manitoba neuroscience network

12th ANNUAL RESEARCH SYMPOSIUM

June 6th, 2025 8AM-8PM

Venue:

Winnipeg Art Gallery Qaumajuq

300 Memorial Boulevard
Winnipeg, Manitoba
Canada R3C 1V1

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We respect the Treaties that were made on these territories, we acknowledge the harms and mistakes of the past, and we dedicate ourselves to move forward in partnership with Indigenous communities in a spirit of Reconciliation and collaboration.

Program At a Glance:

Meeting Chairs:

Dr. Eftekhar Eftekharpour
Professor,
Dept of Physiology and Pathophysiology
University of Manitoba

Dr. Renée Nicole Douville
Associate Professor,
Dept. of Pharmacology and Therapeutics
University of Manitoba

8:00–9:00 Registration Desk Open
– Main Hall, WAG

8:45–8:55 Opening Remarks
– Muriel Richardson Auditorium (MRA)
Welcome: Dr. Eftekhar Eftekharpour
Opening remarks: Dr. Tabrez Siddiqui, President and Executive Director, Manitoba Neuroscience Network

PERSPECTIVES FROM LIVED EXPERIENCE

8:55–9:10 A Fight for Brandon: Living with Batten Disease Hayley Smith
MSc student, Department of Pharmacology and Therapeutics, University of Manitoba

SESSION 1: A FOCUS ON ALS (SPONSORED BY ALS CANADA)

9:10–10:10 Proteopathy in ALS and Dementia: from basic science to novel therapeutical approaches
Keynote Dr. Jean-Pierre Julien
Professor, Cervo Brain Research Centre, Department of Psychiatry & Neuroscience, University of Laval

10:10–10:30 Targeting Oxidative Stress in Neurodegeneration
Dr. Geoffrey Tranmer
Professor, College of Pharmacy, University of Manitoba

10:30–10:45 Refreshment & exercise break– MRA lobby and Main Hall
*** Check out the Nerdoscience activity stations!***

SESSION 2: FEATURED FACULTY TALKS

10:45–11:05 Using human genetics and genomics to understand polyglutamine disorder biology and inform future therapeutic approaches
Dr. Galen Wright
Assistant Professor, Department of Pharmacology and Therapeutics, University of Manitoba

11:05–11:25 Sex Matters:
Predictive Mapping of Autophagy and Mitophagy Alterations in Alzheimer's Disease via Behavioral-Molecular Integration

Dr. Saeid Ghavami
Professor, Department of Human Anatomy and Cell Science, University of Manitoba

11:25–11:45 Mapping Cellular Reactions to Laser Interstitial Thermal Therapy (LITT) in Mouse Brain Tumor

Dr. Thomas Klonisch
Professor, Department of Human Anatomy and Cell Science, University of Manitoba

11:45–12:05 MIRAGE: Mental Imagery and the Regulation and Generation of Emotions
Dr. Steven Greening
Associate Professor, Department of Psychology, University of Manitoba

12:05–1:00 Buffet lunch – Main Hall

SESSION 3: FEATURED TRAINEEs/ASSOCIATES TALKS (SPONSORED BY STEM CELL NETWORK)

1:00–1:05 Welcome remarks – Stem Cell Network Video

1:05–1:15 Repeated Injury Impacts Immune and Neural Stem Cell-Mediated Repair in the Zebrafish Forebrain. Sanjana Grover

1:15–1:25 D-serine facilitates aggressive migration and stemness of recurrent glioblastoma cells by interacting with host endothelial cells. Ryan Mota

1:25–1:35 Transcriptomic and Protein Expression Analysis of Early Differentiating Neurons in the Developing Mouse Cerebellar Nuclei Farshid Ghiyamihoor

1:35–1:45 Ablation of Neuregulin-1 elicits Multiple Sclerosis-like pathology including progressive brain demyelination, inflammation and cognitive impairment in adult mice Elisabet Jakova

1:45–1:55 Cholesterol Metabolism as a Driver of Chemoresistance in Glioblastoma
Shahla Shojaei

1:55–2:05 Correlates of Accelerated Brain Aging in Prodromal Dementia: A Longitudinal Metabolic Imaging Study Jarrad Perron

2:05–2:15 A novel ultrasensitive quaking-induced conversion assay for all forms of sporadic and inherited prion diseases Jennifer Myskiw

2:15–2:25 Regulatory role of Thioredoxin-1 in health and neurodegeneration
Md Imamul Islam

2:30–4:30 Poster session, refreshments &exercise break – Main Hall

4:30–5:00	Awards Ceremony – MRA Send-off: Dr. Eftekhar Eftekharpour
	NETWORKING MIXER
5:00–6:30	Neuroscience Art Silent Auction-MRA lobby Cash bar and Networking- Ilavut Entrance Hall *** Nerdoscience activity station prizes

Acknowledgement: The MNN would like to thank the St. Boniface Hospital Research Division of Neurodegenerative and Neurodevelopmental Disorders for in-kind contributions of administrative assistance by Kelly Jorundson, Protiti Khan, and Eftekharpour and Douville Lab voluntee

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Keynote Lecture: Sponsored by ALS Canada

Proteinopathy in ALS and dementia: From basic science to novel therapeutic approaches.

Jean-Pierre Julien, Ph.D.,
CERVO Brain Research Centre,
Department of Psychiatry and Neuroscience,
Laval University

Biography:

Jean-Pierre Julien is Professor in the Department of Psychiatry and Neuroscience at Université Laval. He obtained his Ph.D. (Biochemistry) from McGill University where he discovered with his mentor Walter Mushynski the phosphorylation of neurofilaments. This was followed by postdoctoral studies in molecular genetics at the National Institute for Medical Research in London UK. This is where he cloned for the first time the mouse and human neurofilament genes. In 1986, he became Assistant Professor at Université de Montréal - Institut du Cancer de Montréal (now CRCHUM). Three years later, he was recruited at McGill University in the Department of Neurology and Neurosurgery (1989-2003). In 2003, he moved to Université Laval (Research Centre of CHUQ) where he was offered a Canada Research Chair in neurodegeneration. In 2013, his laboratory moved to CERVO Brain Research Centre where he acted as director of Axis Integrative Neuroscience.

Professor Julien has been a pioneer in the creation of genetically modified mice to study the regulation and functions of neurofilaments. He was the first to discover that neurofilament disorganization can cause neurological diseases and he has never stopped to work on the topic of neurofilament function. Two years ago, he reported the creation of a unique mouse model of a rare disease called giant axonal neuropathy suggesting a key role of neurofilament disorganization in neurodegenerative changes. He also made seminal contributions in the field of ALS. His studies with chimeric mouse models led to the unexpected discovery, published in Science (2003), that non-neuronal cells contribute to motor neuron loss in ALS. This breakthrough prompted several laboratories to focus on the role of glial and immune cells in ALS, and to test stem cell therapies for ALS. Subsequently, he proposed a new model of pathogenesis for ALS based on the secretion of misfolded proteins. This led him to develop immunization approaches to target toxic proteins in ALS. Thus, he was the first to experiment with immunization for the treatment of ALS caused by

SOD1 mutations and more recently for targeting TDP-43 proteinopathy. In the past decade, Dr. Julien has been working on the development of new treatments for ALS, such as immunotherapies and natural product-based therapies to target inflammatory pathway signaling. He has published several articles demonstrating the therapeutic effects of the medicinal plant *Withania somnifera* and derived compounds in mouse models of ALS and dementia. Finally, his recent studies provided evidence that TDP-43 constitutes a therapeutic target in chronic cerebral hypoperfusion modeling vascular dementia and in pathogenesis induced by the toxicity of cerebrospinal fluid from sporadic ALS patients.

His career productivity is exceptional with 245 articles that are highly cited (31,202). He has supervised more than 50 students and postdoctoral fellows, many of whom have pursued an academic career in Canada or abroad. He has also contributed to the advancement of research by organizing the annual international ALS Symposium of Fondation Andre-Delambre for 15 years (2005-2019), which was held alternately at Université Laval and McGill University. Dr. Julien has received several awards including the Sheila Essey Award for ALS research from the American Academy of Neurology, the Léo-Pariseau Award from ACFAS and the Jonas Salk Award. He is a Fellow of the Royal Society of Canada and of the Canadian Academy of Health Sciences.

Proteinopathy in ALS and dementia: From basic science to novel therapeutic approaches.

Abstract:
Amyotrophic lateral sclerosis (ALS) is a fatal neurologic disease characterized by progressive degeneration of motor neurons that leads to paralysis and death within 3-5 years following onset. Only few drugs are available for ALS and they confer only modest slowing of disease. Over 40 genes have been identified so far as risk factors in ALS. Despite the diversity of causative genes, in both familial and sporadic ALS cases a pathological hallmark of degenerating neurons is the presence of cytoplasmic aggregates of a protein called TDP-43, a nuclear protein involved in RNA splicing. Such cytoplasmic TDP-43 aggregates are also found in the brain of patients suffering of frontotemporal dementia and vascular dementia. In disease, the loss of nuclear TDP-43 can cause aberrant gene expression via defective RNA splicing and chromatin decondensation whereas cytoplasmic TDP-43 accumulations can cause gain of toxic functions affecting proteostasis, mitochondria and translational suppression of neurofilament mRNAs. Aiming to mitigate TDP-43 pathology, we have generated an antibody called E6 against the RRM1 domain of TDP-43. This antibody is unique in that it recognizes cytoplasmic TDP-43, but not nuclear TDP-43.

From this monoclonal antibody, we have derived a single chain antibody (scFv) which can be delivered to neurons via AAV-mediated transduction. To achieve pan-neuronal expression in the CNS of scFv-E6, we recently generated an AAV vector bearing a recombinant capsid designed to achieve efficient transduction in large neuronal populations after intravenous injection. The effects of AAV-mediated delivery of scFv-E6 were investigated in three mouse models with TDP-43 pathology: (1) transgenic mice expressing mutant TDP-43^{G348C}, (2) mice subjected to chronic hypoperfusion (CCH) by unilateral occlusion of common carotid artery (UCCAO) modelling vascular dementia and (3) a mouse model of sporadic ALS based on infusion intracerebroventricularly (i.c.v.) of cerebrospinal fluid from ALS patients. In all three mouse models of ALS/dementia, the intravenous injection of AAV vector encoding scFv E6 prevented cognitive and motor defects, and it mitigated TDP-43 pathology. Furthermore, our results suggest that TDP-43 may be a key therapeutic target in vascular dementia and in pathogenic pathways triggered by the CSF from sporadic ALS patients. An alternative to viral-mediated delivery scFv antibody is possible with the injection intrathecally or i.c.v. of full length E6 antibody. Thus, the CSF-inoculated E6 antibody reduced motor and cognitive impairments, mitigated TDP-43 proteinopathy and prevented neurofilament disorganization in cortical and spinal neurons of mice infused i.c.v. with ALS-CSF. In conclusion, immunotherapy approaches targeting TDP-43 should be considered as potential future treatment for forms of ALS and dementias with TDP-43 proteinopathy.

