a bath and on a field while herding sheep, has very few assumptions and yet explains most of what the immune system seems to do right, as well as most of what it appears to do wrong, covering such areas as transplantation, autoimmunity, and the immunobiology of tumors. The model has been the subject of a BBC "horizon" film and has featured in three other films about immunity, and countless articles in both the scientific and the lay press. Having suggested an answer to the first question the immune system asked (how do I know when to respond?), Polly turned to the second question, namely "once it decides to respond, how does the immune system know what kind of response to make?" A first answer to this question seems to be that “local tissues send instructions to immune cells, guiding them to make the right kind of response”. This has major implications for autoimmunity, cancer immunotherapy, and vaccine design.

In her spare time, Polly trains border collies for competitive shepherding trials, composes songs that are not really worth listening to, and is working on the next major question in the immune system (how do autoimmune diseases occur?). Finally, she is also a sheep breeder and is part of a small group bringing Gotland sheep into the USA, using frozen embryos from New Zealand and semen from Sweden on her humane-certified farm. She is past President of the Gotland Sheep Breeders Association of North America, current Vice President of the Frederick Sheep Breeders Association, on the Board of the Maryland Sheep Breeders Association and a Member of the National Sheep Improvement Program.



Message from the Chair

On behalf of the Department of Physiology, it gives me great pleasure to welcome everyone to the annual “Frontiers in Physiology (FIP) Research Day” held in-person this year! This Annual Symposium highlights the outstanding research and leadership efforts of our graduate trainees, who have organized and run this event for the past 42 years. Today will showcase the exciting and innovative work conducted by our trainees, who represent the future of Physiology in Canada, and around the world. We are extremely proud of the accomplishments of our graduate cohort and their dedication to science and research, and look forward to a great day of presentations.

It is also my pleasure to welcome Dr. Polly Matzinger to the Department of Physiology. Dr. Matzinger is a world-renowned immunologist, who began a new paradigm in Immunology with the publication of her “Danger Model” of immunity and has won several awards, as well as been featured in several films because of her work, including a one-hour BBC documentary on the Danger model titled “Turned on by Danger”. She is currently the chief of the Ghost lab, and the section on T cell Tolerance and Memory at the National Institutes of Health.

A special thanks and acknowledgement is owed today to Eman Nishat, Joseph Lee and Kelvin Lee, FIP co-Chairs and Vice-Presidents of the Graduate Association of Students in Physiology (GASP). This team has managed to put together an outstanding program. A special thank you also to Ms. Yasaman Mostafaie, GASP President, and to everyone else who helped make this year’s research day possible.

We hope you enjoy the day. We invite you to take full advantage of this opportunity for cross-platform interaction and collaboration between members of our exceptional scientific community.

Thank you for helping make this day an enormous success!

Best regards,

Scott P. Heximer
Chair, Department of Physiology
Temerty Faculty of Medicine
University of Toronto



Message from the Vice-Presidents

On behalf of the Graduate Association of Students in Physiology (GASP), we are very excited to present to you the 42nd Annual Frontiers in Physiology (FIP) Symposium. Today, trainees from all four platforms and three professional programs will showcase their cutting-edge research. Through this exciting event, we hope to facilitate the exchange of scientific ideas amongst students and faculty members, promote collaboration within the department and unite the research powerhouse that is the University of Toronto and its affiliated teaching hospitals and research institutions.

This year, we are honoured to welcome Dr. Polly Matzinger as the FIP Keynote Lecturer. Dr. Matzinger is an internationally renowned immunologist who proposed the “Danger Model” theory of how the immune system works. In 2002, Discover magazine recognized Dr. Matzinger as one of the 50 most important women in science. We are very excited to learn more about her research. On behalf of the Department of Physiology, we would like to take this opportunity to thank Dr. Matzinger for accepting our invitation and welcome her to the University of Toronto.

In addition to our esteemed keynote speaker, the success of FIP 2022 is dependent on the combined efforts of several individuals and groups. We would like to extend our gratitude to everyone involved in the planning, organizing and running of FIP. We are especially grateful to everyone on the FIP Planning Committee for their hard work and dedication in planning FIP as well as to everyone on the Graduate Association for Students in Physiology (GASP) for their support. We are also thankful for the support of the Department of Physiology, and all of the platforms for their contributions to this year’s event. 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. Last but certainly not least, thank you to all of our institutional and commercial sponsors for their support. Without such support of research and training, FIP 2022 would not be possible.

We look forward to celebrating the diverse achievements of the Department of Physiology with you today!

Sincerely,

Eman Nishat, Joseph Lee, Kelvin Lee
Vice-Presidents, Graduate Association for Physiology
Frontiers in Physiology 2022 Co-Chairs



Oral Presentation Session 1

8:50 AM – 10:00 AM

Great Hall

01-1: Developing an In Vitro Model of Alzheimer's Disease

Jennifer Kao, Ankit Awasthi, Andrew Mocle, Alex Jacob, Emily Kramer, Ai Tian, Julien Muffat, Sheena Josselyn, Paul Frankland

01-2: Transcriptomic Profiling Across the Spinal Cord Dorsal Horn of Peripheral Nerve Injury Model Reveals Conserved Gene Signatures and Predicts Treatments for Neuropathic Pain

Shahzad Ghazisaeidi, Milind M. Muley, YuShan Tu, Mahshad Kolahehdouzan, Ameet S. Sengar, Arun K. Ramani, Michael Brudno, Michael W. Salter

01-3: Hypothesis-Driven Genome-Wide Association Studies Provide Novel Insights into Genetics of Reading Disabilities

Kaitlyn M. Price, Karen G. Wigg, Else Eising, Yu Feng, Kirsten Blokland, Margaret Wilkinson, Elizabeth N. Kerr, Sharon L. Guger, GenLang Consortium authors, Simon Fisher, Maureen W. Lovett, Lisa J. Strug, Cathy L. Barr

01-4: Discrepancy Between NMDA Receptor Effects at Synapse and Dendrite in Patient Derived GRIN1 Mutant Mouse Leads to Unexpected Treatment Opportunity

Sridevi Venkatesan, Amy J Ramsey, Evelyn K Lambe

01-5: Temporal Dynamics of Neuronal Excitability in the Lateral Amygdala Mediates Allocation to an Engram Supporting Conditioned Fear Memory

Annelies Hoorn, Sylvie Lesuis, Asim Rashid, Paul Frankland, Sheena Josselyn



Poster Presentation Session 1

10:10 AM – 11:00 AM

Music Room

P1-1: Poor Infant Lung Function is Linked to Early Lower Respiratory Tract Infections in Infancy

Maria Medeleanu, Myrtha E. Reyna, Ruixue Dai, Piushkumar J Mandhane, Elinor Simons, Stuart E. Turvey, Per M. Gustafsson, Theo J. Moraes, Padmaja Subbarao

P1-2: Nasal Microbiome Alpha Diversity in Infants With or Without Viral Presence

Yu Chen Qian, Ruixue Dai, Kelsey Fehr, Vanessa Breton, Myrtha E. Reyna, Stuart E Turvey, Piush Mandhane, Mike Surette, Marek Smieja, Elinor Simons, Meghan Azad, Theo Moraes, Padmaja Subbarao

P1-3: NINJ1-mediated Plasma Membrane Rupture Leads to Tumour Lysis Syndrome

Keane Paul Baldoviso Fuerte, Allen Volchuk, Neil Michael Goldenberg

P1-4: The Characterization of LTBP2 in Cardiac Fibrosis

Fahad Ehsan

P1-5: Sodium-Glucose Cotransporter 2 Inhibitors and its Cardiorenal Benefits in Individuals with Diabetes Mellitus: Cardiovascular and Kidney Disease Biomarker Analysis

Luxcia Kugathanan, David Cherney

P1-6: Ex-vivo Organ Perfusion Model of Kidney Fibrosis in the Mouse

Jorge Castillo-Prado, Ian Rogers

P1-7: The exocytotic protein, secretagoin, is essential for circadian glucagon-like peptide-1 secretion

Andrew Biancolin, Hyerin Jeong, Arjuna Srikrishnaraj, Patricia Brubaker

P1-8: Multifaceted impact of Ω 3-polyunsaturated fatty acids on Kv1.2 channels and inhibitory neurotransmission

Tian Kong, Jason Arsenault, Bassam Tawfik, Lu-Yang Wang

P1-9: Relationship Between Actigraphy Measures and Diurnal Mood Measures in Day Treatment of Major Depressive Disorder

Nastasia Kujbid, Ryan Klein, Sean Hill, John Strauss, Jeff Daskalakis, Judith Laposa, Stefan Kloiber, Marco Battaglia, Robert Levitan

P1-10: NINJ1 forms megapores within the plasma membrane during pyroptosis that are targeted by the amino acid glycine

Jazlyn P. Borges, Allen Volchuk, Bridget Kilburn, Neil M. Goldenberg, Benjamin E. Steinberg



Poster Presentation Session 1

10:10 AM – 11:00 AM

Music Room

P1-11: Mechanism Underlying General Anesthetic Drug Induced Cognitive Deficits

Li Ju, Arsène Pinguelo, Anthony Ariza, Dianshi Wang, Beverley A. Orser

P1-12: A Novel Peptide Interferes with the Interaction Between Radixin and α 5GABAA Receptors

Setareh Malekian Naeini, Anthony Ariza, Beverley A. Orser

P1-13: Characterizing Critical Language Sites in Children and Adolescents Using MEG and rTMS

Sara Sino, Vivek Sharma, Hansel M. Greiner, Kishore Vedala, Jennifer Vannest, Hisako Fujiwara, Jeffrey R. Tenney, Brady J. Williamson, Darren S. Kadis

P1-14: Identification of Resin Degrading Enzymes from Human Neutrophils

Aya Ragheai, Russel Gitalis, Yoav Finer, Michael Glogauer

P1-15: Generation of Non-immunogenic Macrophages for Allogenic Cell Therapies

Jean Kit Tang, Huijuan Yang, Jeff Harding, Kristina Nagy, Sheena Bouch, Martin Post, Ian Rogers, Andras Nagy

P1-16: Antenatal Synthetic Glucocorticoid Exposure Modifies the Response to Post-natal Infection at the Blood-brain Barrier

Margaret Elizabeth Eng, Stephen G. Matthews

P1-17: Effects of Antenatal Glucocorticoids on miRNA Levels in the Prefrontal Cortex of the Newborn Guinea Pig Brain

Danna Ellner, Bona Kim, Alisa Kostaki, Stephen G. Matthews

P1-18: Cerebral Small Vessel Disease and Thickness of the Cerebral Cortex in Adults

Ariana Tang, Jean Shin, Tomas Paus, Zdenka Pausova

P1-19: Removal of Epigenetic Barrier Enables ESC to Access Trophoblast and Primitive endoderm Fates and Self-assemble into Blastoids

Jessica-Lynne Welton, Brian Cox

P1-20: Exposure to Synthetic Glucocorticoids Modifies the microRNA Cargo of Epididymal Extracellular Vesicles: Implications for Intergenerational Transmission

Christopher Casciaro, Hirotaka Hamada, Alisa Kostaki, Stephen G. Matthews



Oral Presentation Session 2

11:10 AM – 12:20 PM

Great Hall

O2-1: Upper Small Intestinal Protein Sensing Regulates Food Intake and Glucose Tolerance in Male Rats

Daniel Barros, Rosa J.W. Li, Yu-Mi Lim, Song-Yang Zhang, Anna Gao, Tony K.T. Lam

O2-2: The Effect of a Plant Phytohormone and Purine Signaling in Hypothalamic NPY Neuronal Models

Calvin V. Lieu, Denise D. Belsham

O2-3: The Saturated Fat Palmitate Alters MicroRNA Profiles of Exosomes Derived From Hypothalamic NPY/AgRP-expressing mHypoE-46 Cells

Emma K. McIlwraith, Denise D. Belsham

O2-4: Impact of Age on Functional Cholinergic Synapses in Prefrontal Cortex and an Effective Treatment to Restore Nicotinic Signalling

Saige Power

O2-5: Allocation to a Hippocampal Engram Supporting a Contextual Fear Memory is Mediated by Neuronal Excitability in Pre-configured Neuronal Ensembles

Andrew Mocle, Adam I. Ramsaran, Alexander D. Jacob, Lina M. Tran, Blake A. Richards, Paul W. Frankland, Sheena A. Josselyn



3-Minute Thesis Competition

12:20 PM – 12:50 PM

Great Hall

3MT-1: Getting a Lay of the Land

Sara Sino

3MT-2: Sugary Urine Has Never Sounded Sweeter

Luxcia Kugathasan

3MT-3: TRPM2: A Potential Target for Saving Ischemic Stroke Patients?

Xinyang Zhang

3MT-4: Brain to Bone: How Does the Brain Control the Healing of the Jaw Bones?

Aya Ragheai

3MT-5: Can We Heal the Human Heart?

Jasnoor Verma



Poster Presentation Session 2

3:10 PM – 4:00 PM

Music Room

P2-1: Inflammasome Activation in Pulmonary Arterial Hypertension: The Role of Gasdermin D

Anna Foley, Sonja Sulstarova, Allen Volchuk, Benjamin E Steinberg, Neil Goldenberg

P2-2: Spectrin Breakdown Triggers Endothelial Dysfunction in Pulmonary Arterial Hypertension

Sonja Sulstarova, Neil Goldenberg

P2-3: Elucidating Heterogeneity Between Left and Right Ventricle-derived Cardiac Fibroblasts

Michael Bradley Dewar, Haisam Shah, Dylan Langburt, Fahad Ehsan, Alison Hacker, Scott Heximer

P2-4: Sarco(endo)plasmic Reticulum Membrane Protein REEP5 Regulates Subcellular Structure and Function in the Heart

Michelle Di Paola, Uros Kuzmanov, Cristine J. Reitz, Allen C.T. Teng, Anthony O. Gramolini

P2-5: Evidence that STAT3 is a Negative Transcriptional Regulator of NPY in Mouse-NPY/AgRP Immortalized Hypothalamic Neurons

Wenyuan (Kevin) He, Andy Tran, Denise D. Belsham

P2-6: The Role of microRNAs in Bisphenol A-mediated Regulation of Neuropeptide Y and Neuronatin

Kimberly Mak, Neruja Loganathan, Emma K. McIlwraith, Denise D. Belsham

P2-7: Efficient Generation of Induced Pluripotent Stem Cell Derived Pancreatic Progenitor Cells from a Non-invasive, Accessible Tissue Source - The Plucked Hair Follicle

Amatullah Fatehi, Ian Rogers

P2-8: Modular Organization of Quantal Heterogeneity at a Central Synapse

Raphael Chan, Maria Gurma, Adam Fekete, Stefan Herlitze, Melanie D. Mark, Lu-Yang Wang

P2-9: Ryanodine Receptor Inhibitor Dantrolene Reduces Hypoxic-Ischemic Brain Injury in Neonatal Mice

Andrea Ovcjak, Steve P. Miller, Zhong-Ping Feng, Hong-Shuo Sun

P2-10: Investigating the Vesicular Transport Mechanisms of Myelin Proteolipid Protein 1 (PLP1) in Optic Nerve Myelination

Chun Hin (Daniel) Chow, Shuzo Sugita



Poster Presentation Session 2

3:10 PM – 4:00 PM

Music Room

P2-11: Social Isolation's Effect on Learning and Memory

Naomi Niederhoffer, Paul W Frankland, Sheena A Josselyn

P2-12: SNARE Proteins in Retinal Synaptic Vesicle Release

Maggie Huang, Shuzo Sugita

P2-13: Characterization of Patient Subtypes in Pediatric Mild Traumatic Brain Injury