Faculty Presentation

Targeting Oxidative Stress in Neurodegeneration

Geoffrey K. Tranmer

Professor, College of Pharmacy, Rady Faculty of Health Sciences
University of Manitoba

Amyotrophic lateral sclerosis (ALS) is a progressive motor neurodegenerative disease of the brain and spinal cord, resulting in severe weakness and death due to respiratory failure, often within 2-5 years of first diagnosis. One of the commonly known causes of ALS is a mutation in the SOD1 gene, which acquires a toxic gain of function due to a change in redox disturbances caused by reactive oxygen species, while abnormal misfolding aggregates of SOD1 play a role in the pathogenesis of ALS. Since, the discovery of ALS in 1869, only two FDA-approved drugs, Edaravone, an antioxidant, and Riluzole, an antiglutamatergic, have been widely available and clinically used, although with minimal effects on disease course. Further, preclinical studies of both these drugs in SOD1 ALS mice models have not shown any significant survival benefits. Therefore, our overall goal is to develop novel boron-based pyrazole (B-Pyr) drug candidates (Edaravone analogues) that could slow down the progression of neurodegeneration (in ALS mouse models) by targeting oxidative stress. To date, a longitudinal preclinical study with a novel B-Pyr drug candidate has demonstrated a satisfactory safety profile and statistically significant preclinical, proof-of-concept efficacy in a humanized SOD1 mice model of ALS. Overall, we will discuss results that validate the therapeutic potential of our B-Pyr scaffold for increasing survival, slowing down the progression of incurable ALS and preventing ALS-induced weight loss via chemoselective targeting of oxidative stress.

Faculty Presentation

Using human genetics and genomics to understand polyglutamine disorder biology and inform future therapeutic approaches

Kevin Lucy Namuli,[1,2] Britt I. Drögemöller,[3] **Galen E.B. Wright,[1,2,3]**

1Department of Pharmacology and Therapeutics, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada. 2PrairieNeuro Research Centre, Kleysen Institute for Advanced Medicine, Health Sciences Centre and Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada; 3Department of Biochemistry and Medical Genetics, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada.

Background: Polyglutamine (polyQ) disorders, such as Huntington disease (HD) and several spinocerebellar ataxias, are severe neurological disorders caused by repeat expansions of the glutamine codon. These conditions lack effective treatments, with therapeutic research focused on pathogenic gene knockdown.

Objectives: We aimed to profile these genes using diverse human genomic data to guide therapeutic strategies by identifying new biology and assessing on-target effects of knocking these genes down.

Methods: We conducted an unbiased phenome-wide study to identify human traits and diseases linked to polyQ disorder genes (Open Targets L2G>0.5). Network analyses explored shared trait associations and overlapping biological processes among these genes. Lastly, we assessed the theoretical druggability of polyQ disorder genes using recently identified features predictive of clinical trial success and compared them to repeat expansion (HD) modifier genes.

Results: We identified 215 human phenotype/polyQ disorder gene associations from 3,095 studies, indicating potential adverse effects from gene knockdown. Shared trait associations among polyQ disorder genes suggested overlapping biological processes despite distinct functions. Drug target profile analysis revealed unfavorable risk profiles for polyQ disorder genes, particularly ATN1, ATXN1, ATXN7, and HTT, due to genomic

features such as constraint, molecular interactions, and tissue specificity. PolyQ disorder genes also showed significantly more safety-related risks than HD genetic modifier genes (P=7.03x10-3).

Conclusion: Our analyses emphasize the pleiotropic nature of polyQ disorder genes, highlighting their potential risks as drug targets due to safety concerns. These findings reinforce the importance of exploring alternate therapeutic strategies, such as targeting genetic modifier genes, to mitigate these challenges.

Faculty Presentation

Sex Matters: Predictive Mapping of Autophagy and Mitophagy Alterations in Alzheimer's Disease via Behavioral-Molecular Integration

Aida Adlimoghaddam[1,2,3], Iman Beheshti[4], Benedict C Albensi[5,6*], **Saeid Ghavami[4,7*]**

[1]Department of Neurology, Dale and Deborah Smith Center for Alzheimer's Research and Treatment, Neuroscience Institute, [2]Department of Pharmacology, [3]Department of Medical Microbiology, Immunology and Cell Biology, Southern Illinois University School of Medicine, Springfield, IL, USA. [4]Department of Human Anatomy and Cell Science, University of Manitoba, Winnipeg, MB, Canada. [5]Barry and Judy Silverman College of Pharmacy, Nova Southeastern University, Ft. Lauderdale, FL, USA. [6]Department of Pharmacology and Therapeutics, University of Manitoba, Winnipeg, Canada. [7]Paul Albrechtsen Research Institute, CancerCare Manitoba, Winnipeg, MB, Canada.

1. Motivation/problem statement: Macroautophagy and mitophagy are essential regulators of remain unclear. The gap in understanding how these processes link to cognitive decline across sexes limits the development of personalized interventions. Furthermore, predictive tools to connect behavioral outcomes with cellular pathology are lacking in current neurodegenerative models.

2. Methods/approach: We examined autophagy (LC3B-II, SQSTM1) and mitophagy markers (BNIP3L, BNIP3, BCL2L13) in the cortex and hippocampus of male and female 3xTg-AD mice via Western blotting. Transmission electron microscopy (TEM) quantified mitochondrial and mitophagosome abundance. Cognitive performance was evaluated through novel object recognition and placement tests. To bridge behavioral and molecular data, we applied supervised machine learning algorithms (regression models) to predict mitophagy and mitochondrial phenotypes based on behavioral metrics. All analyses used sex-balanced cohorts (n=6–8 per group), included proper controls, and were validated in replicated experiments.

3. Results: Females displayed autophagosome accumulation in the cortex, alongside elevated BNIP3L and BCL2L13, correlating with cognitive decline and mitochondrial disruption. Males exhibited increased BNIP3 dimers and a positive association between LC3B-II and cognitive function. TEM confirmed sex-specific mitophagy profiles. Critically, machine learning accurately predicted mitochondrial and mitophagosome abundance from behavioral performance, validating a robust link between functional and cellular endpoints.

4. Conclusion/implications: This study reveals sex-specific autophagy and mitophagy signatures driving divergent cognitive outcomes in AD. Importantly, integrating machine learning establishes a novel predictive framework, advancing personalized neurodegeneration research and opening new avenues for biomarker-based therapeutic strategies.

Keywords: Alzheimer's disease, autophagy, mitophagy, sex differences, machine learning

Faculty Presentation

Mapping Cellular Reactions to Laser Interstitial Thermal Therapy (LITT) in Mouse Brain Tumor

Thomas Klonisch[1-4], Jeremy Spence[1], Santhosh S. Anandhan[1], Nimrat Kaur[1], Farhana Begum[1], Dana Henderson[1], Jennifer Chan[5,6], Sabine Hombach-Klonisch[1,2]

1Department of Human Anatomy and Cell Science, 2Pathology, 3Medical Microbiology & Infectious Diseases, University of Manitoba; 4CancerCare Manitoba, Winnipeg, MB, Canada; 5Cumming School of Medicine, Department of Pathology and Laboratory Medicine, 6Arnie Charbonneau Cancer Institute, University of Calgary, AB, Canada.

Introduction: Glioblastoma accounts for 48%-60% of adult high-grade primary brain tumors and has a 5-year net survival of only 4.8%. LITT is a minimally-invasive hyperthermic ablation method indicated for inoperable brain tumors. We have established a preclinical murine model of LITT in glioblastoma to study the cellular effects and explore novel drug treatments in the future.

Methods: Syngeneic glioma cell-line CT2A is orthotopically implanted into C57BL/6 mice using a stereotactic frame. Tumor burden is monitored using T2-weighted MRI, with post-operative scans for treatment confirmation. LITT using a custom 1064nm fibreoptic laser or a surgical sham with conventional tissue damage are performed. Brains and blood plasma samples were collected at relevant timepoints for further analysis.

Results: LITT conditions were optimized to ensure well-tolerated and effective ablation of tumor tissue. Multiplexed immunofluorescence highlighted spatiotemporal changes to resident and immune cell populations including activation of resident microglia and phagocyte recruitment. Astrocytes at the LITT site differed in marker expression suggesting distinct subpopulations. Single-cell spatial transcriptomics also identified well-defined gene clusters indicative of distinct cell populations in LITT brains. Analysis of plasma by mass spectrometry will determine if brain LITT can evoke systemic protein changes which may be relevant in underpinning immunomodulatory mechanisms.

Conclusion: Successful establishment of this LITT murine model provides a valuable platform for investigating LITT effects on the tumor, tumor-microenvironment, and the systemic proteomic milieu. This unique preclinical model empowers the discovery of combined LITT-drug treatments for improved therapeutic efficacy.

Faculty Presentation

MIRAGE: Mental Imagery and the Regulation and Generation of Emotions

Steven Greening

University of Manitoba, Winnipeg, Canada

Abstract

From tales of ghosts told around the campfire to calming one's fears by recalling pleasant moments of the past, the human imagination is a potent tool for expressing and regulating emotions. Both prominent theories and clinical interventions involving the cognitive control of emotion invoke mental imagery as an important process, yet relatively less empirical research has been done to quantify the role of imagery in various emotion processes. In my talk, I will describe a series of studies beginning to remedy this gap in knowledge by combining Pavlovian fear conditioning with manipulations of emotion regulation via mental imagery. These studies evaluate the role of mental imagery in the regulation and generation of fear conditioned reactivity. Fear reactivity was measured using the convergent measures of self-report, psychophysiology (i.e. skin conductance), and functional magnetic resonance imaging (fMRI). The fMRI results include both univariate and multivariate analyses to quantify the brain processes associated with imagery-based emotion regulation, and to compare it to externally directed attentional processes. The findings will be discussed with reference to the Mental Imagery in the Regulation And Generation of Emotions (MIRAGE) framework, which incorporates the biased competition model of attention with aspects of the depictive theory of imagery to evaluate how mental imagery contributes to emotion regulation.

Poster # 1

Disruption of Thioredoxin-1 System is Associated with Wide-Spread Transcriptomic Profiles and DNA Methylation changes in Human SH-SY5Y Neuroblastoma Cells

Tobi Olanipekun, Md Imamul Islam, Soheila Karimi_abdolrezaee, and Eftekhar Eftekharpour

Dept. Physiology and Pathophysiology, University of Manitoba

Thioredoxin reductase 1 (TrxR1) is a central enzyme in the thioredoxin-1 system, which, together with thioredoxin (Trx1) and NADPH function as a central hub in neural cells for maintaining protein homeostasis and antioxidant defense. In the neuron. We have shown that Trx-1 deficiency is linked to structural nuclear lamina and epigenetic alterations. This study investigates the impact of Trx deficiency on DNA methylation and gene expression profiles in the human SH-SY5Y neuroblastoma cell line.

Using a crispr-knockout approach in neuroblastoma cells, we generated a stable TrxR1 depletion that results in Trx1 oxidation and effectively disrupts Trx1 system. Knockdown and scrambled control SHSY5Y cells were cultured in normal and under serum deprivation for 6 hours, after which genomic-DNA, RNA, and protein were extracted for analysis. Global DNA methylation level was assessed using an ELISA kit for 5-methylcytosine, and Western blotting was used to assess the levels of DNA methyl transferase enzymes, DNMT1 and DNMT3a. Transcriptomic profiling was carried out by RNA sequencing followed by Differential gene expression analysis using DESeq2, with downstream gene ontology and KEGG pathway enrichment analyses to identify affected biological processes and pathways.

Disruption of the thioredoxin system by the knockdown of TrxR1 showed reduced global DNA methylation levels in comparison to control cells, but upon serum deprivation, TrxR1KD cells maintain higher levels of global DNA methylation. On the other hand, there was decreased levels of DNMT1 and 3a in TrxR1KD cells, which was further reduced under serum deprivation induced oxidative stress. Transcriptomic profiling revealed substantial changes due to the loss of TrxR1 with downregulation of neuronal biological processes and pathways, such as synapse organization, modulation of chemical synaptic transmission and regulation of trans-synaptic signaling.

These findings highlight the broad impact of Thioredoxin system in a wide range of molecular pathways and provide a mechanistic link to neurodegenerative diseases such as Alzheimer's Disease that are associated with Thioredoxin deficiency.

Trainee Podium Presentation

D-serine facilitates aggressive migration and stemness of recurrent glioblastoma cells by interacting with host endothelial cells

Ryan Mota[1,2], Ping Lu[1,2], Emma Martell[3], Alejandra Llanes Cuesta[1,2], Tanveer Sharif[3], Chris Anderson[1,2]

[1]Department of Pharmacology and Therapeutics, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada. [2]PrairieNeuro Research Centre, Kleysen Institute for Advanced Medicine, Health Sciences Centre, Winnipeg, Canada. [3]Department of Pathology, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada

Abstract

Introduction:

Glioblastoma (GBM) cells infiltrate deep brain structures by exploiting perivascular pathways, utilizing stem-like properties and malignant traits to access nutrient-rich environments that support tumor expansion. Our findings suggest that NMDA receptors (NMDARs) in brain endothelial cells play a key role in transducing signals initiated by parenchymal cells, facilitating recurrent GBM progression.

Hypothesis:

D-serine enhances GBM migration and stemness by interacting with host endothelial cells, thereby promoting tumor aggressiveness.

Methods:

Patient-derived recurrent GBM cells and human cerebral microvascular endothelial cells (hCMECs/D3) were co-cultured in a transwell system to assess the role of endogenous D-serine in GBM migration and stemness. Pharmacological inhibitors, CRISPR/Cas9-mediated gene silencing, and enzymatic modulation of D-serine metabolism were employed to determine the contribution of GBM-derived D-serine and endothelial NMDARs to these processes.

Results:Endothelial cells significantly potentiated GBM migration and stemness in co-culture. D-serine release from GBM cells was confirmed using D-amino acid oxidase, serine racemase (SR) inhibition with phenazine methosulfate (PMS), and CRISPR-mediated SR silencing, all of which significantly reduced migration and stemness markers. Pharmacological NMDAR antagonism and endothelial GluN1 silencing further mitigated these effects. In vivo, PMS administration reduced tumor volume at 4- and 6-weeks post-injection and extended survival in GBM-bearing mice.

Conclusion:

Our findings demonstrate that brain endothelial cells enhance GBM malignancy through D-serine-mediated activation of endothelial NMDARs. This pathway supports GBM migration and stemness, presenting a novel therapeutic target for recurrent GBM treatment.

Poster #**2**

The Role of the Nuclear Transitory Zone (NTZ) in Cerebellar Development

Hassan Marzban

University of Manitoba, Winnipeg, Canada

Abstract

The nuclear transitory zone (NTZ) is a transient yet strategically critical structure that emerges during the earliest stages of cerebellar development. Located at the rostral end of the cerebellar primordium, the NTZ has traditionally been viewed as a pathway for neuronal migration. However, recent studies have uncovered its broader and more active role as a developmental organizer that integrates molecular signaling, cellular specification, and connectivity formation. Our work demonstrates that the NTZ contains diverse populations of excitatory and inhibitory neurons derived from multiple progenitor domains, including the rhombic lip, ventricular zone, and potentially the mesencephalon. These neuronal subsets exhibit regionally restricted expression of key developmental regulators such as such as MEIS2, LMX1A, TBR2, and OTX2, many of which appear in the NTZ prior to their expression elsewhere in the cerebellum. Moreover, the NTZ is the earliest cerebellar structure to receive afferent projections from the trigeminal system, underscoring its potential role in scaffolding early sensorimotor circuits. The convergence of multiple molecular cues and its strategic connectivity position the NTZ as a critical hub for regulating the emergence of cerebellar nuclei neurons, Purkinje cells, and interneurons. In this presentation, I will highlight our recent findings on NTZ structure, gene expression patterns, and its temporal-spatial contribution to cerebellar morphogenesis. Understanding the developmental logic of the NTZ offers new insights into cerebellar circuit assembly and may have important implications for neurodevelopmental disorders linked to cerebellar dysfunction.

Poster #**3**

ATM restoration in a clinically relevant mouse model of ataxia telangiectasia prevents progressive ataxia and reduces leukemia and lymphoma outcomes.

Joanna Joe[1], Molly Pind[1], Paul Mathews[2], Geoffrey Hicks[1]

[1]University of Manitoba, Winnipeg, Canada. [2]UCLA / Lundquist Institute, Los Angeles, USA

Abstract

Ataxia Telangiectasia (AT) is an autosomal recessive neurodegenerative disorder caused by a deficiency in the AT Mutated (ATM) protein. AT is marked by progressive motor dysfunction, immune deficiency, and cancer predisposition. We have developed a novel Atm-deficient mouse model with a clinically relevant nonsense mutation in the Atm gene, and notably, the first model to exhibit both ataxia and neurodevelopmental phenotypes. Using this model, we tested the novel small molecule read-through (SMRT) compounds, which were shown to restore low levels of ATM production and offer therapeutic benefits. However, before clinical trials, the mechanism behind this effect needs to be confirmed.

To address this, we will generate an inducible murine expression model of AT to test if restoring even low levels of Atm can rescue its deficiency throughout the animal. The model is created by adding an estrogen ligand-binding region upstream of the Atm gene such that ATM translocation into the nucleus will be tamoxifen-inducible (Atm-ERT). The mouse genome is edited using an Easi-CRISPR strategy with ssDNA as a template. This model will help us demonstrate that restoration of even low levels of ATM protein has therapeutic effects on the cancer and immune deficiency phenotype, while our collaborators will study its effects on ataxia.

This model represents the final pre-clinical step before SMRT compounds can enter clinical trials. Further applications of this model include identifying leukemic and restorative determinants of microRNA regulation in the ATM signaling pathway.

Poster #**4**

SPATIAL MODULATION OF IMMUNE CELL DISTRIBUTION BY TRANSCUTANEOUS AURICULAR VAGUS NERVE STIMULATION (TAVNS) IN A PRECLINICAL MODEL OF ULCERATIVE COLITIS

Fatemeh Hesampour[1], Charles Bernstein[2], Jean-Eric Ghia[1,2]

[1]Department of Immunology, University of Manitoba, Winnipeg, Canada. [2]Department of Internal Medicine, University of Manitoba, Winnipeg, Canada

Abstract

Introduction: Ulcerative colitis (UC) features reduced vagal tone and heightened pro-inflammatory immune cell infiltration. Given the vagus nerve's immunomodulatory role, vagus nerve stimulation (VNS) is a promising therapeutic strategy. We previously showed that non-invasive transcutaneous auricular VNS (taVNS) protects against UC-like colitis in mice; however, the underlying immune targets remain unclear. **Objective:** To assess the impact of taVNS on granulocytes, macrophages, NK cells, and $CD4^+$/$CD8^+$ T cells in gut-associated and peripheral immune compartments during experimental colitis.**Methods:** Male C57BL/6 mice received daily 10-minute taVNS starting one day before colitis induction with 5% dextran sulfate sodium (DSS) in drinking water for five days; controls received water only. Stimulation continued throughout. A sham group received no stimulation. Disease activity index (DAI) and distal colon damage were evaluated. Flow cytometry quantified $Ly6G^+$ granulocytes, $F4/80^+$ macrophages, $NK1.1^+$ NK cells, and $CD3^+CD4^+$/$CD8^+$ T cells in the cecum, spleen, mesenteric lymph nodes (MLNs), and proximal, mid, and distal colon. **Results:** TaVNS significantly improved DAI and macroscopic outcomes in DSS-treated mice, with no impact in controls. In colitic mice, taVNS selectively decreased $Ly6G^+$ cells in the proximal colon and spleen, and $F4/80^+$ cells in the mid-colon. $NK1.1^+$ cell frequency was increased in the spleen following taVNS, with no changes observed in other tissues or in non-colitic mice. $CD4^+$ and $CD8^+$ T cell distributions remained unaffected across all groups.**Conclusion:** taVNS modulates innate immune subsets in colitic mice by reducing intestinal and systemic granulocytes and macrophages, while increasing splenic NK cells, supporting its anti-inflammatory potential in UC.

Poster #**5**

Contribution of astrocytic N-methyl-D-aspartate (NMDA) receptors in regulation of cortical neuronal function via purinergic signaling

Meher Kantroo, Noushin Ahmadpour, Michael Stobart, Jessica Meza-Resillas, Anna Muzaleva, Bruno Di Gaetano, Michael Jackson, Jillian Stobart

University of Manitoba, Winnipeg, Canada

Abstract

Introduction

Astrocytes bidirectionally communicate with nearby astrocytes and neurons via calcium signaling. These cells express N-methyl-D-aspartate receptors (NMDARs) which can cause rise in astrocytic calcium levels. Increases in astrocyte calcium is associated with release of purines like Adenosine triphosphate (ATP) and adenosine, which can modulate neuronal activity. The mechanisms by which astrocytic NMDARs can modulate neuronal activity via purines is yet to be investigated.