Prashanth Velayudhan, Anne L Wheeler

P2-14: The Involvement of PD-L2 in Retinal Axon Guidance

Xiaoyan (Shirley) Chen

P2-15: Decidual Natural Killer Cells: Uniquely Tolerant Cells That Support Placentation

Morgan Zych, Brian Cox

P2-16: Using Acellular Pancreatic Scaffolds to Direct Differentiation of Pancreatic Progenitors to Functional Pancreatic Tissue

Marwa Sadat, Amanda Fantin, Jorge Castillo-Prado, Dr. Ian Rogers

P2-17: Impaired Placental Antioxidant Function and Iron Homeostasis Promotes Trophoblast Cell Death via Ferroptosis in Pregnancies Complicated by Fetal Growth Restriction

Ruizhe Liu, Chanh Park, Sruthi Alahari, Isabella Caniggia

P2-18: Structural Studies of Proteins, NSD1, and FEM1C, Involved in Post Translational Modifications

Ksenia Providokhina, Jinrong Min

P2-19: 3D Culturing of Renal Progenitor Cells on ECM Scaffolds Results in Maturation of Cells Toward Kidney Lineages

Tonya Bongolan, Theresa Chow, Jennifer Whiteley, Laura Mazilescu, Matyas Hamar, Maria Ryaboshapkina, Anna Jonebring, Ryan Hicks, Markus Selzner, Ian M. Rogers



Oral Presentation Session 3

4:10 PM – 5:20 PM

Great Hall

O3-1: Breaking Through the Defense: Biofilm Disruption Using Genetically Modified Macrophages

Sajad Sadat, Michael L. Litvack, Deepa Raju, Vanessa Hsing, P. Lynne Howell, Martin Post

O3-2: Hypoxia Increases P-glycoprotein (P-gp) Activity in Developing Human Brain Endothelial Cells: Implications for Fetal Brain Protection

Hafsah Mughis, Phetcharawan Lye, Guinever Imperio, Enrrico Bloise, Stephen Matthews

O3-3: Differential DNA Methylation Following Antenatal Corticosteroids in Newborn Brain and Blood

Bona Kim, Alisa Kostaki, Stephen G. Matthews

O3-4: Integrin Alpha 11 Plays a Critical Role in Cardiac Fibrosis and Infarct Formation Following Myocardial Infarction

Patrick Meagher, Xavier Lee, Jean-Francois Desjardin, Kim Connelly

O3-5: A Novel Approach Combining Imaging with Biochemical Assessments to Manage Mechanical Ventilation in ARDS: A Literature Review

Megan Abbott, Haibo Zhang



Oral Presentation Abstracts

O1-1: Developing an In Vitro Model of Alzheimer's Disease

Jennifer Kao^{1,2}; Ankit Awasthi³; Andrew Mocle⁴; Alex Jacob¹; Emily Kramer⁴; Ai Tian³; Julien Muffat³; Sheena Josselyn^{1,3,4}; Paul Frankland^{1,3,4}

¹ Department of Physiology, University of Toronto, Toronto, Canada; ² Temerty Faculty of Medicine, University of Toronto, Toronto, Canada; ³ Program in Neurosciences and Mental Health, Hospital for Sick Children, Toronto, Toronto, Canada; ⁴ Department of Psychology, University of Toronto, Toronto, Canada

Recent advances in cerebral organoid (CO) technology have allowed researchers to study the human molecular landscape in detail and recapitulate the environment from which human-specific neurological diseases manifest. We use this technology to model Alzheimer's disease (AD), a condition of progressive cognitive dysfunction and significant memory loss that is thought to arise from an early, abnormal hyperactive neurocircuitry in the brain. We hypothesize (1) that AD CO models will capture the underlying neural hyperactivity seen in patients, and (2) that the underlying hyperactivity will reduce our ability to optogenetically-stimulate CO neurons into artificial patterns that are reminiscent of short-term memory formation. Using live calcium imaging, we firstly capture hyperactivity in the secondary and primary motor cortices of transgenic AD mice (e.g., 22% more spike events). We then cultured normal COs for 6 months, characterized changes in its electrophysiological maturity over time, and analyzed its endogenous neural patterning using a variety of unsupervised clustering methods. Our COs show transient ventricle-like areas (SOX2+) surrounded by maturing neuronal (CTIP2+) compartments. Live calcium imaging of maturing COs revealed a significant neural activity spike at months 3-4, but a decline after month 5. Coincidentally, month 5 COs showed cortical integration of GAD67+ inhibitory interneurons and increased coordinated neuronal activity, with a greater proportion of active neurons belonging to endogenous groups of coactive neurons (i.e., ensembles). As ensembles are the substrate from which memories are encoded, identifying them in COs is a necessary and promising first step to modeling the intricate neural circuitry affected by AD.

O1-2: Transcriptomic Profiling Across the Spinal Cord Dorsal Horn of Peripheral Nerve Injury Model Reveals Conserved Gene Signatures and Predicts Treatments for Neuropathic Pain

Shahzad Ghazisaeidi^{1,2}; Milind M. Muley²; YuShan Tu²; Mahshad Kolahehdouzan^{1,2}; Ameet S. Sengar²; Arun K. Ramani³; Michael Brudno^{3,4}; Michael W. Salter^{1,2}

¹ Department of Physiology, University of Toronto, Toronto, Canada; ² Program in Neuroscience & Mental Health, Hospital for Sick Children, Toronto, Canada; ³ Centre for Computational Medicine, Hospital for Sick Children, Toronto, Canada; ⁴ Department of Computer Science, University of Toronto, Toronto, Canada

Neuropathic pain is the most devastating and poorly treated subtype of chronic pain. Treatment of neuropathic pain remains challenging primarily due to the lack of effective translation from pre-clinical to clinical settings. Recent advances in genomics has helped characterize pain pathways. However, to date, no investigation has utilized transcriptomic data to predict and test potential therapies to close the translational gap. Here, we hypothesize that RNA-sequencing is an effective tool for the repurposing of drugs. Males and females C57BL6 mice and Sprague Dawley rats, were subjected to Spared Nerve Injury (SNI). Seven days post-surgery, spinal cord dorsal horn was harvested for RNA sequencing. Data were analyzed with the edgeR package. Genes with LogFC>|0.5| and adjusted p-value <0.01 were considered differentially expressed genes (DEGs). A protein-protein interaction (PPI) network was constructed and druggable nodes were identified using a drug-gene interaction database. One potential drug, Fostamatinib-R406, was tested in vivo using intrathecal injection. We have identified 93 genes that were upregulated after nerve injury in both male and female rodents. We then constructed a "pain interactome" and identified FDA-approved drugs predicted to modulate key nodes. From this drug list, we selected Fostamatinib-R406 (a spleen tyrosine kinase inhibitor), for testing in male and female rats subjected to SNI. Here we report that intrathecal injection of Fostamatinib-R406 resulted in a significant reversal of mechanical hypersensitivity in both sexes (p<0.03). Using RNA-sequencing data, we were able to screen drug databases in silico for putative candidate compounds which may modulate neuropathic pain behaviour. Our results with Fostamatinib-R406 provides support for this new strategy and could lead to the repurposing of compounds for the treatment of pain.



Oral Presentation Abstracts

O1-3: Hypothesis-Driven Genome-Wide Association Studies Provide Novel Insights into Genetics of Reading Disabilities

Kaitlyn M. Price^{1,2,3}; Karen G. Wigg¹; Else Eising⁸; Yu Feng¹; Kirsten Blokland²; Margaret Wilkinson²; Elizabeth N. Kerr^{5,6}; Sharon L. Guger⁵; GenLang Consortium authors⁸; Simon Fisher^{8,9}; Maureen W. Lovett^{2,6}; Lisa J. Strug^{4,7}; Cathy L. Barr^{1,2,3}

¹ Division of Experimental and Translational Neuroscience, Krembil Research Institute, University Health Network, Toronto, Canada; ² Program in Neuroscience and Mental Health, Hospital for Sick Children, Toronto, Canada; ³ Department of Physiology, University of Toronto, Toronto, Canada; ⁴ Genetics and Genome Biology, Hospital for Sick Children, Toronto, Canada; ⁵ Department of Psychology, Hospital for Sick Children, Toronto, Canada; ⁶ Department of Pediatrics, University of Toronto, Toronto, Canada; ⁷ Departments of Statistical Sciences and Computer Science, Faculty of Arts and Science and Division of Biostatistics, Dalla Lana School of Public Health, University of Toronto, Toronto, Canada; ⁸ Language and Genetics Department, Max Planck Institute for Psycholinguistics, Nijmegen, Niederlande; ⁹ Donders Institute for Brain, Cognition and Behaviour, Radboud University, Nijmegen, Netherlands

Background: Reading, a uniquely human process, involves the interpretation of written language. Difficulties in reading or reading disabilities (RD) come about when a child struggles with a part of this process, specifically the sounding out/recognition of words. The etiology of RD is not well known; however, researchers hypothesize that genetic variants lead to disruptions in neuronal migration, which affects connectivity in language centers in the brain. To contribute to understanding the genetics of reading, we previously performed a genome-wide association study (GWAS). We found top variants were in genes involved in neuronal migration or implicated in other neurodevelopmental disorders, such as Autism Spectrum Disorder (ASD). Methods: Using this information, we then performed a hypothesis-driven (HD) analysis (HD-GWAS) where we upweighted variants, separately, based on their implication in either neuronal migration or ASD. This analysis was conducted on a family-based RD-selected cohort from Toronto and a large meta-analysis from the GenLang Consortium. Results: For the Toronto sample, no variants reached statistical significance; however, we identified that the joint contribution of ASD genes significantly contributed to word reading. For the GenLang sample, we identified a significant variant on chr 21 (q21.1). This locus was previously identified by traditional GWAS and not up-weighted, confirming the robust association. Discussion: Our results suggest that ASD risk genes are enriched in word reading; however, this finding was limited to the Toronto sample. The relationship between RD and ASD has not been thoroughly investigated, but both are language-based disorders, suggesting a possible common neurobiological link.

O1-4: Discrepancy Between NMDA Receptor Effects and Synapse and Dendrite in Patient Derived GRIN1 Mutant Mouse Leads to Unexpected Treatment Opportunity

Sridevi Venkatesan¹; Amy J Ramsey²; Evelyn K Lambe^{1,3,4}

¹ Department of Physiology, University of Toronto, Toronto, Canada; ² Department of Pharmacology and Toxicology, University of Toronto, Toronto, Canada; ³ Department of Psychiatry, University of Toronto, Toronto, Canada; ⁴ Department of Psychiatry Obstetrics and Gynaecology, University of Toronto, Toronto, Canada

GRIN1 neurodevelopmental disorder is a rare disease caused by mutations in the obligate GluN1 subunit of the NMDA receptor (NMDAR). Y647S+/- mutation in the transmembrane region of GluN1 causes intellectual disability and seizures in a patient, with unknown effects on NMDAR function and synaptic integration. To determine appropriate treatment strategies, we sought to identify the nature of NMDAR deficits using transgenic mice of both sexes heterozygous for the Y647S mutation compared to littermate controls. Patch-clamp electrophysiology in prefrontal layer 5 pyramidal neurons revealed seemingly paradoxical results. Some aspects of NMDAR signaling are diminished, but others are amplified/prolonged. Electrically evoked synaptic NMDAR EPSCs are significantly smaller in Y647S mice, yet whole-cell currents evoked by bath-applied NMDA are significantly larger. This contradictory pattern is also observed on examining dendritic plateau potentials that require NMDARs for synaptic integration. The amplitude of plateau potentials is smaller in Y647S mice, but their duration is significantly and unexpectedly prolonged. We hypothesize that this pattern in Y647S mice arises from a combination of deficient synaptic NMDARs along with an impairment in typical NMDAR recruitment of calcium-activated potassium channels to act as brakes on postsynaptic activity. Consistent with this hypothesis, a drug potentiating calcium-activated potassium channels (NS309) is successful in reducing whole-cell currents evoked pharmacologically with NMDA. NS309 also restores appropriate timing to dendritic plateau potentials in Y647S mice. These findings give insight into dynamic interactions between NMDARs and proximal ion channels and identifies a new research direction for GRIN disorder treatment.



Oral Presentation Abstracts

O1-5: Temporal Dynamics of Neuronal Excitability in the Lateral Amygdala Mediates Allocation to an Engram Supporting Conditioned Fear Memory

Annelies Hoorn^{1,2}; Sylvie Lesuis¹; Asim Rashid¹; Paul Frankland^{1,2,3,4}; Sheena Josselyn^{1,2,3,4}

¹ Program in Neurosciences & Mental Health, The Hospital for Sick Children, Toronto, Canada; ² Department of Physiology, University of Toronto, Toronto, Canada; ³ Department of Psychology, University of Toronto, Toronto, Canada; ⁴ Institute of Medical Science, University of Toronto, Toronto, Canada

Memories are encoded by ensembles of neurons (engrams) that are active during learning. Neurons important in an engram (engram cells) 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 being biased to be allocated to an engram. Previous findings show that neurons with increased excitability during training also have increased excitability for ~6 h. Because of this, two separate but similar training episodes within a 6 h time period tend to be co-allocated to a similar population of neurons and remembered together. Here we examined the temporal dynamics of neuronal excitability important for allocation to an engram. We focused on the lateral amygdala (LA) and cued fear memory. We expressed both an excitatory and inhibitory opsin in the same sparse, random subset of LA neurons. At different times before fear conditioning, we optically activated this sparse subset of neurons to allocate them to the engram. To examine whether these neurons were indeed critical components of the engram, we tested mice both in the absence of light and with optical stimulation to inhibit this same population of neurons. We find that optogenetic stimulation of neurons up to 6 h, but not 12 h or 24 h, before training biases their allocation to the engram. These findings indicate that excitability in the LA is temporally defined and plays a critical role in neuronal selection to a fear engram.

O2-1: Upper Small Intestinal Protein Sensing Regulates Food Intake and Glucose Tolerance in Male Rats

Daniel Barros^{1,2}; Rosa J.W. Li^{1,2}; Yu-Mi Lim^{1,3}; Song-Yang Zhang¹; Anna Gao¹; Tony K.T. Lam^{1,2,4,5}

¹ Toronto General Hospital Research Institute, UHN, Toronto, Canada; ² Department of Physiology, University of Toronto, Toronto, Canada; ³ Medical Research Institute, Kangbuk Samsung Hospital, Sungkyunkwan University, School of Medicine, Seoul, Republic of Korea; ⁴ Department of Medicine, University of Toronto, Toronto, Canada; ⁵ Banting and Best Diabetes Centre, University of Toronto, Toronto, Canada

Direct intestinal protein infusions reduce food intake and improve glucose homeostasis in lean rodents and humans; however, the underlying mechanisms and its effects in obesity and diabetes require further investigation. In this study, we infused casein hydrolysate as a protein source into the upper small intestine (USI) of male rats and performed fasting-refeeding experiments and intravenous glucose tolerance tests to assess food intake and glucose tolerance, respectively. USI casein infusion suppressed food intake in chow-fed rats but not in rats after short-term high-fat (HF) feeding. Meanwhile, USI casein infusion improved glucose tolerance in both chow and HF rats. We then evaluated whether the effects of USI casein sensing on glucose tolerance were mediated by the intestinal Calcium-sensing receptor (CaSR), an amino acid/peptide-sensitive receptor. Chemical inhibition of CaSR blocked the effect of casein on glucose tolerance in chow and HF rats. In a separate cohort of rats, we knocked down CaSR expression in the USI by ~ 40%, and it abolished the ability of casein to improve glucose tolerance in chow and HF rats. Overall, we demonstrate the effect of USI casein sensing on food intake and glucose homeostasis in chow and HF rats and the role of CaSR in casein-mediated effects on glucose tolerance. Further work is required to assess the role of CaSR in USI protein sensing to regulate food intake and in obesity and diabetes.



Oral Presentation Abstracts

O2-2: The effect of a plant phytohormone and purine signaling in hypothalamic NPY neuronal models.

Calvin V. Lieu¹; Denise D. Belsham^{1,2,3}

¹ Department of Physiology, University of Toronto, Toronto, Canada; ² Departments of Medicine, University of Toronto, Toronto, Canada; ³ Departments of Obstetrics and Gynaecology, University of Toronto, Toronto, Canada

Compound X is a plant phytohormone that control plant cell differentiation and division. From mammalian-based studies, compound X appears to act as an antioxidant and has anti-aging effects. We hypothesized that compound X can alter the hypothalamic expression of the orexigenic feeding neuropeptide NPY via a mechanism involving P2 purine receptors. To assess the effects of compound X on hypothalamic NPY expression, we treated the clonal, immortalized mHypoE-41, mHypoE-44, and mHypoE-46 Npy-expressing cell lines with increasing concentrations of compound X over a 24 hr timecourse. We measured the mRNA expression of Npy via RT-qPCR. We report that compound X significantly decreases Npy expression in the mHypoE-44 and mHypoE-46 lines and increases Npy mRNA levels in the mHypoE-41 neurons. To investigate the involvement of P2 purine signaling, we cotreated our neurons for 16 hr with 100 μ M of compound X and 100 μ M of endogenous purine agonists, ATP or UDP. ATP increased basal Npy expression in all cell lines tested but was unable to block the compound X-induced suppression of Npy in the mHypoE-44 and mHypoE-46 neurons. There was no further induction of Npy expression in the mHypoE-41 neurons by compound X with the addition of ATP. Cotreatment with UDP reduced the repression of Npy by compound X in the mHypoE-44 line without affecting basal Npy expression. Our results demonstrate the ability of compound X to modulate the expression of NPY in hypothalamic neuronal cell models and contribute to the ever-growing list of signalling molecules that target hypothalamic neurons to modulate feeding and weight control.

O2-3: The Saturated Fat Palmitate Alters microRNA Profiles of Exosomes Derived From Hypothalamic NPY/AgRP-expressing mHypoE-46 Cells

Emma K. McIlwraith¹; Denise D. Belsham^{1,2}

¹ Department of Physiology, University of Toronto, Toronto, Canada; ² Department of Obstetrics and Gynaecology, University of Toronto, Toronto, Canada

Exosomes are nanoparticles containing a collection of RNA, protein, and lipids that are potential contributors to the pathophysiology of obesity. A known disruptor of exosome production and content is the most abundant saturated fat, palmitate. One tissue impacted by palmitate is the hypothalamus, a brain region that governs energy homeostasis and houses appetite-stimulating neuropeptide Y (NPY) and agouti-related peptide (AgRP) neurons and appetite-suppressing pro-opiomelanocortin (POMC) neurons. Our laboratory has demonstrated that palmitate induces these neuropeptides and alters the intracellular miRNA profile. We hypothesized that NPY/AgRP-expressing cells would secrete exosomes and that the miRNA content of these exosomes would be altered following palmitate exposure. We isolated exosomes from the NPY-expressing mHypoE-46 cells following treatment with 50 μ M palmitate. The presence of nanoparticles was confirmed by nanoparticle tracking analysis with an average particle diameter of approximately 100 nm. To assess exosomal miRNA levels, we performed a miRNA microarray and found 37 miRNAs were significantly upregulated. We are currently determining the individual effect of three top candidate miRNAs, miR-762, miR-2137 and miR-6366, on hypothalamic neurons by overexpressing each miRNA and quantifying changes in the miRNA target genes. To determine if the exosomes mediate communication between feeding-related neurons, we exposed POMC-expressing mHypoA-POMC/GFP-2 cells to the NPY neuron-derived exosomes resulting in a reduction in early activation gene c-fos after 1 hour. Additional experiments will uncover other genes regulated by exosomes. These studies will reveal the impact excess fat consumption has on hypothalamic feeding neuron-neuron communication via exosomes and the specific role of miRNAs.



Oral Presentation Abstracts

O2-4: Impact of Age on Functional Cholinergic Synapses in Prefrontal Cortex and an Effective Treatment to Restore Nicotinic Signalling

Saige Power

Cholinergic modulation of the prefrontal cortex is essential for attention and executive function. It has long been suggested that the prefrontal cholinergic system is vulnerable in aging, but the functional impact on cortical synapses is unknown. Here, we systematically interrogate age-related changes in the integrity and pharmacology of prefrontal cholinergic transmission using optogenetic stimulation in brain slices from transgenic mice across a broad adult age range. Our results show specific age-dependent nicotinic deficits: young-adult cholinergic signaling exploits both nicotinic and excitatory-muscarinic receptors, while older-adult responses become predominantly muscarinic. Toward rescue of the nicotinic deficit, we applied the nicotinic positive allosteric modulator (PAM) NS9283. It potentiated younger but failed to restore older responses, suggesting a decline in nicotinic receptor availability. Since PKC signalling can boost nicotinic receptor availability, we asked whether the signaling pathways of intact postsynaptic muscarinic receptors could be harnessed toward nicotinic receptor restoration. While M1 PAM alone proved insufficient, the M1 agonist and cognitive-enhancer xanomeline significantly restored nicotinic responses in brain slices from older mice. Pharmacological investigation confirmed xanomeline potentiation of the nicotinic response is muscarinic- and PKC-dependent. This result highlights a novel approach to treat age-related cognitive decline by harnessing muscarinic signalling to rescue nicotinic deficits at prefrontal cholinergic synapses.

O2-5: Allocation to a Hippocampal Engram Supporting a Contextual Fear Memory is Mediated by Neuronal Excitability in Pre-configured Neuronal Ensembles

Andrew J. Mocle^{1,2}; Adam I. Ramsaran^{1,3}; Alexander D. Jacob^{1,3}; Lina M. Tran^{1,2,4}; Blake A. Richards⁵; Paul W. Frankland^{1,2,3,6}; Sheena A. Josselyn^{1,2,3}

¹ Program in Neurosciences & Mental Health, Hospital for Sick Children, Toronto, Canada; ² Department of Physiology, University of Toronto, Toronto, Canada; ³ Department of Psychology, University of Toronto, Toronto, Canada; ⁴ Vector Institute, Toronto, Canada; ⁵ Mila, Montreal, Canada; ⁶ Child & Brain Development Program, Canadian Institute for Advanced Research (CIFAR), Toronto, Canada.

Groups of neurons that store memories are known as engrams. Artificially increasing or decreasing the excitability of a small population of random neurons increases or decreases, respectively, the probability these individual neurons are allocated to an engram, suggesting allocation is a competitive process between eligible neurons determined by relative neuronal excitability. However, whether endogenous allocation to an engram is similarly based on neuronal excitability and/or modulated by other factors is unknown. Here, we used in vivo calcium imaging to examine the activity dynamics of CA1 hippocampal neurons before, during and after contextual fear conditioning. Putative Engram neurons were identified as those with increased activity in the memory test in the conditioning context. After verifying the classification of Engram neurons, we retrospectively traced single Engram and Non-Engram neurons back to the homecage imaging sessions before training. Engram neurons were more active than Non-Engram neurons during training, and up to 3h before training, indicating the excitability of individual neurons varies over time and that neurons with higher excitability before training tend to be allocated to an engram. We also observed pre-configured functional connectivity between neurons that showed stability over days. Sub-ensembles containing more active Engram neurons before training were preferentially recruited into the engram and their functional connectivity strengthened. During the memory test, Engram sub-ensembles recapitulated their activity patterns from training in a context-dependent manner. These data indicate both neuronal excitability in the minutes to hours before training and pre-configured functional connectivity influence allocation to an engram.



Oral Presentation Abstracts

O3-1: Breaking Through the Defense: Biofilm Disruption Using Genetically Modified Macrophages

Sajad Sadat^{1,2}; Michael L. Litvack¹; Deepa Raju³; Vanessa Hsing¹; 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 cloned out high producing PslG-ALMs and characterized the quantity of enzyme produced. 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 of both lab and clinical 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.

O3-2: Hypoxia Increases P-glycoprotein (P-gp) Activity in Developing Human Brain Endothelial Cells: Implications for Fetal Brain Protection

Hafsah Mughis^{1,2}; Phetcharawan Lye^{1,2}; Guinever Imperio¹; Enrrico Bloise⁴; Stephen Matthews^{1,2,3}

¹ Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, Canada; ² Department of Physiology, University of Toronto, Toronto, Canada; ³ Department of Obstetrics and Gynaecology, University of Toronto, Toronto, Canada; ⁴ Department of Morphology, Federal University of Minas Gerais, Brazil

P-gp is an important multidrug resistance transporter expressed at the developing blood-brain barrier (BBB) that confers protection against entry of harmful molecules into the fetal brain. During normal development, the fetus is exposed to relatively low oxygen concentrations (~ 5% O₂). However, pregnancy complications may lead to even lower intrauterine O₂ levels. There is limited information about the effects of hypoxia on fetal BBB P-gp. We investigated whether hypoxia can alter P-gp levels in human fetal brain endothelial cells (fBECs) derived from 2nd trimester. fBECs (N=6) were cultured to assess P-gp expression and function under different levels of oxygen (1%, 5%, and 20% O₂) at 3 time-points: 6h, 24h, and 48h. Changes in P-gp function were analyzed through accumulation of the fluorescent substrate calcein-AM. Protein and gene expression of P-gp/ABCB1 was assessed using Western Blot and RT-qPCR, respectively. P-gp activity significantly increased under 1% O₂, compared to 20%, at all 3 time-points. A significant increase in function was observed in P-gp function at 5% O₂, relative to 20%, only after 48h. No significant changes in P-gp protein expression were observed, however, ABCB1 mRNA levels were significantly decreased under 1% O₂ at 24h and 48h (P<0.05). This is the first study to show that severe hypoxia can lead to sustained increases in P-gp function in human fBECs, which can potentially decrease the transfer of xenobiotics across the fetal BBB. Further understanding of P-gp regulation will potentially lead to the development of strategies to enhance fetal brain protection, particularly during vulnerable pregnancies.



Oral Presentation Abstracts

O3-3: Differential DNA Methylation Following Antenatal Corticosteroids in Newborn Brain and Blood

Bona Kim^{1,3}; Alisa Kostaki¹; Stephen G. Matthews^{1,2,3}

¹ Dept of Physiology, University of Toronto, Toronto, Canada; ² Dept of Obstetrics and Gynaecology, University of Toronto, Toronto, Canada; ³ Lunenfeld-Tanenbaum Research Institute, Sinai Health Systems, Toronto, Canada

Antenatal corticosteroids (ACS), administered to pregnant people at risk of preterm delivery to promote neonatal survival, may be associated with increased risk of neurobehavioural disorders in exposed children. We previously identified differential methylation of gene pathways regulating intracellular protein trafficking in human blood following ACS. We hypothesized that in the brain, ACS may influence methylation of gene pathways around intracellular neurotransmitter transport. Pregnant guinea pigs were injected with saline or betamethasone (1mg/kg) on gestational days 50/51. Prefrontal cortex (PFC) and blood were collected from term-born offspring (n=7/gp) on post-natal day 1. Genomic DNA was extracted and sequenced using reduced representation bisulfite sequencing. Differentially methylated CpG sites (DMC) were identified using bioinformatic approaches (MethPipe, CompEpiTools). In response to ACS, 1521 DMCs were associated with 144 genes in the PFC, highlighting pathways of synaptic assembly and maintenance, intracellular vesicle transport, and glutamate and GABA signaling. In blood, 1677 DMCs (174 genes) were identified, regulating nervous system development, dendrite morphogenesis, and cell adhesion. Seven genes were differentially methylated in common between both tissues. Through this study, we have identified an altered DNA methylome in the PFC and blood of guinea pig offspring following ACS. Interestingly, genes commonly identified in the PFC and blood were involved in GABAergic synapse regulation (IGSF21) and intracellular transport (DNAH17), identifying potential peripheral markers for brain methylation patterns. We are currently investigating target gene expression in response to ACS to elucidate the relationship between early glucocorticoid exposure and risk of neurobehavioural disorder.

O3-4: Integrin Alpha 11 Plays a Critical Role in Cardiac Fibrosis and Infarct Formation Following Myocardial Infarction

Patrick Meagher^{1,2}; Xavier Lee^{1,2}; Jean-Francois Desjardin²; Kim Connelly^{1,2}

¹ Keenan Research Centre for Biomedical Science, St. Michael's Hospital, Toronto, Canada; ² Department of Physiology, University of Toronto, Toronto, Canada

Cardiac fibrosis (CF), a key feature of cardiac remodeling, defined as the accumulation of excessive amounts of extracellular matrix (ECM), in response to injuries such as myocardial infarction (MI). In the heart, cardiac fibroblast within and surrounding the injured area respond to biochemical and mechanical stimuli transforming into myofibroblasts and begin to secrete collagen. Long term collagen production reduces ventricular compliance and ultimately results in further cardiac dysfunction leading to heart failure. The mechanisms via which mechanical stimuli initiate cardiac fibroblast activation are not well understood. Therefore, we sort to investigate the role of Integrin $\alpha 11$, a collagen I binding mechanoreceptor. Fibroblast conditional $\alpha 11$ knockout (KO) mice demonstrated similar cardiac function to that of Wildtype littermates (WT). However, cardiac collagen I (P<0.05) and III (P<0.0001) content was reduce along with cardiac myocyte size (P<0.0001). In response to ligation of the left anterior descending aorta (LAD) $\alpha 11$ KO mice had significantly increased MI size (P<0.05). The larger MI size resulted in increased mortality in $\alpha 11$ KO mice (57%, P<0.05) following LAD ligation compared to that of WT (~22%). Fractional Area Change (FAC) was significantly worse for $\alpha 11$ KO mice (~9%, P<0.0001) compared to WT (~23%). Moreover, $\alpha 11$ KO mice that survived LAD ligation had significantly more collagen I content (P<0.0001) than that of WT along with, ablated cardiac myocyte hypertrophy. In conclusion, the loss $\alpha 11$ results in increased ventricular dilation, impairs cardiac function and was associated with increased mortality following MI. In mice which, survived MI $\alpha 11$ deletion led to increased CF and blunted cardiac myocyte hypertrophy.



Oral Presentation Abstracts

O3-5: A Novel Approach Combining Imaging with Biochemical Assessments to Manage Mechanical Ventilation in ARDS: A Literature Review

Megan Abbott¹; Haibo Zhang¹⁻⁵

¹ Department of Physiology, University of Toronto, Canada; ² Keenan Research Centre for Biomedical Science, St. Michael's Hospital, Unity Health Toronto, Canada;

³ Department of Anesthesiology and Pain Medicine, University of Toronto, Canada; ⁴ Interdepartmental Division of Critical Care Medicine, University of Toronto, Canada; ⁵ Institute of Medical Sciences, University of Toronto, Canada

Acute respiratory distress syndrome (ARDS) has a ~40% mortality rate with an increasing prevalence exacerbated by the COVID-19 pandemic. Although mechanical ventilation (MV) is lifesaving, it can lead to ventilator-induced lung injury (VILI). Personalized strategies to reduce VILI are necessary, but not currently delivered. We will evaluate the integrated approach of computed tomography (CT), electrical impedance tomography (EIT), and plasma biomarkers (Figure 1) to guide individualized management of MV in ARDS. Analysis of electronic databases between September 2021 – March 2022 with keywords including ARDS, MV, VILI, CT, EIT and biomarkers in animal models and human studies. Animal models significantly correlated VILI to mechanical power and strain thresholds using micro-CT analysis. Human CT had high sensitivity, specificity and diagnostic accuracy, and enabled prediction of survival. EIT has been used in experimental and human ARDS to monitor and guide regional tidal volumes and titration of positive end-expiratory pressure (PEEP) to reduce VILI. Biomarkers reflect changes and the severity of lung injury with high sensitivity, but lack specificity. A method to visualize the distribution of MV with imaging and to stratify patient heterogeneity with biomarkers for personalized care is urgently needed to minimize VILI. CT, EIT and biomarkers have been used separately as useful tools for diagnostic and predictive assessment of ARDS. No study has combined CT and EIT with biomarkers in managing ventilated patients at the bedside. We propose a conjunctive approach that combines these three methods towards precision medicine to improve the outcomes of patients with ARDS.