Methods

In this study, NMDARs in astrocytes have been selectively targeted and knocked down in mice barrel cortex (aNMDAR KD). Immunohistochemistry was performed to characterize aNMDAR KD. Awake in-vivo 2-photon calcium imaging was performed to observe astrocytic and neuronal calcium imaging. Texture discrimination behavior tasks were performed to investigate sensory acuity. Acute pharmacology was done by applying ATP and Adenosine to the barrel cortex and studying possible rescue of aNMDAR KD related impairments. Currently, electrophysiology is being performed to dissect the effect of aNMDAR KD on different types of cortical neurons.

Results

We observed a KD of aNMDARs in tissue. Awake two-photon imaging showed KD dependent calcium impairments in astrocytes and neurons. There were texture discrimination deficits present. Interestingly, upon ATP application, there was a rescue of neuronal and behavioral impairments. Only partial neuronal rescue was observed upon Adenosine application.

Conclusion

This study highlights the relevance of aNMDARs in modulating cortical circuits. We show that aNMDARs are important for sensory information processing. This is relevant for diseases such as schizophrenia where NMDA receptors are poorly activated and thus, can help us shed light on disease mechanisms.

Poster #**6**

Intranasal delivery of human Wharton's jelly-derived mesenchymal stem cells alleviates Aβ-induced Alzheimer's symptoms in rat models by regulating neurotrophic and apoptotic factors

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Abstract

Alzheimer's disease (AD) is the most common cause of dementia in adulthood, followed by cognitive and behavioral deficits. Today, mesenchymal stem cell (MSC)-based therapy is a suitable therapeutic option to improve regenerative medicine approaches against neurodegenerative disorders, including AD. This study aimed to investigate the effects of human Wharton's jelly-derived MSCs (WJ-MSCs) on AD-like rat models (rats treated with amyloid beta 1-42 ($A\beta_{1-42}$)) by evaluating the expression of neurotrophic factors such as brain-derived neurotrophic factor (*BDNF*) and nerve growth factor (*NGF*), as well as the expression of apoptotic factors such as B-cell lymphoma 2 (*BCL2*, an anti-apoptotic factor to inhibit apoptosis) and BCL2-associated X protein (*BAX*, a pro-apoptotic factor to regulate apoptosis). After treatment of AD rat models with WJ-MSCs, behavioral tests (i.e., passive avoidance and Morris water maze) showed cognitive improvements, and amelioration of cells in the CA1 area of the hippocampus was detected by cresyl violet staining. Additionally, real-time polymerase chain reaction (RT-PCR) of the hippocampus indicated an increase in the expression level of the *BDNF*, *NGF*, and *BCL2* genes and a decrease in the expression level of the *BAX* gene. Overall, the WJ-MSCs improved the cognitive function in AD rat models by increasing the neurotrophic and anti-apoptotic factors and decreasing the pro-apoptotic factor.

Trainee Podium Presentation

Repeated Injury Impacts Immune and Neural Stem Cell-Mediated Repair in the Zebrafish Forebrain

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Abstract

Neural Stem Cells (NSCs) play a vital role in brain repair post-injury. While mammals struggle with glial scarring, zebrafish serve as an exceptional regenerative model, exhibiting robust NSC-driven neurogenesis in the adult forebrain after injury. Radial Glial Cells (RGCs), a type of NSC, generate new neurons to replace lost lineages, a process mediated by the immune responses and gene upregulation. However, it is unclear if RGCs sustain long-term neuronal production after repeated injuries. This study investigates the effects of repeated injuries on RGCs, focusing on neuronal production and the pro-regenerative environment, using a paradigm where zebrafish were subjected to injuries weekly, up to four times. Histological analysis with H&E staining at 1-day post-injury (dpi) showed sustained pathology, including widening lesions and increased blood clotting, which resolved by 7 dpi across all groups. Using the *Tg(GFAP:gfp) X Tg(Her4.1:mCherry)* reporter line, I observed declining RGC proliferation and increased quiescence with repeated injuries. Neurogenesis analysis via EdU/HuC/D co-labeling revealed reduced neuronal production, while 4C4 immunostaining indicated elevated microglial activity. RT-qPCR analysis of pro-regenerative genes *gata3*, cxcr5, and *id1* revealed unexpected trends, with gata3 and *cxcr5* downregulated, and id1 upregulated with successive injuries. These findings reveal the neuroregenerative limits of zebrafish RGCs and their link to immune responses and gene expression changes in the injury microenvironment.

Poster #**7**

Investigating FAN1 as a genetic modifier of Rett syndrome in human stem cell-derived neural precursor cells and dorsal forebrain organoids

Alana Slike[1,2,3,4], John Pham[1,5], Abdoulaye Diarra[1,2,3,4], Alana Lamont[5], Noushin Ahmadpour[6], Paris Musaphir[1,2,3,4], Anisa Kahlon[1,2], Britt Drögemöller[3,5], Michael Jackson[1,2,6], Soheila Karimi-Abdolrezaee[3,4,7], Galen Wright[1,2,3,4]

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Abstract

Rett syndrome (RTT) is a rare neurodevelopmental disorder with limited treatment options. RTT is caused by mutations in the *MECP2* gene, and affected individuals exhibit variable clinical severity. Therefore, we consider genetic modifiers, as they can alter disease severity and may inform therapeutic targets. An RTT modifier screen in Mecp2/Y mice found that ablation of *Fan1* improved the RTT phenotype. To determine the translational relevance, we aim to examine whether FAN1 ablation alters human induced pluripotent stem cell (hiPSCs) derived models of RTT at the molecular level.

An isogenic RTT hiPSC pair was edited to remove FAN1, resulting in an RTT *FAN1*KO line. The three hiPSCs lines were differentiated into neural progenitor cells (NPCs). Bulk RNA-seq found that FAN1 knockout in RTT NPCs resulted in 185 DEGs. Gene set enrichment analysis found that ablation of *FAN1* in RTT NPCs led to the upregulation of neuronal processes such as "regulation of neurotransmitter levels" and *"regulation of dendritic extension,"* suggesting that loss of FAN1 may alter aberrant neuronal morphology and activity in RTT.

At the transcriptional level, RTT dorsal forebrain organoids (DFOs) indicate aberrant neuronal processes. Single-nuclei RNA sequencing (snRNA-seq) data of DFOs show downregulation of neuronal processes such as *"regulation of post-synapse organization and regulation of neuron migration"* in RTT inhibitory neurons compared to controls. Further analysis with NEUROeSTIMater found that RTT inhibitory and corticofugal projection neurons showed increased neural activity compared to control. These transcriptional-level analyses have revealed aberrant neuronal processes in RTT that may be ameliorated via *FAN1* ablation

Poster #**8**

Investigation of DNA double-strand break repair pathway choice in Rett syndrome

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Abstract

Introduction: Rett syndrome (RTT) is an X-linked dominant neurodevelopmental disorder caused by mutations in the *MECP2* gene. The largest genetic modifier screen conducted in *Mecp2*-null mice identified genes that were enriched in the repair of double-stranded breaks (DSBs).

Objective: To determine the role of DSB pathway choice as a genetic modifier in RTT.

Methods: Two experimental models are being used: (i) the analysis of a key DSB pathway choice gene (*RBBP8*) in RTT stem cells and in SH-SY5Y neuroblastoma cells (ii) use of a bioinformatic approach to identify and prioritize genes implicated in RTT to inform future therapeutic studies. Rett iPSCs and isogenic controls will be differentiated into NPCs, providing appropriate disease modelling. Working alongside collaborators, siRNA will be used to knockdown *RBBP8* in both cell model types, allowing us to study downstream effects in relation to the DDR.

Results: SiRNA has been used to effectively knockdown *RBBP8* protein expression near 50% across all cell lines. Trends show there may be increased *RBBP8* expression in RTT lines at the mRNA level (in iPSCs via qPCR) and at the protein level (NPCs via ICC). Trends also indicate increased DNA damage in RTT cell lines.

Conclusion: Using approaches that focus on identifying DNA repair-related RTT genetic modifiers and how the *RBBP8* genetic modifier affects DNA repair pathway choice, may provide new therapeutic insights for RTT as limited options exist.

Trainee Podium Presentation

Correlates of Accelerated Brain Aging in Prodromal Dementia: A Longitudinal Metabolic Imaging Study

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Abstract

Normative models of brain aging can be developed using neuroimaging datasets such that an individual's predicted age is estimated from their neuroimaging data. The difference between their brain age and chronological age (brain age gap, BAG) has been reported in neurodegenerative and psychiatric conditions, but literature on longitudinal BAG changes is sparse. We sought to determine the correlates of brain aging in patients with prodromal dementia. 631 subjects ($n_{control}$ = 184, $n_{prodromal}$ = 447) underwent longitudinal brain imaging with fluorodeoxyglucose positron emission tomography. A support vector regression model was trained using regional tracer uptake in controls to predict age. Linear mixed-effects modeling determined significant predictors of BAG in prodromal subjects. Incipient decliners had significantly elevated mean BAG (3.03 ± 0.20 years) compared to resilient-decliners (1.43 ± 0.41 years) and stable (0.45 ± 0.18 years) subjects (Tukey's test, $P < 0.01$). Significant predictors of BAG included cerebrospinal amyloid-beta burden, education, race and sex ($P < 0.036$). Age and executive function showed significant interactions with cohort ($P = 0.0085$). Race had a significant three-way interaction with time and cohort ($P = 0.013$). Post-hoc analysis identified hypometabolism of the dorsal caudate, pregenual area 32 and the medial prefrontal thalamus as drivers of brain aging. We demonstrated that established markers and risk factors of neurodegenerative pathology are meaningfully associated with BAG in a large longitudinal study of brain metabolism. These observations may open avenues for personalized brain health tracking and monitoring response to disease-modifying therapies. We acknowledge the support of NSERC, the University of Manitoba and CIHR.

Poster #**9**

Multiple Sclerosis, Comorbid Depression and the Association with Different Blood Biomarkers.

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Abstract

Introduction: Multiple sclerosis (MS) is a disease characterized by neuroinflammation and neurodegeneration. Serum neurofilament light chain (sNfL) reflects neuronal injury, while glial fibrillary acidic protein (GFAP) indicates astrocytic activation. Both biomarkers are elevated in persons with MS (pwMS) and in those with depression. Understanding whether sNfL and GFAP are particularly elevated in pwMS with comorbid depression may clarify how depression influences MS outcomes.

Methods: We analyzed data from 529 participants in the 3-year longitudinal IMID study, including 52 pwMS with depression, 50 pwMS without depression, 69 individuals with depression but no immune disease, and 89 healthy controls. Baseline sNfL and GFAP levels were compared across groups.