Poster Presentation Abstracts

P1-1: Poor Infant Lung Function is Linked to Early Lower Respiratory Tract Infections in Infancy

Maria Medeleanu¹; Myrtha E. Reyna¹; Ruixue Dai¹; Piushkumar J Mandhane⁴; Elinor Simons⁵; Stuart E. Turvey⁶; Per M. Gustafsson⁷; Theo J. Moraes^{1,2}; Padmaja Subbarao^{1,2}

¹ Translational Medicine Program, The Hospital for Sick Children, Toronto, Canada; ² Department of Pediatrics, Hospital for Sick Children, University of Toronto, Toronto, Canada; ³ Division of Pediatric Respiriology, University of Alberta, Edmonton, Canada; ⁴ Section of Allergy and Immunology, Department of Pediatrics and Child Health, University of Manitoba and Children's Hospital Research Institute of Manitoba, Winnipeg, Canada; ⁵ Department of Pediatrics, British Columbia, Canada; ⁶ Department of Pediatrics, Central Hospital, Skövde, Sweden

Introduction: Lower respiratory tract infections (LRTI) are the leading cause of infant death worldwide and may cause permanent damage the developing infant lung. Whether infant LRTIs can directly cause lung function decline has not been well studied. **Methods:** The CHILD Study is Canadian birth cohort of 3454 mothers, fathers, and infants recruited at birth. We defined LRTI by parent-reported questionnaires (required the presence of i) a cold, ii) a fever and iii) any lower airway symptoms). Infants were classified into two groups (LRTI<18m groups) based on whether a LRTI was reported before 18 months of age. Spirometry and multiple breath washout testing were conducted at the 3,6,12,18 month and 3 and 5 year study visits. Lung function measurements of interest were Forced Expiratory Volume in 0.75 seconds (FEV0.75) z-score, Forced Vital Capacity (FVC) z-score, FEV/FVC z-score and Lung Clearance Index (LCI) z-score. Cross-sectional and longitudinal models were used to explore lung function measurements by LRTI <18 months groups. **Results:** Infants who reported LRTI<18m had significantly lower FEV0.75 z-score ($p=.002$) and FEV/FVC z-scores ($p<0.01$) and slightly higher LCI z-score at the 3 month visit. LRTI <18m was also associated with a -0.11 ($p=0.04$) decrease in FVC z-score from 3m-to-5year visits. All differences in lung function measurements resolved by the 3 and 5 year visits. **Conclusion:** LRTIs are associated with breathing obstruction (FEV0.75 and FEV/FVC z-score) and high lung inhomogeneity (LCI z-score) at the 3 month visit suggesting that poor initial lung function increases the likelihood of having a symptomatic LRTI <18 months of life.

P1-2: Nasal Microbiome Alpha Diversity in Infants With or Without Viral Presence

Yu Chen Qian^{1,2}; Ruixue Dai²; Kelsey Fehr^{3,4}; Vanessa Breton²; Myrtha E. Reyna²; Stuart E Turvey⁵; Piush Mandhane⁶; Mike Surette⁷; Marek Smieja⁸; Elinor Simons⁹; Meghan Azad⁹; Theo Moraes²; Padmaja Subbarao^{1,2}

¹ Department of Physiology, University of Toronto, Toronto, Canada; ² Division of Respiratory Medicine, Department of Pediatrics, Hospital for Sick Children & Research Institute, Toronto, Canada; ³ Children's Hospital Research Institute of Manitoba, Winnipeg, Canada; ⁴ Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, Canada; ⁵ Department of Pediatrics, Child & Family Research Institute, BC Children's Hospital, University of British Columbia, Vancouver, Canada; ⁶ Department of Pediatrics, University of Alberta, Edmonton, Canada; ⁷ Faculty of Health Science, McMaster University, Hamilton, Canada; ⁸ Department of Pathology & Molecular Medicine, McMaster University, Hamilton, Canada; ⁹ Department of Pediatrics and Child Health, Children's Hospital Research Institute of Manitoba, University of Manitoba, Winnipeg, Canada

BACKGROUND: The nasal microbiome is a collection of bacteria living in the nose and sinuses. Viruses are also present in the nasal passage and can compete for similar resources as the nasal microbiome; however, the effect of viral presence on nasal microbiome diversity in infancy is unclear. **AIM:** To identify the association between viral presence and microbiome diversity in infants. **METHODS:** The data used in this study were collected from the CHILD Cohort Study, a large Canadian prospective longitudinal cohort study that follows mothers and children starting from the prenatal stage and continuing into childhood. Anterior nares nasal swabs were obtained at a routine visit from infants at 3 months of age. Bacteria and viruses were identified using 16S rRNA sequencing and polymerase chain reaction (PCR), respectively. Alpha diversity was compared between infants with any and with no viral presence using Wilcoxon rank-sum test. **RESULTS:** Out of 2535 infants, 352 (14%) had any viral presence. Infants with viral presence were older (mean 3.95 months with vs 3.78 months without, $p=0.01$) and had more older siblings (69.3% with older siblings with vs 45.9% without; $p = 0.001$) compared to those without viral presence. Infants with viral presence had lower alpha diversity (p -values < 0.01 in Chao1, Shannon, and Inverse Simpsons) compared to infants without viral presence. These results were further stratified by types of viruses. **SIGNIFICANCE:** Viral presence and microbiome alpha diversity were found to be interrelated in infancy and shaped by factors such as age and number of older siblings.



Poster Presentation Abstracts

P1-3: NINJ1-mediated Plasma Membrane Rupture Leads to Tumour Lysis Syndrome

Keane Paul Baldoviso Fuerte¹; Allen Volchuk²; Neil Michael Goldenberg^{1, 2, 3}

¹ Department of Physiology, University of Toronto, Toronto, Canada; ² Program in Cell Biology, Hospital for Sick Children, Toronto, Canada; ³ Department of Anesthesia and Pain Medicine, Hospital for Sick Children and University of Toronto, University of Toronto

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 tumour cell lines, we now demonstrate that NINJ1 is expressed across many human tumours. In response to cytotoxic drugs, substantial cell lysis occurs, as indicated by lactate dehydrogenase (LDH) release. Knockdown of NINJ1 by siRNA prevents PMR, while still allowing for chemotherapy-induced cell death. Taken together, our data suggest a role for NINJ1 in tumour 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.

P1-4: The Characterization of LTBP2 in Cardiac Fibrosis

Fahad Ehsan

Introduction: Cardiac fibrosis is characterized by the pathological remodelling of the myocardium through excessive extracellular matrix (ECM) protein deposition by cardiac fibroblasts (Cfb). Of the ECM proteins released, Latent transforming growth factor binding protein 2 (LTBP2) stands out to be present in every case of heart failure that we sought to better characterize in infarcted hearts and activated fibroblasts. **Methods:** Published transcriptomic data were analyzed for LTBP2 expression across heart failure models in mice and humans. Subsequently, myocardial infarction was modelled through the ligation of the left anterior descending (LAD) coronary artery causing an infarct in the apical region of the heart. Hearts were collected seven days post LAD ligation and used for immunofluorescence microscopy (IF). Alternatively, primary mice Cfb were plated and treated with TGF-B to study the expression of LTBP2 in activated Cfb. **Results:** Analysis of available transcriptomic data showed a significant increase in LTBP2 expression in ischemic (fold change (FC) > 2.0, p-value < 0.01) and nonischemic (FC > 3.0, p-value < 0.0001) heart failure in both mice and humans compared to sham or healthy control. In infarcted hearts, LTBP2 localized mainly to the apex of infarcted mouse hearts. In-vitro analysis of Cfb showed TGF-B treatment significantly induced LTBP2 expression. **Conclusion:** In this preliminary assessment, LTBP2 is shown to be an early marker for cardiac fibrosis across various heart failure models however, its function remains unknown. Analysis in LTBP2 ^{-/-} are ongoing to understand the role of LTBP2 in cardiac fibrosis and its importance in heart failure.



Poster Presentation Abstracts

P1-5: Sodium-Glucose Cotransporter 2 Inhibitors and its Cardiorenal Benefits in Individuals with Diabetes Mellitus: Cardiovascular and Kidney Disease Biomarker Analysis

Luxcia Kugathasan^{1,2,3}; David Cherney^{1,2,3,4}

¹ Department of Medicine, Division of Nephrology, University Health Network, Toronto, Canada; ² Department of Physiology, University of Toronto, Toronto, Canada; ³ Cardiovascular Sciences Collaborative Specialization, University of Toronto, Toronto, Canada; ⁴ Department of Medicine, University of Toronto, Toronto, Canada

Diabetes mellitus is one of the fastest growing public health crises worldwide and accounts for 6% of global prevalence. Chronically high blood glucose, a hallmark of diabetes, stems from impaired insulin release or action, defined as type 1 diabetes (T1D) or type 2 diabetes (T2D) respectively, and can implicate a myriad of complications. Namely, diabetes is one of the leading causes of chronic kidney disease (CKD) and is a prime risk factor for cardiovascular disease (CVD). Until recently, the most effective treatment plan for diabetes-associated CVD and CKD was to monitor blood pressure and glucose. This shifted when sodium-glucose cotransporter 2 (SGLT2) inhibitors revolutionized clinical practice for people with T2D. In addition to promoting glucosuria, SGLT2 inhibitors were also reported to display protective effects against CVD and CKD progression. Since this discovery was serendipitous, the mechanisms that underpin these observed clinical benefits remain unclear. Since SGLT2 inhibitors have mainly been studied as a T2D therapeutic, the potential benefits in T1D are incompletely understood. My research aims to advance our current state of knowledge by investigating how SGLT2 inhibitors elicit protection against T1D-associated CKD and CVD risk. To investigate this, I will (1) identify CVD and CKD biomarkers with unique expression profiles between non-T1D and T1D patients, and (2) examine whether intervention modifies the T1D-specific marker profile. By exploring mechanisms of SGLT2 inhibitors, health research can expand treatment options for additional patient groups, including those with T1D, CKD, and CVD, in hopes of reducing diabetes-related complications and mortality.

P1-6: Ex-vivo Organ Perfusion Model of Kidney Fibrosis in the Mouse

Jorge Castillo-Prado^{1,2}; Ian Rogers^{1,2,3,4}

¹ Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Canada; ² Department of Physiology, University of Toronto, Toronto, Canada; ³ Soham & Shaila Ajmera Family Transplant Centre, University Health Network, Toronto, Canada; ⁴ Department of Obstetrics and Gynaecology, Mount Sinai Hospital and the University of Toronto, Toronto, Canada

Chronic kidney disease (CKD) affects 11-13% of the population worldwide. A common end-stage hallmark in CKD is interstitial fibrosis. However, we currently lack a practical, easily generated disease model that fully recapitulates all CKD manifestations such as fibrosis. Limited studies have utilized ex-vivo cultures in kidney fibrosis research. In ex-vivo whole-organ culture, tissues or organs are isolated from an animal and maintained in an optimal environment. Tightly controlled conditions of ex-vivo cultures minimize alterations and deviations between experiments. This culture setting enables the practical administration of treatments and live observation of the experiment's progress at the whole-organ level. Two possible means to generate kidney fibrosis are ureteric obstruction (UO) and cisplatin-induced nephrotoxicity. Ureteric obstruction leads to tubular epithelium degeneration and accumulation of extracellular matrix leading to fibrosis. Cisplatin therapy is an anti-neoplastic treatment used in various forms of cancer. It induces side-effects in the kidney including cytotoxicity and interstitial fibrosis. The kidneys utilized in this research were isolated and the renal artery cannulated for media perfusion. The cannulated kidneys were connected to a bioreactor system developed by the Rogers Lab. UO kidneys underwent ureter obstruction. Cisplatin-treated kidneys received a single high dose of cisplatin of 10mg/mL. Preliminary results showed fibrosis appeared in UO kidneys by day 4 and 7 of culture. Also, control kidneys cultured for 21 days have shown cell survival. Future steps include utilizing therapeutic agents to study the clinical feasibility of this system.



Poster Presentation Abstracts

P1-7: The Exocytotic Protein, Secretagoin, is Essential for Circadian Glucagon-like Peptide-1 Secretion

Andrew Biancolin¹; Hyerin Jeong¹; Arjuna Srikrishnaraj¹; Patricia Brubaker^{1,2}

¹ Department of Physiology, University of Toronto, Toronto, Canada; ² Department of Medicine, University of Toronto, Toronto, Canada

The intestinal L-cell hormone, glucagon-like peptide-1 (GLP-1), upregulates insulin secretion in a glucose-dependent manner and is therefore used in the treatment of type 2 diabetes. This anti-diabetic hormone exhibits a circadian pattern in its secretion in rodents and varies by time of day in humans. Recently, we have shown that in vitro knockdown of secretagoin (Scgn), an endocrine secretory protein, results in a loss of circadian GLP-1 secretion. We thus hypothesized that in vivo knockout of Scgn results in decreased circulating GLP-1 levels and an ablated physiological rhythm. Scgn levels were undetectable via RT-qPCR or immunofluorescence in constitutive Scgn knockout (KO) mice, which exhibited increased fasting blood glucose ($p < 0.05$) in association with decreased peak GLP-1 secretion ($p < 0.05$) as compared to wild-type animals. Inducible knockout models were then developed by crossing Scgn-floxed mice with intestinal epithelial- (Villin-creERT2/+) and proglucagon- (Gcg-creERT2/+) cre animals. The intestinal epithelial-specific Scgn knockout mice exhibited increased fasting blood glucose ($p < 0.05$) as well as decreased peak GLP-1 secretion ($p < 0.05$) when compared to controls (male and female – wild-type, cre and/or floxed, with and without tamoxifen as appropriate). The proglucagon-specific Scgn knockout animals demonstrated no changes in fasting blood glucose, but fasting plasma glucagon levels were decreased ($p < 0.05$) and the inhibitory effect of glucose was abolished ($p < 0.05$). Importantly, the physiological rhythm in GLP-1 secretion was ablated. Together, these studies identify secretagoin as an essential regulator of GLP-1 secretion in vivo, and confirm that rhythmic release of GLP-1 plays an important role in the maintenance of glucose homeostasis.