Results: PwMS with and without depression had similarly high sNfL levels (mean logNfL = 2.47, standard deviation (SD) = 0.48 and 0.57, respectively), while individuals with depression but no immune disease had lower levels, with healthy controls having the lowest (1.96, SD = 0.51). GFAP was elevated in pwMS with (4.55, SD = 0.53) and without depression (4.66, SD = 0.66), compared to individuals with depression but no immune disease (4.15, SD = 0.49) and healthy controls (4.12, SD = 0.51).

Conclusion: These findings suggest increased neuroaxonal and astrocytic damage in pwMS with comorbid depression. Investigating sNfL and GFAP in this population may provide insight into the interplay between depression and MS. Next, we will conduct statistical modeling to explore cross-sectional and longitudinal biomarker associations.

Poster #**10**

Pannexin 2 is a constitutively active chloride channel with similar yet distinct properties from Pannexin 1

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Abstract

Pannexins are ion channels which can release molecules up to 1kDa. Previously thought to reside intracellularly, Pannexin 2 (Panx2) properties are relatively unexplored compared to the extensively studied Pannexin 1 (Panx1). We now report prevalent Panx2 expression at the plasma membrane (PM) and the properties of membrane-bound Panx2. Immunocytochemistry was used to evaluate surface localization of Panx2 transfected in HEK293T. Cells were treated with wheat germ agglutinin (WGA, a PM marker) and an anti-calnexin antibody (an endoplasmic reticulum (ER) marker). Panx2 was prevalent at the PM where it co-distributes with WGA. To explore the role and properties of Panx2 as a membrane channel, we performed whole-cell recordings in HEK293T cells expressing Panx2. Ramp-evoked currents suggested that Panx2 may be primarily chloride permeable, as reported for Panx1. When extracellular chloride was replaced using HEPES, current was abolished and reversal potential shifted. This affirms chloride as the major component of constitutive currents in Panx1/2. Due to lack of knowledge regarding Panx2 activation, we sought to examine whether Panx2 shares a mechanism with Panx1. Cell swelling due to hypotonic stimulus activates Panx1, but we show Panx2 is unaffected. Lastly, we have begun to assess the extent which Panx1/2 share sensitivity to pharmacological agents. Panx2 is insensitive to carbenoxolone while DCPIB inhibits both Panx1/2-mediated currents. Unitedly, by studying these properties of Panx1 and Panx2, we show Panx2 distinguishes itself from Panx1. Panx2 conducts current at the PM, indicating it is functional in this subcellular location, and should be recognized as such.

Trainee Podium Presentation

Cholesterol Metabolism as a Driver of Chemoresistance in Glioblastoma

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Abstract

Problem Statement

Glioblastoma (GB) remains the most lethal and treatment-refractory primary brain tumor. Despite Temozolomide (TMZ) being a frontline therapy, its effectiveness is limited by the rapid development of resistance. Emerging evidence highlights that cholesterol metabolism reprogramming plays a crucial role in cancer growth, survival, and therapy resistance, particularly in GB. We investigated the role of cholesterol metabolism in TMZ resistance and assessed the potential of Simvastatin (ST), a lipid-lowering agent, to overcome this resistance alone or in combination with TMZ.

Approach

This study utilized both Temozolomide-resistant and non-resistant U251 cells to investigate mechanisms of drug resistance. Lipidomic profiling was conducted to analyze cholesterol ester composition, while expression levels of cholesterol regulators were quantified via real-time PCR. Finally, the impact of ST alone or combined with TMZ on cholesterol metabolism, apoptosis, and cell viability was determined.

Results TMZ-resistant cells showed elevated total cholesterol and cholesterol ester **CE 22:0** levels, alongside decreased de novo cholesterol synthesis and lower expression of **LDL-R** and SREBP2, reflecting reduced proliferation and mitotic arrest. Resistance was also associated with a shift toward oxidative phosphorylation (OXPHOS). While Simvastatin enhanced TMZ-induced apoptosis in non-resistant cells, it failed to sensitize resistant cells, indicating activation of compensatory metabolic pathways.

Conclusion

TMZ resistance in glioblastoma involves lipid metabolic reprogramming and oxidative metabolic adaptation. Targeting cholesterol metabolism or OXPHOS may offer therapeutic benefit, but statins alone are insufficient and may require combination strategies to effectively overcome resistance.

Poster # **11**

Neuregulin-1 Enhances Hippocampal Neurogenesis and Cognitive Function in a Chronic Cuprizone Model of Multiple Sclerosis.

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Abstract

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system characterized by widespread demyelination, axonal injury, and synaptic disruption. Among affected brain regions, the hippocampus—a critical hub for learning and memory—is especially vulnerable, with damage in this area strongly correlating with cognitive impairment. Adult hippocampal neurogenesis, driven by neural precursor cells (NPCs), plays a key role in maintaining cognitive functions, yet this process is markedly diminished in progressive MS. As such, therapeutic strategies that preserve neuronal architecture and restore NPC-mediated neurogenesis are urgently needed. Previous work from our group identified dysregulated expression of Neuregulin-1 (Nrg-1) in MS lesions. Nrg-1 is essential for neuronal differentiation and has been linked to neurodevelopmental disorders, but its role in adult neurogenesis and cognitive recovery in chronic demyelination remains unclear.

Here, we employed a chronic cuprizone-induced demyelination model in Nes-CreERT2;Rosa26-mGFP mice to track NPCs and mimic progressive MS pathology. Following ten weeks of demyelination, Nrg-1 was administered daily for four weeks. Using both in vitro and in vivo analyses, we assessed the impact of Nrg-1 on myelin repair, neurogenesis, and cognitive function.

Our results reveal that Nrg-1 treatment significantly restored myelin integrity, reduced axonal degeneration, and enhanced neuronal arborization and synaptic density in the hippocampus. Importantly, Nrg-1 reactivated endogenous hippocampal NPCs, leading to increased neurogenesis and oligodendrogenesis. These findings identify Nrg-1 as a promising therapeutic candidate for progressive MS, capable of promoting structural repair and functional recovery in demyelinated neural circuits.

Poster # **12**

Reconstruction of damaged circuit after spinal cord injury through combined stem cell and pharmacological approaches

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Abstract

Extensive neuronal loss, progressive neurodegeneration, and circuit impairment are the key characteristics of spinal cord injury (SCI). The intrinsic capacity of the spinal cord to replenish damaged neurons and reassemble the disrupted spinal circuitry is restricted. Transplantation of exogenous neural precursor cells (NPCs) has shown promise to structurally repair the injured spinal network. However, proper maturation and integration of newly generated neurons from NPC graft into the host spinal circuit has remained challenging. Here, we have developed a combinatorial strategy to augment maturation of newly generated neurons and facilitate their functional integration with the host circuitry. We demonstrate that blockade of inhibitory CSPG/LAR/PTP-s axis and neuromodulation by activation of serotonin receptors 5-$HT_{1/2/7}$ fosters the generation, maturity, and functional connectivity of NPC-derived neurons with the host local spinal network as well as two major descending motor pathways that culminate in recovery of locomotion and sensorimotor integration. Taken together, this novel cellular and pharmacological approach addresses critical gaps in cell-based repair strategies for SCI by optimize neuronal replacement and restoration of functional neural networks within the damaged spinal circuitry.

Poster #**13**

Genetic Contributions to Treatment Resistant Schizophrenia: A Scoping Review

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Abstract

Background: Approximately 30% of individuals with schizophrenia have treatment resistance (TRS), marked by inadequate response to multiple antipsychotic medications. TRS is linked to heightened disability, poor prognosis, and increased mortality. Despite advancements in genetics, the underlying genetic architecture of TRS remains unclear. This scoping review aimed to synthesize current evidence on the genetics of TRS.

Method: Articles were retrieved from PubMed in January 2024 and screened using Covidence. Inclusion criteria encompassed studies reporting on TRS genetics, participants aged 18+, and English-language publications. Excluded were review articles, grey literature, theses/dissertations, case reports, and non-article publications. Screening involved initial title and abstract review followed by full-text assessment, conducted independently by two reviewers with discrepancies resolved by a third reviewer.

Results: Ninety-two studies published between 1998 and 2024 met inclusion criteria, mostly involving individuals of European descent. Studies exhibited variability in TRS definition, including terminology, clinical assessments, and medication histories. Genetic investigations were grouped into three categories: 1) Common variation (n = 72), 2) Rare variants (n = 9), and 3) Functional annotation of genetic variants (n = 16).

Discussion: Inconsistent TRS definitions across studies emphasize the need for a standard characterization of TRS. The predominance of European population data highlights the lack of generalizability across ancestries. Most studies explored specific genetic variants associated with TRS, warranting comprehensive genome-wide research, functional assessment of variants, and gene-environment interactions in TRS.

Poster #**14**

Investigating the roles of CtIP and DNA double-strand break repair as genetic modifiers of Rett syndrome

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Abstract

Introduction: *MECP2* genetic variant type does not fully account for the variability in clinical severity in Rett syndrome (RTT), suggesting the involvement of additional factors, such as genetic modifiers. A recent unbiased genetic modifier screen in *Mecp2*-null mice detected a significant enrichment for homology-directed repair (HDR) genes related to the double-strand break (DSB) pathway. One of the candidate modifier genes RTT was Rbbp8, which encodes CtIP, an important gene involved in DSB pathway choice, which requires additional characterization.

Methods: RBBP8 will be edited in a *MECP2*-null isogenic human pluripotent stem cell pair using CRISPR. These cells will be differentiated into neural progenitor cells (NPCs). This will allow for the two major DSB-related pathways, HDR and non-homologous end joining (NHEJ), to be studied. DNA damage will be assessed using γ-H2AX (a marker of DSBs), RAD51 (HR activity), and Artemis (NHEJ activity) via immunoblotting. Bulk RNA sequencing on NPC mRNA will also assess the dysregulation of DNA repair and other pathways comprehensively.

Results: CRISPR gene editing has successfully introduced a heterozygous RBBP8 TAA stop codon into the respective lines. These cells have been differentiated into NPCs and mRNA has been isolated. Samples are currently being sequenced – preliminary results of these analyses will be presented.

Conclusion: This study will help determine how perturbing CtIP and DSB repair pathway choice influences RTT-related processes in a human cell model of RTT. This will increase our understanding of genetic modifiers in RTT, which could provide new therapeutic insights.

Poster #**15**

The Impact of Thioredoxin on Adult Neurogenesis and Neuronal Maturation in Hippocampal-derived Neural Stem Cells (NSC)

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Abstract

Introduction: Reactive oxygen species (ROS) are generated during cellular metabolism and can help regulate cellular activities. However, excessive production known as oxidative stress can cause irreversible cellular damage and promote cell death. Antioxidant systems, such as thioredoxin-1 (Trx1) regulate ROS levels. These protective mechanisms decline in aging, contributing to development of age-associated diseases.