P1-8: Multifaceted Impact of Ω 3-polyunsaturated Fatty Acids on Kv1.2 Channels and Inhibitory Neurotransmission

Tian Kong²; Jason Arsenault¹; Bassam Tawfik¹; Lu-Yang Wang^{1,2}

¹ Program in Neurosciences & Mental Health, Sick Kids Research Institute, Toronto, Canada; ² Department of Physiology, University of Toronto, Toronto, Canada

Principal neurons encode information by varying their firing rates and patterns fine-tuned through GABAergic interneurons. We have previously 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. We created a stable Kv1.2-GFP CHO cell-line and combined electrophysiological patch clamp technique and confocal fluorescence microscopy to investigate the effect of DHA on Kv1.2 activity, expression, and localization. Electrophysiological recordings of these cells revealed that DHA acutely accelerates Kv1.2 activation and decelerates its deactivation. Using a combination of in silico simulation and site-directed mutagenesis, we demonstrated that DHA produces its allosteric positive effects by directly binding to a deep cavity that shifts the voltage sensor (S4) of Kv1.2. Chronically, an elevation in Kv1.2-GFP membrane expression was observed after 48hr DHA treatment or when dynasore was co-applied 3hr following DHA treatment, suggesting that DHA promotes Kv1.2 trafficking to cytoplasmic membranes from the intracellular pool. 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 and other NDDs linked to over-inhibition.

Poster Presentation Abstracts

P1-9: Relationship Between Actigraphy Measures and Diurnal Mood Measures in Day Treatment of Major Depressive Disorder

Nastasia Kuibid^{1,2}; Ryan Klein^{2,3}; Sean Hill^{1,2,3}; John Strauss³; Jeff Daskalakis⁴; Judith Laposa^{2,3}; Stefan Kloiber^{2,3}; Marco Battaglia^{2,3}; Robert Levitan^{1,2,3}

¹ Department of Physiology, University of Toronto, Toronto, Canada; ² The Centre for Addiction and Mental Health, Toronto, Canada; ³ Department of Psychiatry, University of Toronto, Toronto, Canada; ⁴ Department of Psychiatry, University of California San Diego, La Jolla, CA, United States

Chronotype, one's circadian preference to be awake and active at a specific time of day, reflects intrinsic circadian rhythms. Evening chronotypes experience worse depression severity and higher risk of treatment failure. In patients with Major Depressive Disorder (MDD), receiving Integrated Day Treatment (IDT) at a time asynchronous to chronotype was associated with improved treatment response. The current project expands on the role of circadian rhythms in treatment response, by objectively measuring chronotype using actigraphy. To capture sleep and activity rhythms over treatment, participants with MDD wore passive actigraphy devices daily, for several weeks of IDT. To assess potential diurnal variation in mood, participants self-rated their mood at different times of day, every other day, using hand-held devices, reported as early-day and late-day ratings. The Patient Health Questionnaire-9 and Morningness-Eveningness Questionnaire assessed changes in depression severity and chronotype, respectively. Percent change scores from early (Week 1) to late treatment (Week 3) were calculated for each variable. Spearman correlations to determine the relationship of change scores to each other were completed. Due to small sample size, a correlation coefficient (R) cut-off of 0.5+ was considered a medium effect size of interest. Increased morningness was associated with reduced negative thoughts (R=0.6) and negative mood (R=0.8) early in the day across treatment. This effect was not observed in late-day mood. Decreased overall depression severity at treatment completion was related to increased morningness (R=0.8) and improved sleep efficiency (R=-0.5). This corroborates that the ability of IDT to advance chronotype improves depression, particularly for early-day mood.

P1-10: NINJ1 Forms Megapores Within the Plasma Membrane During Pyroptosis that are Targeted by the Amino Acid Glycine

Jazlyn P. Borges^{1,2}, Allen Volchuk³, Bridget Kilburn¹, Neil M. Goldenberg^{3,4,5}, Benjamin E. Steinberg^{1,2,4,5}

¹ Program in Neuroscience and Mental Health, The Hospital for Sick Children, Toronto, Canada; ² Department of Physiology, Temerty Faculty Medicine, University of Toronto, Toronto, Canada; ³ Program in Cell Biology, The Hospital for Sick Children, Toronto, Canada; ⁴ Department of Anesthesia and Pain Medicine, The Hospital for Sick Children, Toronto, Canada; ⁵ Department of Anesthesiology and Pain Medicine, University of Toronto, Toronto, 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 had been assumed to be a passive event but was recently found to be an active process mediated by nerve injury-induced protein 1 (NINJ1). NINJ1 is postulated to cluster within the plasma membrane to mediate cell rupture; however, the mechanisms by which NINJ1 clusters to mediate membrane rupture and its regulation remain to be determined. We firstly aimed to determine the membrane organization of NINJ1 clusters in primary mouse macrophages using super-resolution microscopy. Using stimulated emission depletion microscopy (STED), we show that during pyroptosis, NINJ1 clusters to form large membrane pores of ~150 nm in diameter. We next aimed to determine whether NINJ1 clustering is blocked by the amino acid glycine, which has been known to provide cytoprotection against membrane rupture through an unknown mechanism for over 30 years. We find that glycine blocks NINJ1 clustering and pore formation, thereby revealing a regulatory mechanism of NINJ1-mediated membrane rupture and identifying the elusive protein target of glycine cytoprotection. Our work reveals novel insight into the mechanisms and regulation of NINJ1-mediated plasma membrane rupture. In turn, this will inform the development of tissue preservation strategies and therapeutics for the numerous pathologies in which lytic cell death has an important role.



Poster Presentation Abstracts

P1-11: Mechanism Underlying General Anesthetic Drug Induced Cognitive Deficits

Li Ju¹; Arsène Pinguelo¹; Anthony Ariza¹; Dianshi Wang¹; Beverley Orser^{1,2,3}

¹ Department of Physiology, University of Toronto, Toronto, Canada; ² Department of Anesthesiology & Pain Medicine, University of Toronto, Toronto, Canada; ³ Department of Anesthesia, Sunnybrook Health Sciences Centre, Toronto, Canada.

Long term cognitive deficits are common adverse consequences after surgery and anesthesia. General anesthetic drugs likely contribute to cognitive deficits. We have previously shown that a single exposure to anesthetic drugs can cause persistent cognitive impairment, mediated by an increase in tonic inhibition in neurons. Interestingly, studies in cell culture showed that this anesthetic-induced increase in tonic inhibition was triggered by factors released from astrocytes. The molecular mechanism for this astrocyte-neuron crosstalk remains unknown. The first aim of my project is to identify the astrocytic receptors that trigger the sustained increase in tonic current in hippocampal neurons due to general anesthetic drugs. Our preliminary results suggest that $\alpha 4$ GABAA receptors ($\alpha 4$ GABAAR) on the astrocytes may play a crucial role. I have maintained previously generated $\alpha 4$ GABAAR knockout colonies and wild type animals which the genotype has been confirmed using PCR. Subsequently, I have dissected and created co-culture of $\alpha 4$ GABAAR knockout astrocytes and CD1 neurons, which the tonic current has been assessed using whole cell patch clamping after etomidate or vehicle treatment, to determine whether astrocyte $\alpha 4$ GABAAR indeed contribute to the molecular pathway. The second aim is to identify factors released into the medium by astrocytes. Preliminary data shows that antagonists of interleukin-1 β (IL-1 β) prevented anesthetic drug-induced increase in tonic current in neurons, however, IL-1 β has not been directly detected in the astrocyte medium. I am optimizing the detection of the presence of IL-1 β in the astrocyte medium using ELISA in order to verify whether IL-1 β signalled the increase of tonic current.

P1-12: A Novel Peptide Interferes with the Interaction Between Radixin and $\alpha 5$ GABAA Receptors

Setareh Malekian Naeini¹; Anthony Ariza¹; Beverley A. Orser^{1,2,3}

¹ Department of Physiology, University of Toronto, Toronto, Canada; ² Department of Anesthesiology & Pain Medicine, University of Toronto, Toronto, Canada; ³ Department of Anesthesia, Sunnybrook Health Sciences Centre, Toronto, Canada

Dysregulation of $\alpha 5$ GABAA receptor ($\alpha 5$ GABAAR) expression contributes to a variety of neurocognitive disorders, including anesthetic-induced cognitive deficits. Our team previously showed that general anesthetic drugs trigger a persistent increase in $\alpha 5$ GABAAR surface expression. Increased surface expression of these receptors leads to an increase in tonic inhibitory neurotransmission, which causes cognitive deficits. $\alpha 5$ GABAARs are anchored to the membrane by radixin, a cytoskeletal protein. Disrupting this interaction is a potential avenue for reducing $\alpha 5$ GABAAR surface expression following general anesthesia. Here, we describe the effects of a novel peptide that reduces radixin- $\alpha 5$ GABAAR interactions in hippocampal slices. Studies were approved by the local animal care committee. Hippocampal slices were prepared from mice aged 8–9 weeks. Slices were preincubated with okadaic acid (1 μ M) for 1 hour to induce phosphorylation. Subsequently, brain slices were sonicated and brain lysates were treated with or without the peptide (0.1 μ M) for 1 hour. Radixin- $\alpha 5$ GABAAR binding was assessed using co-immunoprecipitation and western blots. Co-immunoprecipitation of radixin and $\alpha 5$ GABAAR showed that okadaic acid increased radixin- $\alpha 5$ GABAAR binding. These findings confirm that interaction of radixin with $\alpha 5$ GABAAR is phosphorylation-dependent. Importantly, we found that the peptide markedly reduced radixin- $\alpha 5$ GABAAR binding in hippocampal slices. Our results provide evidence of a potential novel strategy to reduce $\alpha 5$ GABAAR activity by disrupting its interaction with radixin which may be effective in treating disorders associated with $\alpha 5$ GABAAR over-activity.



Poster Presentation Abstracts

P1-13: Characterizing Critical Language Sites in Children and Adolescents Using MEG and rTMS

Sara Sino^{1,2}; Vivek Sharma¹; Hansel M. Greiner^{3,4}; Kishore Vedala^{3,4}; Jennifer Vannest⁵; Hisako Fujiwara^{3,4}; Jeffrey R. Tenney^{3,4}; Brady J. Williamson⁶; Darren S. Kadis^{1,2}

¹ Neurosciences and Mental Health, Hospital for Sick Children, Toronto, Canada; ² Department of Physiology, Faculty of Medicine, University of Toronto, Toronto, Canada; ³ Division of Neurology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States; ⁴ Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, United States; ⁵ Division of Speech Language Pathology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States; ⁶ Division of Radiology, University of Cincinnati, Cincinnati, OH, United States.

INTRODUCTION: We compare MEG connectivity and centrality-based language maps to language rTMS findings. **METHODS:** 26 subjects, ages 5-19, completed MEG (stories) and navigated rTMS (naming). MEG was collected on a 275-channel whole-head system (CTF, Coquitlam, BC), at 1200Hz, and analyzed using FieldTrip routines. Continuous recordings were filtered from 0.1-100Hz, and power line noise suppressed. Artifacts were removed via ICA. Data were epoched from 0-2000ms, from sentence onset, and sources estimated via an LCMV beamformer. Connectivity (coherence, imaginary coherence, PLV, wPLI), and centrality (eigencentrality, betweenness) were computed within canonical frequency bands. Picture naming is widely used in rTMS. We studied 33 sites (standardized) per hemisphere, regularly distributed over the lateral cortex. Each site was assessed 3 times. Pulses were initiated 300ms after picture onset, delivered at 5Hz for 1 second. Sessions were reviewed by two independent raters. Sites were deemed critical for language if both raters identified errors (no response, phonologic, or semantic). MEG and rTMS results were assessed in a common anatomical framework and compared using ROC analyses. Combinations yielding maximal AUC were identified; Youden's J-statistic identified optimal centrality threshold for the preferred schemes. **RESULTS:** rTMS revealed predominantly left lateralized critical language sites across participants. ROC analyses revealed eigencentrality on coherence and PLV in the beta band were in maximal agreement with rTMS. Retaining the top 65% of nodes yielded the optimal balance between sensitivity and specificity. **CONCLUSION:** Sites showing high eigencentrality within the beta band are useful for mapping critical cortical language sites.

P1-14: Identification of Resin Degrading Enzymes from Human Neutrophils

Ava Ragheai^{1,2}; Russel Gitalis^{1,2}; Yoav Finer^{1,2}; Michael Glogauer^{1,3}

¹ Finer Lab, Faculty of Dentistry, University of Toronto, Toronto, Canada; ² Institute of Biomedical Engineering, University of Toronto, Toronto, Canada; ³ Dental Oncology, Princess Margaret Cancer Centre, Toronto, Canada

Background: Neutrophils can break down resin composite and adhesives, as well as compromise resin-dentin interfaces, potentially contributing to recurrent caries, a major cause for restoration failure (Gitalis R., et al., Acta Biomaterialia 2019 and 2020). However, the specific factor(s) from neutrophils responsible for resin degradation is unknown. **Objective:** To determine the contribution of neutrophil elastase (NE) and acyloxyacyl hydrolase (AOAH) in hydrolyzing ester bonds of methacrylate monomers. **Methods:** Purified NE (FroggaBio Inc., ON) and recombinant AOAH, R345E-K379E mutant (Dr. Nagar, McGill University, QC) were incubated (37°C, 5% CO₂, pH=6.8) with common dental methacrylate-based monomers, 2,2-bis[4(2-hydroxy-3-methacryloxypropoxy)-phenyl]propane (BisGMA) or diurethane dimethacrylate (UDMA) in Hanks' Balanced Salt solution (HBSS) for 72 hours. Every 24 hours samples were removed and mixed with methanol to terminate the enzymatic reaction. Degradation byproducts 2,2-bis[4(2,3-hydroxypropoxy)phenyl]propane (bisHPPP), diurethane monomethacrylate and diurethane were quantified using Ultra Performance Liquid Chromatography. **Results:** NE in HBSS increased bisHPPP release (0.56±0.13 vs. 0.003±0.006 µg/mL, p<0.05) and diurethane monomethacrylate release (424927±21100 vs. 1300±98 area of MS peak (mV*sec), p<0.05) compared to HBSS alone after 72 hours. AOAH in HBSS increased bisHPPP release (0.052±0.001 vs. 0.003±0.006 µg/mL p<0.05) and diurethane monomethacrylate (81989±1918 vs. 1300±98 area of MS peak (mV*sec), p<0.05) compared to HBSS alone after 72 hours. **Conclusions:** NE and AOAH hydrolyse dental methacrylates and could contribute to the neutrophils' whole cell degradative activity.



Poster Presentation Abstracts

P1-15: Generation of Non-immunogenic Macrophages for Allogenic Cell Therapies

Jean Kit Tang^{1,2}; Huijuan Yang^{1,2}; Jeff Harding²; Kristina Nagy^{1,2}; Sheena Bouch⁵; Martin Post^{1,5}; Ian Rogers^{1,2}; Andras Nagy^{2,3,4,6}

¹ Department of Physiology, University of Toronto, Canada; ² Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Canada; ³ Institute of Medical Science, University of Toronto, Toronto, Canada; ⁴ Department of Obstetrics and Gynaecology, University of Toronto, Toronto, Canada; ⁵ Peter Gilgan Centre for Research and Learning, Hospital for Sick Children, Toronto, Canada; ⁶ Australian Regenerative Medicine Institute, Monash University, Melbourne, VIC, Australia

Cell-based therapies utilizing hematopoietic cells - given their vast range of functions - have the potential to treat numerous devastating diseases. However, as with all cell-based therapies, the fundamental challenges of safety and allogenic acceptance are crucial hurdles that need to be overcome. We have previously integrated the eight immunomodulatory transgenes of the iACT system into human SafeCell (SC) ESCs to prevent allograft rejection without the use of any immunosuppression. The SC system combined with iACT (SC-iACT cells) technology has proven to be compatible with generating a spectrum of potential therapeutic cells. Here we show that differentiated cells derived from SC and SC-iACT ESCs, not only exhibit myeloid cell morphology but are also capable of differentiating into alveolar macrophage-like cells. While their phagocytosis functionality is still being investigated, we are aiming to optimize the direct myeloid differentiation protocol in order to increase its differentiation efficiency. Ultimately, the generation of functional hematopoietic SC-iACT cells will advance the development of future cell-based therapies while overcoming the challenges of safety and allogenic acceptance.

P1-16: Antenatal Synthetic Glucocorticoid Exposure Modifies the Response to Post-natal Infection at the Blood-brain Barrier

Margaret Elizabeth Eng¹; Stephen G. Matthews^{1,2,3}

¹ Department of Physiology, University of Toronto, Toronto, Canada; ² Department of Obstetrics and Gynaecology, Toronto, Canada; ³ Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, Canada

Antenatal synthetic glucocorticoids (sGCs) administered in the management of pre-term birth can have systemic fetal effects, including acute changes in blood-brain barrier (BBB) drug transporter P-glycoprotein (P-gp; Abcb1). Our lab has previously shown prenatal sGC exposure in pregnant guinea pigs modifies the response of fetal BEC drug transporters to pro-inflammatory cytokines in vitro. We hypothesized that maternal antenatal sGC exposure will modify the BBB P-gp response to post-natal infection. Pregnant guinea pigs (N = 42) received multiple courses (gestation day (GD)s 40, 41 50, 51 and 60, 61) of betamethasone (BETA; 1 mg/kg) or vehicle (saline). BECs derived from post-natal day (PND) 14 offspring were exposed to single stranded RNA (ssRNA-40) virus mimic (0.001 – 1 µg/mL) or Lyovec control, for 4 or 24 hours. P-gp function, and expression of cytokines/interferons were assessed. In BECs from females, there was a significant main effect of prenatal treatment (Two-way ANOVA; * P = < 0.05) on P-gp Activity after 4 hours of ssRNA-40 treatment (not 24 hours). In BECs from males, there was no significant effect of prenatal treatment or ssRNA-40 on P-gp function or interferon/cytokine expression. However, in cells derived from females, there was a significant main effect of prenatal BETA on mRNA levels of IL-6 (increased) and IFN-β1 (decreased). The significant interplay between prenatal sGCs and inflammation at the developing BBB is important in the context of preterm birth, as this population of infants may both receive prenatal sGCs and be at risk for infection/inflammation (e.g., chorioamnionitis) in utero or postnatally.



Poster Presentation Abstracts

P1-17: Effects of Antenatal Glucocorticoids on miRNA Levels in the Prefrontal Cortex of the Newborn Guinea Pig Brain

Danna Ellner¹; Bona Kim^{1,3}; Alisa Kostaki¹; Stephen G. Matthews^{1,2,3}

¹ Department of Physiology, University of Toronto, Toronto, Canada; ² Department of Obstetrics and Gynaecology, University of Toronto, Toronto, Canada; ³ Lunenfeld-Tanenbaum Research Institute, Sinai Health Systems, Toronto, Canada

Synthetic glucocorticoids (sGC) are a common treatment for women at-risk for preterm birth. This is because glucocorticoids act as a signal to induce maturation of major organs. Suboptimal timing, or excessive exposure to glucocorticoids can alter the trajectory of development and result in behavioural differences and negative health implications later in life. Previous animal and human studies have demonstrated that antenatal sGC exposure is associated with altered stress response later in life. The prefrontal cortex (PFC) expresses high levels of glucocorticoid receptors and has been associated with many of the behavioural changes observed to be associated with sGC exposure. Understanding the underlying mechanisms of these processes will lead to a greater awareness of the implications of prenatal exposure to sGCs. In the present study, we hypothesized that antenatal sGC exposure leads to altered expression of miRNA in the PFC of the new born brain. Pregnant guinea pigs were injected with betamethasone (BETA; 1mg/kg; n=7) or saline (n=7) on gestational days 50 and 51, and offspring were euthanized on postnatal day (PND) 1. The medial prefrontal cortex (mPFC) of female PND1 offspring was isolated. Total RNA was isolated and analyzed using a miRNA microarray (GeneChip miRNA 4.0 Array). MiRNA expression levels were analyzed using the Transcriptome Analysis Console 4.0.1 (Thermo Fisher Scientific Inc.). There was no significant effect of antenatal sGC exposure on miRNA levels in the mPFC of newborn guinea pigs. These results are important as synthetic glucocorticoids are an essential treatment used worldwide in the management of preterm delivery.

P1-18: Cerebral Small Vessel Disease and Thickness of the Cerebral Cortex in Adults

Ariana Tang^{1,2}, Jean Shin^{1,2,3}, Tomas Paus⁴, Zdenka Pausova^{1,2,3}

¹ Hospital for Sick Children, Toronto, Canada; ² Department of Physiology, University of Toronto, Toronto, Canada; ³ Department of Nutritional Sciences, University of Toronto, Toronto, Canada; ⁴ Departments of Psychiatry and Neuroscience and Centre Hospitalier Universitaire Sainte-Justine, University of Montreal, Montreal, Canada; ECOGENE-21, Chicoutimi, Canada; Departments of Psychology and Psychiatry, University of Toronto, Toronto, Canada

Background: White-matter hyperintensities (WMH) are focal, bright areas on T2-weighted magnetic resonance images of the brain. WMH are a neuroimaging marker of small vessel lesions in white matter; they may impact the cerebral cortex by reducing thickness. We tested the association between WMH volume and cortical thickness (CT) and explored possible mechanisms with genomic and transcriptomic approaches. Methods: We studied 17,612 individuals (52.5% female, 63 years) from the population-based UK Biobank study. WMH volume was quantified from T2-weighted FLAIR magnetic resonance (MR) images of the brain; mean CT and regional CTs were assessed from T1-weighted MR images of the brain. Regional CTs were quantified at 34 regions of the cerebral cortex delineated by FreeSurfer. Results: Higher WMH volume was associated with lower mean CT ($p=4.7 \times 10^{-22}$). The association varied across the cortex and was of the largest effect size in the insular cortex ($pFDR=1.6 \times 10^{-84}$), which a region of the cortex known to be particularly vulnerable to reduced blood flow. Genome-wide association study of shared variance between WMH volume and insular CT identified a genome-wide significant locus at rs79934840 near KLHL24 ($p=5.7 \times 10^{-9}$). The locus was associated with mRNA expression of KLHL24 in the brain ($p=1.3 \times 10^{-10}$), where the gene was highly expressed in neurons and glial cells. Additionally, WMH volume mediated the association between the locus and insular CT ($p=2.2 \times 10^{-16}$). Conclusions: Small vessel disease in white matter may contribute to thinning of the cerebral cortex in middle-aged and older individuals. Processes involving neurons and glial cells may be involved in this cortical thinning.



Poster Presentation Abstracts

P1-19: Removal of Epigenetic Barrier Enables ESC to Access Trophoblast and Primitive endoderm Fates and Self-assemble into Blastoids

Jessica-Lynne Welton¹; Brian Cox¹

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

A blastoid model generated from a single cell can provide clues into the Blackbox of early lineage specification. Current murine models well illustrate lineage self-assembly but circumvent fate specification by combining fully differentiated trophectoderm (TE) and embryonic (EPI) lineages. The objective is to reprogram ESCs to a "plastic" state to enable access to restricted trophoctoderm (TE) and primitive endoderm (PrE) lineages. Closer replication of in vivo development may provide greater opportunity to study the first essential lineage choices in development. Epigenetic reprogramming is used, via specific histone deacetylase (HDAC) 1-3 chemical inhibition of ESCs, to promote a permissive and accessible chromatin state. HDAC-inhibited ESCs grown in non-adherent cultures aggregated in blastocyst-like structures with spatial organization. Results of transcript (rt-qPCR) and protein expression (immune-fluorescence) analysis confirm that HDAC1-3 inhibited ESCs access the 2-cell (2C) state prior to accessing the restricted trophoblast fate. Significant increase in expanded potential marker Zscan4, is seen after 3 days of HDAC inhibition. After a longer HDAC inhibition duration (6 days), trophoblastic (Cdx2 & Eomes) and PrE lineage (Sox17) markers significantly increase during aggregate culture. Optimal culture media produced a high percentage of blastocyst-like structures (>10%). In summary, I demonstrate evidence that HDAC1-3 inhibited ESCs have the capacity to access alternative TE and PrE fates and self-assemble into blastocyst-like structures.

P1-20: Exposure to Synthetic Glucocorticoids Modifies the microRNA Cargo of Epididymal Extracellular Vesicles: Implications for Intergenerational Transmission

Christopher Casciaro¹; Hirotaka Hamada¹; Alisa Kostaki¹; Stephen G. Matthews^{1,2,3,4}

¹ Department of Physiology, University of Toronto, Toronto, Canada; ² Department of Obstetrics and Gynaecology, University of Toronto, Toronto, Canada; ³ Department of Medicine, University of Toronto, Toronto, Canada; ⁴ Alliance for Human Development, Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, Canada

Introduction: Specialized extracellular vesicles (epididymosomes) are crucial in shaping the molecular landscape of sperm. It has been suggested that the miRNA cargo within epididymosomes becomes altered after environmental adversity, and this altered miRNA complement can influence gene expression within the embryo. We hypothesized that the miRNA profile of caput and cauda epididymosomes will be altered following sGC exposure, paralleling changes that we have previously demonstrated in sperm. **Methods:** Adult male guinea pigs were exposed to the sGC dexamethasone (Dex) in drinking water or normal water (N=6/group) every other day for 48 days. Caput and cauda epididymal fluid was ultracentrifuged to isolate epididymosomes. Epididymosomes were imaged and size/concentration were evaluated. RNA was extracted from caput and cauda epididymosomes, and miRNA expression was assessed by miRNA 4.0 microarray. **Results:** Dex-treatment did not affect epididymosome size or concentration. miRNA expression analysis revealed no significant changes in the caput following Dex-treatment. In the cauda, 111 miRNA were down-regulated while 141 were up-regulated. Examples of miRNA that were significantly downregulated by Dex included miR-125 and miR-199. These miRNAs were also affected in the cauda sperm of Dex-treated animals. **Conclusion:** These findings demonstrate that Dex-exposure influences the miRNA cargo of epididymosomes in the cauda, and parallel changes observed in sperm. This is highly clinically relevant as a deeper understanding of the molecular mechanisms responsible for the transmission of paternal experiences could help in the development of new approaches to break the cycle of intergenerational transmission, and identify novel predictive biomarkers of embryo, fetal and postnatal health.



Poster Presentation Abstracts

P2-1: 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, Toronto, Ontario; ² Department of Physiology, The University of Toronto, Toronto, Canada; ³ Department of Anesthesia and Pain Medicine, University of Toronto, Toronto, Canada; ⁴ Department of Anesthesiology, University of Toronto, Toronto, Canada

Pulmonary arterial hypertension (PAH) is characterized by substantial pulmonary vascular inflammation and elevated circulating levels of cytokines. A critical component of this inflammation is the inflammasome, a multi-protein complex found in immune and vascular cells. Inflammasome assembly activates caspase-1, which cleaves several proteins, including gasdermin D (GSDMD). Cleaved GSDMD subunits form a pore in the cell membrane, through which cytokines such as IL-1 β are secreted, ultimately causing pyroptotic cellular rupture and intracellular DAMP release. Given the importance of IL-1 β and these other inflammatory molecules in PAH pathogenesis, we wished to study the role of GSDMD in PAH. Following 4 weeks of hypoxia, Gsdmd^{-/-} mice had significantly lower right ventricular systolic pressure than controls ($p=0.03$). Plasma levels of IL-1 receptor antagonist (a marker of IL-1 β production) were higher in WT than Gsdmd^{-/-} mice. To explore the mechanism of inflammasome activation in the context of hypoxia, we examined the effect of hypoxia on primary BMDM during inflammasome LPS priming. Notably, hypoxia potentiated upregulation of NLRP3 inflammasome protein in response to LPS. Furthermore, triggering of inflammasome activation was increased by hypoxia, with an increase in cleaved caspase-1 and secreted IL-1 β . Hypoxia itself stimulated upregulation of pro-IL-1 β mRNA, 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. Mechanistically, we have shown that hypoxia potentiates inflammasome activation, and may itself serve to prime inflammasomes. Together, these data demonstrate that GSDMD may be a therapeutic target in PAH.

P2-2: Spectrin Breakdown Triggers Endothelial Dysfunction in Pulmonary Arterial Hypertension

Sonja Sulstarova^{1,2}; Neil Goldenberg^{1,2,3}

¹ Department of Physiology, University of Toronto, Toronto, Canada; ² Peter Gilgan Research Centre, The Hospital for Sick Children, Toronto, Canada; ³ Department of Anesthesiology and Pain Medicine, University of Toronto, Toronto, Canada

Pulmonary hypertension (PH) is a severe disease characterized by elevated pressure in the pulmonary vasculature that exceeds 25 mmHg at rest. A subset of PH, pulmonary arterial hypertension, is characterized by vascular remodelling, perivascular inflammation, and endothelial cell dysfunction that results in altered production of nitric oxide (NO), a potent vasodilator. Recent research has suggested that loss of caveolin-1 (Cav-1), a phosphoprotein that binds and sequesters eNOS (endothelial nitric oxide synthase) in caveolae, invaginations in the plasma membrane that act as signalling platforms, may be responsible for the dysregulated production of NO. Formation of caveolae and localization of cav-1 in the plasma membrane has been shown to be dependent on the presence of spectrin, a cytoskeletal protein that is comprised of tetramers formed by alternating alpha and beta subunits which associate with actin filaments. We propose that spectrin breakdown, known to occur in PAH, triggers the loss of cav-1, driving endothelial cell dysfunction, and ultimately resulting in the onset and progression of PAH. Using a monocrotaline (MCT) rat model, we have shown that PAH is associated with an increased presence of spectrin breakdown products in lung lysates compared to normal controls. Furthermore, in vitro studies of human pulmonary arterial endothelial cells (HPAECs) have demonstrated that spectrin knockdown disrupts both the distribution and expression of caveolin-1 in the plasma membrane. Taken together, these data demonstrate a novel mechanism by which endothelial dysfunction arises in PAH.



Poster Presentation Abstracts

P2-3: 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, Alison²; Heximer, Scott^{2,3}

¹ Department of Physiology, University of Toronto, Toronto, Canada; ² Translational Biology and Engineering Program, Ted Rogers Centre for Heart Research, Toronto, Canada; ³ Heart & Stroke/Richard Lewar Centre of Excellence for Cardiovascular Research, University of Toronto, Toronto, Canada

Right ventricle fibrosis is frequently associated with cardiovascular disease, and leads to impaired electrical conduction and reduced ventricular compliance of the heart. Despite numerous reports on the heterogeneity of cardiac fibroblasts, there have been no direct comparisons between those from the left ventricle (LV) and right ventricle (RV). To compare these populations, fibroblasts were isolated from the LV and RV of uninjured mice using fluorescence activated cell sorting, and used for transcriptomics analysis. Bulk RNA-sequencing (RNA-seq) revealed 442 differentially expressed genes ($p < 0.05$, $n = 4$) with numerous fibrosis-related genes such as IGFBP3, CCL11, COL8A1, CTGF, ASPN, and POSTN being the most significantly different. Overall, the genes are evenly split regarding which ventricle displays greater expression; 230 genes are higher in the LV while 212 are higher in the RV. We next performed single-cell RNA-seq to elucidate the driving factors behind these differences. Analysis of a combined 8092 fibroblasts from the LV and RV identified 7 sub-populations. A single population, marked by expression of POSTN, COL8A1, and CTGF, was significantly larger in the LV than RV ($p < 0.05$), supporting the differences seen in our bulk RNA-seq data. Comparisons with published datasets suggest this is a group of fibroblasts that express greater levels of pro-fibrotic genes and are primed for differentiation into myofibroblasts after injury. These findings demonstrate that LV and RV-derived fibroblasts display baseline differences in both their gene expression and sub-population ratios. Understanding these differences is critical for determining whether distinct therapies are required for the treatment of LV and RV fibrosis.