Rationale: Alzheimer's Disease (AD) is characterized by progressive neuronal loss and reduced hippocampal neurogenesis. Whether decreased neurogenesis in AD patients can be attributed to Trx1 depletion remains to be examined.

Hypothesis: We hypothesize that lower levels of Trx1 in the brain are responsible for decreased neurogenesis.

Methods: We generated a transgenic mouse model with a neuron-specific deletion of Trx1 (Trx-nKO). This model displays significant Trx1 reduction in the brain, along with sensory and motor deficits. The status of hippocampal neural stem and precursor cells (NSPCs) under in vitro and in vivo conditions was examined.

Results: Neurosphere formation assays revealed no differences in number of NSPCs as determined by the number of neurospheres. However, the Trx-nKO mice displayed smaller neurospheres which indicates a lower proliferative capacity. Immunocytochemical analysis of neurogenesis showed a higher number of doublecortin-positive immature neurons in Trx-nKO mice, while beta-tubulin-III-positive mature neurons were more abundant in controls. I am currently assessing the expression of these markers in brain sections.

Conclusion: This study may suggest a role for Trx1 in neuronal maturation. Further examination and understanding the precise mechanisms by which Trx1 influences NSPCs behavior could provide valuable insight into developing therapeutic strategies for neurodegenerative diseases.

Poster #**16**

Polygenicity of Cognitive Ability and Educational Attainment in Multiple Sclerosis

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Abstract

Introduction: Approximately 40-65% of people with multiple sclerosis (PwMS) experience cognitive impairment, which can adversely affect quality of life. Cognitive ability and educational attainment are both heritable traits and their cumulative genetic weight can be measured using polygenic scores (PGS). Differences in PGS for both traits must be further investigated to assess its heterogeneity in cognitive impairment for PwMS.

Objectives: In PwMS, we aimed to determine whether the (a) cognitive ability PGS is associated with information processing speed, and (b) educational attainment PGS is associated with education level.

Methods: We used existing data from 2663 PwMS from studies in Canada (IMID study, N = 213), USA (CombiRx trial, N = 602), and UK Biobank (N = 1848). Information processing speed was measured using the Symbols Digit Modalities Test (Canada), Paced Auditory Serial Addition Test (USA), and Digit Symbol Substitution Test (UK), respectively. Education level was dichotimized (< high school vs. ≥ high school). We generated the PGS for cognitive ability and educational attainment using SBayesR and tested their association with their respective outcomes in PwMS using linear regression. Results from the three cohorts were pooled using a fixed effect meta-analysis.

Results: In PwMS, higher cognitive ability PGS was associated with higher processing speed (beta per 1-SD=0.56, SE=0.23, P=0.01). Likewise, higher educational attainment PGS was associated with attaining more than a high school education (beta per 1-SD=0.39, SE=0.06, P<0.001).

Conclusions: Polygenicity for higher cognitive ability and educational attainment were associated with information processing speed and education level in PwMS, respectively.

Trainee Podium Presentation

Transcriptomic and Protein Expression Analysis of Early Differentiating Neurons in the Developing Mouse Cerebellar Nuclei

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Abstract

Introduction: The cerebellar nuclei (CN) play crucial roles in sensorimotor integration and cognitive function, and their developmental trajectory may influence neurodevelopmental disorders such as ataxia and autism spectrum disorder (ASD). Traditionally, glutamatergic and GABAergic CN neurons are thought to arise after embryonic day 9 (E9) from the rhombic lip and ventricular zone, respectively. Here, we identify a previously less understood population of CN neurons that emerges before E9 in the nuclear transitory zone (NTZ)—the precursor to mature CN—marked by expression of α-synuclein (SNCA) protein.

Methods: Using SncaGFP transgenic mice, in which GFP is co-expressed with SNCA, we tracked the emergence and maturation of $SNCA^+$ NTZ neurons via EdU proliferation assay and immunofluorescence protein labeling. Then we analyzed single-cell RNA sequencing (scRNA-seq) data (GSE118068) to profile postmitotic $SNCA^+/MEIS2^+$ neurons at embryonic days E10 and E12.

Results: $SNCA^+$ NTZ neurons first appear by E8.75 and are the only $NeuN^+$ differentiated cerebellar neurons between E10 and E12, co-expressing MEIS2 and OTX2. Transcriptomic analysis revealed a distinct molecular profile, with differential gene expression and gene ontology enrichment highlighting involvement in axonogenesis at E10 and in neuronal differentiation and axon guidance by E12. Enriched cellular components included the growth cone, axon terminus, and pre- and postsynaptic membranes.

Conclusion: We identified an early-born, differentiated population of $SNCA^+/MEIS2^+$ NTZ neurons that predates canonical CN neuron development. Their distinct temporal emergence and gene expression profile suggest a foundational role in initiating cerebellar circuit formation, with potential implications for neurodevelopmental synaptic disorders such as ASD.

Trainee Podium Presentation

Ablation of Neuregulin-1 elicits Multiple Sclerosis-like pathology including progressive brain demyelination, inflammation and cognitive impairment in adult mice

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Abstract

Multiple Sclerosis (MS) is a complex immune-mediated disease that affects the central nervous system, leading to neurodegeneration and neurological impairments such as cognitive decline and anxiety. Early in the disease, neural precursor cells (NPCs) contribute to myelin and hippocampal repair. However, we have identified the ability of NPCs to promote repair diminishes in chronic MS lesions resulting in cognition decline. We have linked this to dysregulation of Neuregulin-1 (Nrg-1) levels in both human MS lesions and animal models. Nrg-1 is important for NPC regulation, myelination, and immune homeostasis in the CNS, suggesting that its depletion may hinder remyelination and drive neurodegeneration and cognitive deficits in chronic MS. To explore this, we employed a loss-of-function approach to assess Nrg-1 a susceptibility gene for developing MS.

We used a tamoxifen-inducible Cre/Lox Nrg1 conditional knockout (cKO) mouse model to conduct longitudinal neurobehavioral assessments of spatial memory and anxiety. In vivo and in vitro experiments further examined the impact of Nrg-1 deletion on neurodegeneration, inflammation, demyelination, and NPC function in adult mice.

Nrg-1 ablation led to spontaneous demyelination and progressive neuroinflammation in the brain and the spinal cord. Mice exhibited significant hippocampal memory impairments and increased anxiety. In addition, NPCs from Nrg-1 cKO mice showed reduced self-renewal capacity, proliferation, and stem cell activity compared to wild-type controls.

These findings highlight the critical role of Nrg-1 in the homeostasis of NPCs and myelination in the CNS, and its dysregulation contributes to lesion development and neurodegeneration, underscoring its importance in MS pathology.

Poster #**17**

Sex Matters: Predictive Mapping of Autophagy and Mitophagy Alterations in Alzheimer's Disease via Behavioral-Molecular Integration

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Abstract

1. Motivation/problem statement: Macroautophagy and mitophagy are essential regulators of remain unclear. The gap in understanding how these processes link to cognitive decline across sexes limits the development of personalized interventions. Furthermore, predictive tools to connect behavioral outcomes with cellular pathology are lacking in current neurodegenerative models.

2. Methods/approach: We examined autophagy (LC3B-II, SQSTM1) and mitophagy markers (BNIP3L, BNIP3, BCL2L13) in the cortex and hippocampus of male and female 3xTg-AD mice via Western blotting. Transmission electron microscopy (TEM) quantified mitochondrial and mitophagosome abundance. Cognitive performance was evaluated through novel object recognition and placement tests. To bridge behavioral and molecular data, we applied supervised machine learning algorithms (regression models) to predict mitophagy and mitochondrial phenotypes based on behavioral metrics. All analyses used sex-balanced cohorts (n=6–8 per group), included proper controls, and were validated in replicated experiments.

3. Results: Females displayed autophagosome accumulation in the cortex, alongside elevated BNIP3L and BCL2L13, correlating with cognitive decline and mitochondrial disruption. Males exhibited increased BNIP3 dimers and a positive association between LC3B-II and cognitive function. TEM confirmed sex-specific mitophagy profiles. Critically, machine learning accurately predicted mitochondrial and mitophagosome abundance from behavioral performance, validating a robust link between functional and cellular endpoints.

4. Conclusion/implications: This study reveals sex-specific autophagy and mitophagy signatures driving divergent cognitive outcomes in AD. Importantly, integrating machine learning establishes a novel predictive framework, advancing personalized neurodegeneration research and opening new avenues for biomarker-based therapeutic strategies.

Poster #**18**

Uncovering the genetic relationship between dementia and age-related hearing loss

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Abstract

Age-related hearing loss (ARHL) is an important modifiable midlife risk factor for dementia. Recent studies have explored a potential genetic relationship between dementia and age-related hearing loss (ARHL). However, due to variability in phenotype definitions and analysis methods, these studies have not reached a clear consensus on the specific genetic factors underlying both conditions. This study will use genotype data and highly specific cognitive and audiological phenotypes from the Canadian Longitudinal Study on Aging to (1) measure the degree of causality between ARHL and dementia (analysis tool: Mendelian randomization), and (2) identify genetic variants, genes and pathways that may affect both ARHL and dementia (analysis tool: MTAG). Investigation of the relationship between cognitive impairment and ARHL subtypes identified a highly significant association with estimates of metabolic hearing loss (P=3x10^{-9}; OR[95%CI]=1.016[1.011,1.022]), whereas no significant association was observed with sensory hearing loss (P=0.35; OR[95%CI]=0.99[0.988,1.004]). Identifying correlated genetic variants and/or a causal link between these conditions will enhance our understanding of the biological mechanisms underlying the genetics of dementia and ARHL. Additionally, our findings may guide the development of improved dementia prediction tools through the incorporation of measures of hearing and genetic data.

Poster #**19**

Using Drosophila melanogaster as a Model Organism to Determine the Phenotypic Spectrum of Pathogenic *KAT6A* and *KAT6B* Variants

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Abstract

There are over 500 reported cases of KAT6 syndromes worldwide. Pathogenic variants in the *KAT6A* gene cause Arboleda-Tham syndrome and pathogenic variants in the *KAT6B* gene cause KAT6B-related disorders, including genitopatellar syndrome and Say-Barber-Biesecker-Young-Simpson syndrome. Individuals present with a range of features including intellectual disability, speech delay, distinct facial features and congenital anomalies. Individuals with KAT6B syndromes also present with genital and skeletal features. Most *KAT6A* and *KAT6B* (*KAT6A/B*) variants are *de novo* and present in a heterozygous state. Truncating variants likely lead to loss-of-function and haploinsufficiency. Most missense variants are classified as variants of uncertain significance and variant function is difficult to predict. Currently, interpretations of *KAT6A/B* variant function have been drawn from clinical characterization. Thus, functional studies will help identify variant impact and help understand the pathophysiology of KAT6 syndromes. We will be conducting ubiquitous and tissue-specific overexpression studies in *Drosophila melanogaster* using the GAL4/UAS system to determine a genotype-phenotype correlation in KAT6 syndromes. Our results show that overexpression of *KAT6A/B* truncating variants leads to a spectrum of functional outcomes, ranging from non-disruptive to partial and complete loss-of-function. Overexpression of *KAT6B* missense variants exhibits partial to complete loss-of-function, which contrasts with overexpression of *KAT6A* missense variants, which maintain a non-disruptive function. Notably, overexpression of *KAT6A* missense variants in the developing wing suggests that these variants may exhibit gain-of-function activity. Overall, this project aims to provide insight into KAT6 disease mechanisms and bridge the gap between basic science researchers and clinicians.