P2-4: Sarco(endo)plasmic Reticulum Membrane Protein REEP5 Regulates Subcellular Structure and Function in the Heart

Michelle Di Paola^{1,2}; Uros Kuzmanov^{1,2}; Cristine J. Reitz^{1,2}; Allen C.T. Teng^{1,2}; Anthony O. Gramolini^{1,2}

¹ Translational Biology and Engineering Program, Ted Rogers Centre for Heart Research, Toronto, Canada; ² Department of Physiology, University of Toronto, Toronto, Canada

While many key regulators of cardiac function have been identified, considerable details of cardiac development and function remain unclear. Using large-scale proteomics studies, we have identified hundreds of unstudied cardiac myocyte-enriched protein candidates, conserved through evolution, and differentially expressed at the transcriptomic and proteomic levels during myocyte development and in models of heart failure. Our top-ranked candidate of interest is Receptor Expression-Enhancing Protein 5 (REEP5). REEP5 is abundantly expressed in both cardiac and skeletal muscle, and its expression in cardiomyocytes is required to maintain sarco(endo)plasmic reticulum (SR/ER) integrity. Depletion of Reep5 results in decreased muscle cell contraction and disrupted Ca²⁺ signaling. In zebrafish models, genetic knock-out of reep5 results in cardiac functional defects and reduced heart rate. These data show that Reep5 expression is essential for proper formation and maintenance of the SR/ER. To assess the importance of REEP5 as a regulator of ER-stress, apoptosis, and organelle structure, in vivo knock-down of Reep5 is achieved using recombinant adeno-associated virus serotype 9 (rAAV9)-mediated gene delivery into neonatal mice. Following knock-down, cardiac tissues or isolated adult cardiomyocytes are harvested for biochemical and functional assessments. 2 weeks post-rAAV9 injection, a 73% knock-down of REEP5 is observed by immunoblotting (unpaired t-test, $p = 0.0216$, $n = 4$). Using these tools, we will characterize the mechanisms and function by which REEP5 serves to maintain SR/ER integrity within the cardiomyocyte at the subcellular level, and under conditions of cellular stress. This will provide a detailed understanding of the role REEP5 plays in maintaining ER homeostasis and organelle integrity.



Poster Presentation Abstracts

P2-5: Evidence that STAT3 is a Negative Transcriptional Regulator of NPY in Mouse-NPY/AgRP Immortalized Hypothalamic Neurons

Wenyuan He¹; Andy Tran¹; Denise D. Belsham^{1,2,3}

¹ Department of Physiology, University of Toronto, Toronto, Canada; ² Department of Obstetrics and Gynaecology, University of Toronto, Toronto, Canada; ³ Department of Medicine, University of Toronto, Toronto, Canada

Introduction/Aim: Neuropeptide Y (NPY) is co-expressed in a subset of agouti-related peptide (AgRP) neurons in the arcuate nucleus of the hypothalamus (ARC), where it is a potent orexigenic signal. Signal transducer and activator of transcription 3 (STAT3) is implicated as a negative transcriptional regulator of the Npy gene (Npy). Leptin, an activator of STAT3 signalling, downregulates Npy mRNA and protein levels in the ARC. STAT3 knockout in ARC-NPY neurons also elevates Npy mRNA in the whole hypothalamus. However, STAT3 and Npy promoter interactions were only studied for the rat promoter and in models where leptin paradoxically upregulates Npy, and the identified binding sites are not conserved in humans and mice. Thus, our hypothesis was that STAT3 is a direct transcriptional regulator of Npy in mouse ARC-NPY/AgRP hypothalamic neurons. **Methods/Results:** Immortalized mHypoE-46 NPY/AgRP-expressing hypothalamic neurons were used, and RT-qPCR was used to examine mRNA levels. We examined the mRNA of suppressor of cytokine signalling 3 (Socs-3), which STAT3 positively regulates. Treatment of 100 ng/mL mouse recombinant interleukin-6, a STAT3 activator, repressed Npy mRNA between 2 and 24 hours, correlated with upregulated Socs-3 mRNA. In contrast, 24 hours treatment of STAT3 inhibitor stattic (1-10 μ M) and cucurbitacin I (10 μ M) independently upregulated Npy mRNA. Putative STAT binding sites were identified in the 5' untranslated region of Npy, with one region that is largely conserved between rat, mouse, and human. Chromatin immunoprecipitation is underway to determine binding. **Conclusions:** These results demonstrate that STAT3 is a negative transcriptional regulator of murine Npy in ARC-NPY/AgRP neurons.

P2-6: The Role of microRNAs in Bisphenol A-mediated Regulation of Neuropeptide Y and Neuronatin

Kimberly Mak¹; Neruja Loganathan¹; Emma K. McIlwraith¹; Denise D. Belsham^{1,2,3}

¹ Departments of Physiology, University of Toronto, Toronto, Canada; ² Department of Obstetrics and Gynaecology, University of Toronto, Toronto, Canada; ³ Department of Medicine, University of Toronto, Toronto, Canada

The growing prevalence of obesity overlaps with the increasing usage of endocrine-disrupting chemicals such as bisphenol A (BPA). BPA targets hypothalamic feeding-related neurons that express neuropeptide Y (NPY), an appetite-promoting neuropeptide, and neuronatin (NNAT), a weight-related proteolipid. Our lab has identified miR-708-5p as a microRNA (miRNA) that mediates BPA-induced dysregulation of Npy and Nnat; however, miRNAs that directly target the Npy 3'UTR remain unknown. Given the role of miRNAs in gene regulation, we hypothesized that hypothalamic miRNAs altered by BPA can directly target Npy and Nnat, and that beneficial free fatty acids, such as oleate and docosahexaenoic acid (DHA), would ameliorate BPA-induced dysregulation of Npy, Nnat and their corresponding miRNAs. To examine BPA-induced changes in miRNA profiles, an Affymetrix GeneChip miRNA 4.0 array was performed on an NPY/AgRP clonal hypothalamic cell line, mHypoA-59, treated with 100 μ M BPA for 24 hours. mmu-miR-135a-2-3p and mmu-miR-7032-3p were significantly altered by BPA and were predicted to target Npy and/or Nnat using in silico prediction tools, including TargetScan, miRwalk and miRDB. mHypoA-59 cells treated with mimics and inhibitors of these miRNA candidates will be assessed to determine their effects on Npy and Nnat mRNA levels. Preliminary data suggested that oleate and DHA mitigated BPA-induced dysregulation of miR-708-5p. Hence, the effects of oleate and DHA on BPA-mediated changes in Npy and Nnat warrant further investigation. Understanding how BPA influences hypothalamic miRNAs will provide insights into alternative regulatory pathways involved in energy homeostasis and may contribute to the discovery of novel therapeutic targets or biomarkers for obesity.



Poster Presentation Abstracts

P2-7: Efficient Generation of Induced Pluripotent Stem Cell Derived Pancreatic Progenitor Cells from a Non-invasive, Accessible Tissue Source - The Plucked Hair Follicle

Amatullah Fatehi, Ian Rogers

The advent of induced pluripotent stem cell (iPSC) technology has allowed for a new era of regenerative medicine in which the patient's own cells can be used as starting material for disease-modeling and cell replacement therapies. These patient-specific, stem cell-based therapies are advantageous because they decrease the risk of adverse reactions due to immune rejection. However autologous therapies are still far away from being a commercially viable option due to the cost and complexity of scaling out manufacturing for each patient. Also, the effects of cellular aging and senescence can lead to less-than-ideal results in the therapeutic process. The use of plucked hair follicles as starting material for iPSC generation substantially decreases the infrastructure required at the site of collection and promises the availability of highly proliferative keratinocytes. This would allow for the first non-invasive and accessible way of generating patient-specific iPSC cell lines. The goal of the project is to generate patient matched iPSC cell lines from cryopreserved, plucked hair follicles using integration-free vectors. The lines will be used towards pancreatic progenitor cell differentiation and the ability of the primary cell lines to generate validated pluripotent cell lines will be evaluated based on sex differences and donor age.

P2-8: Modular Organization of Quantal Heterogeneity at a Central Synapse

Raphael Chan¹; Maria Gurma¹; Adam Fekete²; Stefan Herlitze³; Melanie D. Mark³; Lu-Yang Wang^{1,2}

¹ Department of Physiology, University of Toronto, Toronto, Canada; ² Program in Neurosciences and Mental Health, Hospital for Sick Children Research Institute, Toronto, Canada; ³ Ruhr-University Bochum, Bochum, Germany

Functional synaptic heterogeneity is widely observed but poorly understood. We use the mature calyx of Held nerve terminal in mice as a model to address this question. The calyx contains main stalks with a varying number of bouton-like swellings which inversely correlate with release probability (Pr) of the whole terminal (Grande and Wang, 2011). Stalks contain large clusters of Ca^{2+} channels tightly coupled to synaptic vesicles (SVs) to support high Pr , whereas swellings contain small clusters of Ca^{2+} channels loosely coupled to SVs to yield low Pr (Fekete et al., 2019). Whether these different morphological modules with distinct Ca^{2+} channel topologies impacts unitary quantal release is not known. To address this issue, we examined miniature excitatory postsynaptic currents (mEPSCs) in morphologically diverse synapses and their Ca^{2+} dependence, complemented by analyses of the distribution of immunolabelled postsynaptic AMPARs relative to fluorescently tagged knock-in Ca^{2+} channels (Mark et al., 2011) in stalks and swellings. Preliminary data indicate that mEPSC amplitude inversely correlates with the number of swellings, where small clusters of Ca^{2+} channels are matched with small clusters of postsynaptic AMPARs. We propose that the same nerve terminal harbours modular constructs for distinct topographical arrangement of presynaptic Ca^{2+} channels and postsynaptic AMPARs between low and high Pr sites, potentially underpinning the heterogeneity of quantal size at a central synapse.

Poster Presentation Abstracts

P2-9: Ryanodine Receptor Inhibitor Dantrolene Reduces Hypoxic-Ischemic Brain Injury in Neonatal Mice

Andrea Ovcjak¹; Steve P. Miller³; Zhong-Ping Feng¹; Hong-Shuo Sun^{1,2}

¹ Department of Physiology, University of Toronto, Toronto, Canada; ² Department of Surgery, University of Toronto, Toronto, Canada; ³ Department of Paediatrics, University of Toronto, Toronto, Canada

Background and Hypothesis: Ryanodine receptors (RyR) located on the membrane of the endoplasmic reticulum, are potent regulators of intracellular calcium levels upon activation. Dysregulated Ca²⁺ homeostasis is characteristic of hypoxic-ischemic (HI) brain injury, triggering deleterious cascades that ultimately lead to neurodegeneration. RyRs have thereby been implicated in the Ca²⁺ imbalance that occurs after HI insult. Accordingly, we hypothesized that RyR antagonist, dantrolene, would reduce neuronal death and confer neuroprotection against neonatal HI brain injury. **Methods:** To evaluate the effect of dantrolene in vitro, cortical neurons were subjected to oxygen-glucose deprivation (OGD) with or without dantrolene (50 μM) treatment. To evaluate the in vivo effects of dantrolene, the Rice-Vannucci HI brain injury model was carried out on postnatal day 7 mouse pups. Assessments of brain infarction volume and short- and long-term neurobehavior were carried out and compared between Sham, HI-dantrolene (i.p., 5 mg/kg) and HI-vehicle (0.05% DMSO) mice. TUNEL staining and Western blot were employed to investigate the molecular mechanisms underlying the effects of dantrolene, and Fura-2 Ca²⁺ imaging was carried out to validate dantrolene's ability to inhibit RyR-mediated Ca²⁺ release. **Results and Conclusion:** Dantrolene treatment reduced infarction volume and improved neurobehavioral deficits up to 3 weeks post HI-insult. Dantrolene reduced OGD- and HI-induced neuronal death, and was shown to downregulate apoptotic signaling (caspase-3, caspase-9, GSK-3β, HIF-1α), while promoting pro-survival signaling (PKC). Our study suggests that RyRs mediate the ionic imbalance induced by HI and represent a potential target for drug development.

P2-10: Investigating the Vesicular Transport Mechanisms of Myelin Proteolipid Protein 1 (PLP1) in Optic Nerve Myelination

Chun Hin Chow¹; Shuzo Sugita¹

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

Optic nerves are responsible for the transduction of electrical information from the retina to the brain. During myelination, oligodendrocytes transport Proteolipid protein 1 (PLP1) to the myelin sheath by vesicular transport via Soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) protein. Nevertheless, the critical SNARE isoforms involved in this process remain unknown. Studies of oligodendrocyte progenitor cell cultures indicate syntaxin-3/4 and SNAP23/29 are the leading candidates. However, in vivo animal models are still lacking. Here, we study the roles of syntaxin-3 and SNAP29 in oligodendrocytes by creating oligodendrocyte-conditional knockout of these SNAREs in adult mice 4- to 6-week-old. The rotarod test that characterizes overall central nervous system myelination. Optic nerve myelination is characterized behaviorally via optokinetic test and structurally by optical coherence tomography. We confirm that Cre recombinase successfully translocates to the nucleus upon tamoxifen injection in the white matter of the spinal cord. SNAP29 in situ hybridization suggests SNAP29 expression in myelin structures, similar to syntaxin-3 previously reported. However, preliminary data shows that conditional knockout of syntaxin-3 has no difference in the behavioral tests, optic nerve head size, and thickness of retinal ganglion cell complex compared to control mice (n=3 for each group). Nevertheless, current results come from mice 2- to 5-weeks after tamoxifen injection. As myelin in adult is relatively stable, a longer timescale will be necessary to track changes in myelination. Overall, we attempt to elucidate the essential SNAREs in oligodendrocytes protein trafficking in the myelinating process of the optic nerves.



Poster Presentation Abstracts

P2-11: Social Isolation's Effect on Learning and Memory

Naomi N. Niederhoffer^{1,2,3}; Paul W. Frankland^{1,2,3,4,5,6}; Sheena A. Josselyn^{1,2,3,4,5,6}

¹ Program in Neurosciences & Mental Health, Hospital for Sick Children, Toronto, Canada; ² Department of Physiology, University of Toronto, Toronto, Canada; ³ Collaborative Program in Neuroscience, University of Toronto, Toronto, Canada; ⁴ Institute of Medical Sciences, University of Toronto, Toronto, Canada; ⁵ Department of Psychology, University of Toronto, Toronto, Canada; ⁶ Brain, Mind & Consciousness Program, Canadian Institute for Advanced Research, Toronto, Canada

The COVID-19 pandemic has dramatically changed people's social lives, with many experiencing social isolation (SI). SI is linked with many detrimental health consequences, but little is understood about the effect of SI on learning and memory. Acute stress can impact the process of allocation to an engram, the specific neurons that hold the memory for a particular event, thereby disrupting the memory. Both female and male mice that have been socially isolated for 1- or 3-weeks, but not for 24-hours, before training show significant memory impairments. SI's effect is on memory encoding, as isolation after training does not lead to impairment. These SI-induced memory deficits are seen in both auditory fear conditioning and novel object recognition. These deficits cannot be explained by differences in anxiety or depression. To visualize the changes in the lateral amygdala engram that may be underlying this fear memory impairment, we are using a viral vector strategy that allows the activated neurons to be tagged and visualized. Additionally, we are using an iDisco approach to look at changes at learning due to SI throughout the brain. Lastly, we plan to use both natural and artificial interventions to overcome these memory deficits, by re-activating the engram neurons underlying this memory and by resocialization of the isolated mice to attempt to restore group-housed levels of freezing. This project has important relevance to understanding the mechanism of the detrimental consequence of SI on human health and memory.