Poster #**20**

Incorporating novel audiogram classification strategies to identify genes and pathways involved in subtype components of age-related hearing loss

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Abstract

Background: Age-related hearing loss (ARHL) is heterogenous group of phenotypes affecting one-third of the population over 65. Both environmental and genetic factors play a role in ARHL. However, the specific genetic factors that underlie each phenotype remain unclear.

Objective: To uncover genetic factors underlying distinct ARHL phenotypes.

Methods: We obtained genomic and audiologic data from 26,622 healthy older individuals participating in the Canadian Longitudinal Study on Aging. By adopting a mathematical approach, we calculated metabolic and sensory estimates for each audiogram. To identify genetic variants, genes, and pathways associated with each phenotype, we performed Genome-Wide Association Studies (GWAS) and functional enrichment analyses.

Results: Our analyses revealed that, in addition to metabolic and sensory estimates increasing with age, females exhibited higher metabolic estimates and males exhibited higher sensory estimates. GWAS revealed that a missense variant in *ARHGEF28* was significantly associated with the metabolic phenotype (P=2.67x10^{-9}); while a missense variant in *KLHDC7B* was significantly associated with the sensory phenotype (P=2.37x10^{-12}). The RhoA activity regulation pathway was implicated in the metabolic phenotype, while sensory processing of sound by hair cells pathway was implicated in the sensory phenotype.

Conclusions: In this large-scale genomic study, we identified biological processes involved in distinct sensorineural hearing loss phenotypes. These findings have improved our understanding of the biological mechanisms underlying ARHL.

Poster #**21**

Identifying the Role of Glypican 6 on the Development of the Embryonic Mouse Neocortex

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Abstract

The neocortex is the site of higher-order cognitive functions such as language and abstract thinking. During embryonic development, neural progenitor cells (NPCs) within the germinal layers of the developing neocortex undergo both proliferative and differentiative divisions to give rise to the late-born neurons that constitute the mature six-layered neocortex. Of these NPCs, the basal progenitors (BP) in the subventricular zone have received much attention in neocortical developmental studies owing to their distinct proliferative and neurogenic capacities. Proper balance between BP proliferation and differentiation depends on the delicate orchestration of various intrinsic and extrinsic factors. In particular, placental growth differentiation factor 15 (GDF15) plays a role in neocortical development by governing the abundance of mitotic BPs. However, much of the mechanism regarding how placental GDF15 mediates the development of the neocortex remains to be elucidated. Preliminary findings in our lab have suggested *Glypican 6* (*Gpc6*) to be a potential downstream regulatory target of GDF15. In this study, we investigated the difference in *Gpc6* expression in the developing neocortex of WT and GDF15KO mouse embryos. We also present evidence of deviations in BP and neuron distribution in the neocortex upon ectopic expression of *Gpc6*. Collectively, our findings suggest that GDF15 regulate NPC proliferation via *Gpc6* in the developing mouse neocortex.

Poster #**22**

Establishing iPSCs from primary fibroblast cell cultures from Ursus maritimus to model neural development in vitro

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Abstract

Polar bears (PB), known for their high intelligence and large brain-to-body ratios, represent a captivating subject in evolutionary biology research. Furthermore, PBs are particularly susceptible to the effects of climate change and environmental pollution in their rapidly warming sea ice habitats. This research project focuses on bridging the understanding of neural development in PBs to the broader effects of environmental toxins on these processes.

To investigate these effects, we have generated primary fibroblast cell cultures from PBs. We confirmed their genetic and structural integrity via DNA barcoding and karyotypic analysis, and they have been sub-cultured for over 20 passages.

Building on these fibroblast cultures, we aim to develop a PB-specific induced pluripotent stem cell (iPSC) model. iPSCs can differentiate into all three germ layers, allowing us to recapitulate critical developmental steps and study cellular and molecular changes. Additionally, these iPSCs enable the replication of complex organ development including forming cerebral organoids, which will serve as a platform to study how environmental pollutants impact PB brain development and health. These insights will support conservation strategies by identifying molecular vulnerabilities to environmental stressors. We have made several attempts to generate PB-iPSCs, with promising results: colonies formed and were confirmed as pluripotent through TRA-1-60 and alkaline phosphatase live-cell staining.

This study represents important impacts on Arctic conservation and evolutionary biology. By following a One Health approach, we aim to identify and prevent health risks across humans, animals, and environments, creating a paradigm shift in how we study and preserve endangered species.

Poster # **23**

Unlocking the genetic link: Mindfulness meditation in mitigating stress and psychiatric disorders

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Abstract

Background: Mindfulness meditation reduces stress by modulating cortisol levels. Genetic variation in the hypothalamic-pituitary-adrenal (HPA) axis influences individual stress responses. However, limited research has examined the interaction between mindfulness practices and genetic factors for stress and psychiatric disorders. This study aimed to determine whether mindfulness meditation mitigates stress responses and how it correlates with genetic risks for psychiatric conditions.

Methods: We conducted a scoping review on the effects of meditation on stress responses using COVIDENCE. Secondly, we performed a genetic correlation analysis using six genome-wide association studies (GWAS) to examine shared genetic architecture between stress-related traits or diseases with psychiatric disorders.

Results: Our scoping review identified 169 studies; 19 met the inclusion criteria, and suggested that meditation lowers corticotropin-releasing hormone and cortisol levels, enhancing emotional resilience, and modulating genetic pathways via FKBP5 and *BDNF*. Genetic correlation analysis revealed notably significant ($P<0.05$) correlations between post-traumatic stress disorder (PTSD) with major depressive disorder (MDD, Rg = 0.87), schizophrenia (Rg = 0.40), and bipolar disorder (Rg = 0.34). Similarly, stress disorders and MDD (Rg = 0.77), schizophrenia (Rg = 0.32), and bipolar disorder (Rg = 0.29).

Discussion: The scoping review found mindfulness meditation improves stress regulation, while genetic correlation analysis revealed stress-related traits are positively associated with psychiatric disorders. These findings suggest long-term mindfulness meditation practices may help reduce genetic risk factors for stress and associated psychiatric disorders, offering potential for new mental health treatments and preventive strategies. To expand these findings, clinical studies involving diverse populations are warranted.

Poster # **24**

Single Nuclei RNA-Sequencing Reveals Genetic and Cellular Insights into Cisplatin-Induced Sensorineural Hearing Loss

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Abstract

Background: Cisplatin chemotherapy is limited by off-target toxicities, such as ototoxicity (sensorineural hearing loss). Genetics play an important role in cisplatin-induced ototoxicity (CIO). We hypothesize that genetic variants modulate cisplatin-induced gene expression changes within the cochlea, leading to CIO. To explore this, we profiled single-cell gene expression in the cochlea.

Methods:Postnatal-day-6 CBA/CaJ mice received intraperitoneal injections of 3mg/kg cisplatin or saline (*n*=6/group). Cochlear ducts were dissected four-hours post-cisplatin administration. Single nuclei were isolated using the Chromium Nuclei Isolation Kit, followed by single-nuclei RNA-sequencing (snRNA-seq) using the Single Cell Gene Expression + RNA Profiling Kit. Data was processed using CellRanger and analyzed with Seurat, DESeq2 and MILO-R to detect changes in gene expression and cell-type proportions.

Results: We sequenced 15,510 (control) and 12,194 (cisplatin-treated) nuclei. Cisplatin reduced the abundance of spindle cells, outer hair cells (OHCs), type I neurons (T1Ns), supporting cells (SCs), immune cells and bone cells, and increased certain macrophage clusters (MILO-R:LogFC<=-3,SpatialFDR<0.1). Among these, 159 differentially expressed genes were identified (DESeq2:*P*adj<0.05).

Conclusions: Our pilot study identified key genes, pathways and cell types associated with CIO. The stria vascularis, organ of Corti and spiral ganglion have been implicated in CIO, with spindle cells, OHCs, and T1Ns residing in these structures. Reduced SCs and increased macrophages underscore the need to further investigate their roles. Pinpointing cochlear cells significantly associated with CIO provides novel insights into its mechanisms. A follow-up study will examine the 1-hour post-cisplatin timepoint to investigate gene expression changes preceding cell death, guiding future snRNA-seq and snATAC-seq experiments.

Poster #25

Targeting TRPM2 as a novel therapeutic intervention for Alzheimer's Disease

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Abstract

Introduction: Cognitive decline in Alzheimer's Disease (AD) is caused by accumulation of Aβ oligomers (AβOs), microglia-mediated neuroinflammation and consequent synapse failure. TRPM2, a Ca^{2+}-permeable channel, drives AβO-induced synaptotoxic cascades, including those contributed by microglia. We previously demonstrated that TRPM2 knockout in an AD mouse model is associated with attenuated cognitive decline. Here, we assessed whether JNJ-28583113 (JNJ), a potent and selective TRPM2 blocker, can prevent AβO-induced synaptotoxicity and neuroinflammation.

Methods: We tested the effects of JNJ to block: 1) TRPM2 currents in TRPM2-expressing HEK293 cells, 2) AβO-induced synaptotoxicity in hippocampal slices, 3) AβO-stimulated GSK3β activity (pGSK3βSer9/total) in hippocampal slices, 4) AβO-stimulated nitric oxide (NO) production from cultured microglia. We also examined the differential vulnerability of apical and basal synapses to AβOs.

Results: JNJ completely inhibits TRPM2 currents in a Ca^{2+}-dependent manner. When applied to hippocampal slices, JNJ prevents the synaptotoxic effects of AβOs on LTP, while having no effect on basal synaptic transmission. Interestingly, although apical synapses in the stratum radiatum are vulnerable to the AβOs, basal synapses in the stratum oriens are resistant to their disruptive effects. In cultured microglia, JNJ prevents AβO-induced NO release, providing a candidate mechanism underlying the synaptoprotective properties of JNJ. Our pilot assays showed that alterations in pGSK3βSer9 are detectable by WB and can be used to evaluate JNJ effects on AβO-stimulated GSK3β activity.