P2-12: SNARE Proteins in Retinal Synaptic Vesicle Release

Maggie Huang^{1,2}; Shuzo Sugita^{1,2}

¹ Department of Physiology, University of Toronto, Toronto, Canada; ² Division of Fundamental Neurobiology, Krembil Research Institute, University Health Network, Toronto, Canada

The leading cause of blindness worldwide is retinal degeneration and synaptic changes commonly precede photoreceptor degeneration. Therefore, the molecular events that determine how photoreceptors communicate is important for vision. Photoreceptors communicate with each other by releasing the neurotransmitter glutamate. Glutamate is released through vesicle fusion, requiring the assembly of a fusion complex called the SNARE complex. The SNARE complex traditionally consists of the proteins syntaxin-1, SNAP-25, and synaptobrevin-2. However, this complex is less well studied in the visual system. Nonconventional photoreceptor synapses have SNARE complexes containing syntaxin-3 and possibly SNAP-23 to mediate neurotransmitter release. Elucidating the role of these SNARE proteins and SNARE complex mediators will be important in understanding photoreceptor synapses, and to combat synaptic change mediated retinal degeneration. We hypothesize non-traditional SNARE proteins such as syntaxin-3 and SNAP-23 along with traditional mediators such as Munc18-1 play a role in photoreceptor neurotransmitter release. To study the role of these proteins in photoreceptor synapses, we developed an *in vivo* model to selectively remove these proteins from photoreceptor cells in mice. We find SNAP-23 is an unlikely candidate for mediating glutamate release, as SNAP-23 removal leads to no changes in both morphology and function. However, removal of syntaxin-3 or Munc18-1 dramatically alters both large-scale retinal morphology and synaptic integrity leading to decreased responses to light and visual acuity. These results suggest syntaxin-3 and Munc18-1 play an important role in photoreceptor glutamate release.



Poster Presentation Abstracts

P2-13: Characterization of Patient Subtypes in Pediatric Mild Traumatic Brain Injury

Prashanth S Velayudhan^{1,2}; Anne L Wheeler^{1,2}

¹ Program in Neurosciences and Mental Health, The Hospital for Sick Children, Toronto, Canada; ² University of Toronto, Department of Physiology, Toronto, Canada

Background: Mild traumatic brain injuries (mTBIs) are prevalent in the pediatric population. The heterogeneity of patients, injuries, and symptoms has been a major barrier to improving the clinical management of mTBI. Limitations in datasets and clustering algorithms have prevented previous studies from characterizing this heterogeneity across the full scope of variables known to be relevant to mTBI. In this study, I will use similarity network fusion (SNF), a novel data-integration technique that facilitates patient clustering across high-dimensional and diverse datasets, to characterize subtypes of pediatric mTBI patients in a large and deeply-phenotyped longitudinal cohort dataset. Objectives: 1) Identify subtypes of mTBI patients, 2) Examine how those subtypes change over time. Methods: I will use SNF to find subtypes of the 432 9-10-year-old children in the Adolescent Brain Cognitive Development dataset that have reported a previous mTBI. Inputs will include acute symptoms, demographics, medical history, brain imaging measures, and psychosocial factors. Variables driving subtype generation will be identifying using normalized mutual information scores. I will also compare the subtypes of mTBI patients at baseline and 2-year follow-up timepoints. Hypotheses and Expected Outcomes: 1) If mTBI heterogeneity depends on several diverse datatypes, SNF will reveal novel subtypes of mTBI patients, 2) If the subtypes are driven by persistent factors non-specific to injury, they will be similar between baseline and follow-up timepoints. Significance: The results of this work can inform data-driven patient stratification for clinical trials, reveal subtype-specific targets for future intervention studies, and improve the accuracy of mTBI prognostic models.

P2-14: The Involvement of PD-L2 in Retinal Axon Guidance

Xiaoyan (Shirley) Chen

Axon guidance is an important process during embryonic development and is essential for synaptic activities in adulthood. Comprehending the mechanisms that regulate axon guidance and the development of neural circuit formation may help to find a cure for neural disorders and regenerate the nervous system. A previous study demonstrated that the Repulsive guidance molecule b (RGMb) is a guidance molecule which inhibits axon outgrowth in retinal ganglion cells (RGC) through the negative regulation in the Wnt pathway via the degradation of Wnt receptor Low-density lipoprotein receptor-related protein 5 (LRP5). However, we could not confirm the direct interaction of RGMb and LRP5. Suggestive evidence shows the expression of Programmed cell death-1 ligand 2 (PD-L2) in the developing central nervous system (CNS) such as the retina and it is responsible for maintaining proper RGC numbers as a programmed cell death ligand. Yet, no study shows the role of PD-L2 in axon guidance. PD-L2 is also known to be the ligand of RGMb. In this study, we investigated the new function of PD-L2 in developing visual system and our data showed PD-L2 overexpression increased axon length in RGC primary culture. The axon length was suppressed by PD-L2 overexpression in RGMb/Wnt knockdown condition. These results imply a new function of PD-L2 and the potential interaction of PD-L2 with RGMb and Wnt signaling in the developing CNS.



Poster Presentation Abstracts

P2-15: Decidual Natural Killer Cells: Uniquely Tolerant Cells That Support Placentation

Morgan Zych¹; Brian Cox¹

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

Pregnancy presents the maternal immune system with the seemingly conflicting tasks of maintaining tolerance to a hemi-allogeneic fetus and maintaining requisite inflammation for tissue remodeling and defense against pathogens. During the first trimester the predominant immune cell type at the maternal-fetal interface is decidual natural killer cells (dNKs). Recent transcriptomic analyses have comprehensively phenotypically characterized these cells in isolation, but gaps remain in understanding their mechanisms of interaction. We developed co-culture systems consisting of completely allogeneic trophoblast organoids and primary dNKs encapsulated together in Matrigel domes to attempt to model dNKs' interactions with other cell types. dNKs and trophoblast organoids could be maintained together in co-culture for over one month, with organoid growth necessitating passaging in each of four co-culture setups. Co-culture with dNKs was associated with increased organoid size after 7 days, suggesting that dNKs may have growth-promoting secretory programs. Significant increases in IL-6, IL-8, and G-CSF contents in culture media were also associated with co-culturing of trophoblast organoids and dNKs. Further interrogation of these co-culture systems is expected to lend insights into the mechanisms of the maintenance of normal pregnancy and suggest immune-based therapies for conditions including recurrent spontaneous abortion and preeclampsia.

P2-16: Using Acellular Pancreatic Scaffolds to Direct Differentiation of Pancreatic Progenitors to Functional Pancreatic Tissue

Marwa Sadat^{1,2}; Amanda Fantin^{1,2}; Jorge Castillo-Prado^{1,2}; Ian Rogers^{1,2,3}

¹ Lunenfeld Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Canada; ² Department of Physiology, University of Toronto, Toronto, Canada; ³ Department of Obstetrics and Gynaecology, University of Toronto, Toronto, Canada

An enticing therapeutic avenue for treatment of pancreatic disorders is the development of a bioengineered pancreas through the combination of decellularized donor organs and stem cells to generate tissue for replacement of the diseased organ. Current in vitro culture systems are unable to promote sufficient differentiation of stem cells to mature tissue, thus we predict that incorporating the extracellular matrix (ECM) by using an acellular pancreatic scaffold will allow for organ-specific differentiation without the addition of growth factors. Ultimately, we hope to repopulate these acellular murine pancreatic scaffolds with progenitor cells derived from human induced pluripotent stem cells (iPSCs), to test whether the murine ECM is capable of supporting maturation of these human cells. We have shown that acellular murine pancreatic lobe recellularized with definitive endoderm cells differentiated from human embryonic stem cells, begin to express markers of mature pancreatic tissue after 14 days, including PDX1, NKX-6.1, and CK19, demonstrating that the ECM does direct differentiation. Currently, we plan to recellularize whole acellular murine pancreatic lobes with differentiated human iPSCs to determine at what stage the ECM is sufficient to promote differentiation into mature functional pancreatic tissue. Using our ex vivo perfusion system, we will maintain these recellularized organs for several weeks to monitor their maturation and test their function through a glucose-stimulated insulin secretion test (GSIS). These studies will not only aid in our understanding of the maturation potential of the ECM but also in furthering efforts for generation of patient-specific organs for transplantation and disease modelling.



Poster Presentation Abstracts

P2-17: Impaired Placental Antioxidant Function and Iron Homeostasis Promotes Trophoblast Cell Death via Ferroptosis in Pregnancies Complicated by Fetal Growth Restriction

Ruizhe (Nicole) Liu^{1,2}; Chanho Park¹; Sruthi Alahari¹; Isabella Caniggia^{1,2,3}

¹ Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, Canada; ² Department of Physiology, University of Toronto, Toronto, Canada; ³ Department of Obstetrics & Gynaecology, University of Toronto, Toronto, Canada

Objective: Ferroptosis, a newly identified form of programmed cell death characterized by iron-dependent oxidative stress and attenuated glutathione peroxidase 4 (GPx4) enzymatic activity, is a primary contributor to the accumulation of lipid peroxidation products that impairs cell homeostasis leading to cell death. Fetal growth restriction (FGR) is a pregnancy-related complication characterized by placental hypoxia that, in part, contributes to heightened trophoblast cell death. To date, the role of ferroptosis in FGR remains elusive. The objective of the present study is to characterize ferroptosis in FGR. **Methods:** Placentae from FGR (n=15) and age-matched controls (AMC, n=15) were obtained following informed consent from Mount Sinai's RCWIH. Transmission electron microscopy was conducted to visualize ferroptosis morphologies in trophoblasts. Western blotting was employed to determine expression of iron storage protein ferritin (FTN), iron transport protein transferrin receptor-1 (Tfr-1), and GPx4. Ferrous iron content was measured in mitochondrial isolates from AMC and FGR placentae. Colorimetric assays were conducted for antioxidant activity and lipid peroxidation in AMC and FGR. **Results:** Hallmarks of ferroptotic cell death, including diminished mitochondrial cristae and nuclear decondensation were identified in FGR syncytiotrophoblasts (STBs) and cytotrophoblasts (CTBs). FTN and Tfr-1 expressions were decreased in FGR placentae compared to AMC (p<0.05). Increased iron content was found in FGR mitochondria (p<0.05). FGR placentae have decreased GPx4 protein levels and activity and increased lipid peroxidation (p<0.05). **Conclusion:** In FGR, disrupted iron homeostasis and attenuated antioxidant function increase ferroptotic susceptibility in trophoblast cells, thereby contributing to the heightened cell death rates typical of this pathology.

P2-18: Structural Studies of Proteins, NSD1, and FEM1C, Involved in Post Translational Modifications

Ksenia Providokhina¹; Jinrong Min¹

¹ Department of Physiology, Mars Centre, University of Toronto, Toronto, Canada

Post translational modifications (PTMs) are biochemical reactions that modify proteins by controlling their conformation, activity, and stability. Histone methylation is a vital PTM that is required for genome programming during development. NSD1 is a SET domain methyltransferase, a protein that adds one to two methyl groups to a lysine residue. Mutation in NSD1 can cause Sotos Syndrome, a neurological disease caused by trimethylation of H3K36. Ubiquitination, another key PTM, is a protein degradation signal through which the ubiquitin proteasome system (UPS) breaks down unwanted proteins. FEM1C, a CRL2 substrate receptor of UPS, specifically recognizes proteins with an arginine at the C-terminus. Dysfunction in C-degron-mediated ubiquitination leads to diseases like Alzheimer's or ALS. To understand the functions of these proteins better, we aim to determine the protein structures of a NSD1 mutant (Y1869C) and of FEM1C in complex with an inhibitor. Both proteins were obtained through recombinant DNA cloned into E. coli expression vectors, purified through affinity and size exclusion chromatography, and co-crystallized with their corresponding binding partners. Crystal diffraction data will be collected by a synchrotron light source and processed by HKL3000. Structures will be solved by molecular replacement using available structures as starting models. In this study, once NSD1 protein crystals are achieved as FEM1C were, their molecular structures will be compared to the previously solved structures to understand: 1, How a critical residue mutation in NSD1 affects its enzymatic activity; 2, How the inhibitor disrupts the ability of FEM1C in recognizing its substrate.



Poster Presentation Abstracts

P2-19: 3D Culturing of Renal Progenitor Cells on ECM Scaffolds Results in Maturation of Cells Toward Kidney Lineages

Tonya Bongolan^{1,2}; Theresa Chow²; Jennifer Whiteley²; Laura Mazilescu³; Matyas Hamar³; Maria Ryaboshapkina⁴; Anna Jonebring⁴; Ryan Hicks⁴; Markus Selzner⁴; Ian M. Rogers^{1,2}

¹ Department of Physiology, University of Toronto, Toronto, Canada; ² Women's and Infant's Health, Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Canada; ³ Toronto General Hospital Research Institute, Toronto, Canada; ⁴ AstraZeneca, Sweden

The extracellular matrix (ECM) provides a structural scaffold upon which cells organize to perform specific functions. It also acts as a reservoir for growth factors to mediate paracrine signaling which results in tissue homeostasis, development, and repair. Incorporating ECM components in 3D culture systems has proven to better model solid organs in vitro than 2D monolayer cultures. Because of this, the Rogers Lab is investigating the use of ECM scaffolds derived from kidney decellularization for use in culturing renal progenitor cells. Following the 2D differentiation of human induced pluripotent stem cells into renal progenitors, cells are pipetted onto slices of porcine kidney ECM scaffolds and cultured at air liquid interface to induce epithelialization. We found that cells continue to differentiate toward mature cell types found in the kidney, including cytokeratin+/LTL+ proximal tubule cells and CD31+ endothelial cells. To further enhance the ECM niche prior to recellularization, we soaked ECM scaffolds with growth factors derived from porcine kidneys. We found that ECM scaffolds re-bind growth factors isolated from the native tissue. This resulted in changes in gene expression of recellularized cells, with cells grown on ECM containing reconstituted growth factors exhibiting a more mature phenotype than cells grown on ECM without reconstituted growth factors. Future experiments will determine the roles of specific growth factors during recellularization culture. We hope to apply the knowledge gained from this project to regenerative medicine applications, including disease modeling and drug screening.



Organizers and Contributors

Planning Committee Co-Chairs

Kelvin Lee

Eman Nishat

Joseph Lee

Planning Committee

Ariana Tang
Maria Medeleanu
Luxcia Kugathanan
Yuchen Qian

Raina Ladha
Maria Gurma
Xavier Lee
Tina Nguyen
Mariam Kiwan

Frank Mazza
Harrison Levine
Gabrielle Jacobson
Octavia We

Graduate Association for Students in Physiology

Delphine Ji
Aya Ragheai
Ishnoor Singh
Alicia Gibbs
Mark Rzepka

Kimberly Mak
Christopher Casciaro
Li Ju
Yasaman Mostafaie
Radu Gugustea
Raina Ladha

Kelvin Lee
Eman Nishat
Joseph Lee
Sajad Sadat
Riley Pontello

Department of Physiology

Dr. Scott Heximer, Chair
Dr. Andrea Jurisicova
Dr. Denise Belsham

Dr. Helen Miliotis Lazongas
Jenny Katsoulakos
Paula Smellie

Rosalie Pang
Julia Tausch
Justin Kim

Graphics & Web

Robert L. Kolaja

Keerththana Ramakrishnan

Joseph Lee

Special thank you to all abstract and presentation judges.



Special Thanks

Frontiers in Physiology 2022 Sponsors



Scientists Helping Scientists™ | www.s