Conclusion: TRPM2 channels are important players in AβO-synaptotoxicity and neuroinflammation. Moreover, JNJ, a TRPM2 inhibitor, attenuates microglial responses and synaptic failure. Thus, targeting TRPM2 may be beneficial in treating AD.

Poster #**26**

Antimuscarinic drugs exert β-arrestin-biased agonism at the muscarinic acetylcholine type 1 receptor

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Abstract

Pirenzepine (PZ) and muscarinic toxin 7 (MT7) are muscarinic acetylcholine type 1 receptor (M_1R) antagonists, and act via M_1R to promote neuritogenesis in rodent primary dorsal root ganglia (DRG) sensory neurons, in part, through β-arrestin-dependent activation of extracellular signal-regulated protein kinase 1/2 (ERK1/2). To understand the therapeutic effects and mechanism of M_1R antagonist, we tested the hypothesis that PZ and MT7 possess β-arrestin-biased agonism at M_1R to drive activation of ERK and enhance neurite outgrowth. PZ and MT7 dose-dependently recruited β-arrestin2 to M_1R and increased ERK phosphorylation in both HEK293 cells and DRG neurons. DRG neurons of different sub-types express M_1R, and ERK activation by MT7 was only observed in M_1R-positive neurons. These pharmacological effects occurred in the absence of activation of G protein signaling or receptor internalization. PZ phosphorylated M_1R at specific serine/threonine residues (T230, S251, T254, S321, T354, S356) and deletion mutation of these sites suppressed PZ and MT7 induction of β-arrestin pathway. With regard to PZ signaling, mutation at S251 and T254 was sufficient to impede β-arrestin binding and ERK activation. β-arrestin-biased activity of PZ and MT7 involved the mobilization of casein kinase 2 (CK2) and this occurred in the absence of Gαq or G protein receptor kinase (GRK) activity. Pharmacological or siRNA-based inhibition of CK2 blocked PZ-induction of β-arrestin association, ERK activation and neurite outgrowth in DRG neurons. In conclusion, PZ/MT7 activated M_1R toward the β-arrestin signaling pathway in HEK293 cells and DRG neurons to augment ERK activation and neurite outgrowth via engagement of CK2.

Poster # **27**

Development of peripheral-tissue biomarkers to predict disease trajectory of patients with human Transmissible Spongiform Encephalopathies (TSEs)

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Abstract

Transmissible Spongiform Encephalopathies (TSEs) are a group of fatal, rapidly progressive neurodegenerative disorders caused by the misfolding of prion proteins into an infectious form (PrPSc). Human TSEs are classified into three categories: sporadic, genetic, and acquired. In Canada, the most common form is sporadic Creutzfeldt-Jakob disease (sCJD), which can only be definitively diagnosed post-mortem through brain biopsy. Currently, cerebrospinal fluid (CSF) obtained via lumbar puncture is the primary pre-mortem diagnostic method. Although invasive, this procedure enables real-time quaking-induced conversion (RT-QuIC) assays, which offer high sensitivity for prion detection. This study aims to evaluate less invasive pre-mortem peripheral tissue samples—such as tears, saliva, and nasopharyngeal mucosa—as potential biomarkers for TSEs using RT-QuIC. The primary objective is to assess the diagnostic utility of these sample types while maintaining sensitivity and specificity comparable to CSF-based assays. Participant recruitment is ongoing and includes symptomatic individuals with probable sCJD, asymptomatic carriers of genetic TSE mutations, and family members of diagnosed patients, who will serve as non-CJD controls. Although no effective treatments currently exist for CJD, the ability to monitor disease progression through less invasive methods may support the development of future therapeutic interventions and the potential for earlier diagnoses.

Trainee Podium Presentation

A novel ultrasensitive quaking-induced conversion assay for all forms of sporadic and inherited prion diseases

Jennifer Myskiw[1], Rebecca Fox[1,2], Dominic Kielich[1], Lise Lamoureux[1], Olivia Ferriera[1,2], Jessy Slota[1], Ben Bailey-Elkin[1], Stephanie Booth[1]

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Abstract

Human prion diseases are a group of fatal, progressive neurodegenerative disorders characterized by the accumulation of misfolded prion proteins (PrPSc) in the brain. These diseases can arise sporadically (about 85% of cases), be inherited (10-15%), or be acquired through contaminated food or medical procedures (less than 1%). Their broad clinical phenotypes often complicate diagnosis, as symptoms can overlap with those of other neurodegenerative conditions. The introduction of the quaking-induced conversion (QuIC) assay has revolutionized prion disease diagnostics by enabling the detection of PrPSc in cerebrospinal fluid (CSF). This assay uses patient CSF to seed the formation of PrPSc amyloids in a reaction containing recombinant correctly folded prion protein (rPrP), typically derived from the hamster prion protein. Diagnostic laboratories worldwide report sensitivities of 87-96% and specificities of 98-100% for sporadic cases. However, QuIC often fails to detect certain inherited and rare sporadic prion disease variants. In this study, we present a modified QuIC assay that incorporates an alternative shaking method and a novel recombinant substrate derived from the prion protein of the North American deer mouse (*Peromyscus maniculatus*). This adaptation achieves 100% sensitivity across all tested sporadic, inherited, and rare variant cases, including those previously refractory to standard testing, representing a significant advancement in prion disease diagnostics. We also demonstrate that while truncated hamster rPrP, commonly used in diagnostic settings, exhibits high sensitivity for sporadic cases, it shows limited sensitivity for detecting certain rare prion seeds, such as those associated with fatal familial insomnia (FFI).

Trainee Podium Presentation

Md Imamul Islam [a], Shakila Sultana [a], Nirmala Padmanabhan [a 1], Mahamud-Ur Rashid [b], Tabrez J. Siddiqui [a 1], Kevin M. Coombs [b], Peter F. Vitiello [c], Soheila Karimi-Abdolrezaee [a], Eftekhar Eftekharpour

Abstract

Neuronal cell death remains the principal pathophysiologic hallmark of neurodegenerative diseases and the main challenge for treatment strategies. Thioredoxin1 (Trx1) is a major cytoplasmic thiol oxidoreductase protein involved in redox signaling, hence a crucial player in maintaining neuronal health. Trx1 levels are notably reduced in neurodegenerative diseases including Alzheimer's and Parkinson's diseases, however, the impact of this decrease on neuronal physiology remains largely unexplored. This is mainly due to the nature of Trx1 redox regulatory role which is afforded by a rapid electron transfer to its oxidized protein substrates. During this reaction, Trx1 forms a transient bond with the oxidized disulfide bond in the substrate. This is a highly fast reaction which makes the identification of Trx1 substrates a technically challenging task. In this project, we utilized a transgenic mouse model expressing a Flag-tagged mutant form of Trx1 that can form stable disulfide bonds with its substrates, hence allowing identification of the Trx1 target proteins. Autophagy is a vital housekeeping process in neurons that is critical for degradation of damaged proteins under oxidative stress conditions and is interrupted in neurodegenerative diseases. Given Trx1's suggested involvement in autophagy, we aimed to identify potential Trx1 substrates following pharmacologic induction of autophagy in primary cortical neurons. Treatment with rapamycin, an autophagy inducer, significantly reduced neurite outgrowth and caused cytoskeletal alterations. Using immunoprecipitation and mass spectrometry, we have identified 77 Trx1 target proteins associated with a wide range of cellular functions including cytoskeletal organization and neurodegenerative diseases. Focusing on neuronal cytoskeleton organization, we identified a novel interaction between Trx1 and RhoB which was confirmed in genetic models of Trx1 downregulation in primary neuronal cultures and HT22 mouse immortalized hippocampal neurons. The applicability of these findings was also tested against the publicly available proteomic data from Alzheimer's patients. Our study uncovers a novel role for Trx1 in regulating neuronal cytoskeleton organization and provides a mechanistic explanation for its multifaceted role in the physiology and pathology of the nervous system, offering new insights into the molecular mechanisms underlying neurodegeneration.

Poster # **29**

AI-mediated design of small protein binders that target infectious prion fibrils

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Abstract

Prion diseases are fatal, infectious, and incurable neurological disorders caused by the accumulation of misfolded prion proteins in the brain. Molecules that specifically recognize infectious prion amyloid fibrils are sought after for research, diagnostic, and therapeutic applications. Here, we designed small protein binders against prion fibril structures using RFdiffusion, an open-source deep-learning framework for protein designed based on denoising diffusion probabilistic models (DDPM'S). RFdiffusion was used to design between 1500-4000 proteins against a panel of 10 truncated prion fibril target structures, identifying over 160 proteins that bind and cap prion fibrils in silico. Of these, we selected 30 promising prion-binding proteins for in vitro characterization, which were expressed and purified from *E. coli* using a C-terminal TwinStrep tag. Dot blot assays were used to assess binding to an array of 8 monomeric or fibrillar PrP targets x 15 concentrations, which were used to computing EC50's between each binder protein and PrP target. Each prion binder showed higher affinity towards fibrillar PrP than monomeric PrP. We also characterized the ability of binder proteins to interfere with prion seeding activity in quaking-induced conversion (QuIC) assays, identifying several binders that showed promising inhibition of infectious prion seeds. In summary, RFdiffusion was used to design several proteins that show promising binding affinities towards prion fibrils. We will further characterize the abilities of these prion-binding proteins to inhibit prion infection in brain slice cultures and mouse bioassays, which could eventually lead to therapeutic applications against prion disease.

Poster #**30**

An overview of Creutzfeldt-Jakob disease Testing at the National Microbiology Laboratory, 2016-2024

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Abstract

Prion disease is caused by misfolding of the host Prion protein (PrP) into an infectious, disease-causing conformation which accumulates in the brain of affected individuals, leading to rapid neurodegeneration, and invariably death. The most common human prion disease, sporadic Creutzfeldt - Jakob disease (sCJD), develops spontaneously at a rate of roughly 2 per 1,000,000 population per year. The Prion Disease Section operates out of the National Microbiology Laboratory, and provides Canada-wide diagnostic testing services to clinicians confronted with suspected cases of CJD. Importantly, PDS supports CJD diagnosis using the end-point quaking induced conversion (EP-QuIC) test, which operates with a specificity/sensitivity approaching 100%, and directly detects prion seeding activity in patient CSF. Positive CSF tests can be corroborated through post-mortem analysis of autopsied tissue, which directly detects the accumulation specific prion glycoforms in the brain. This information, when interpreted alongside genetic test results describing the patient's genetic status at codon 129 of the prion protein, helps identify the specific prion strain present in individual cases.

Here we present diagnostic data retrieved from cases between 2016-2024 and evaluate CSF test performance over this time-period among a cohort of autopsy confirmed cases and across CJD subtypes. We determine that for CJD diagnosis, EP-QuIC outperforms surrogate biomarker tests. We also observe that CJD incidence does not vary significantly across Canadian Provinces, despite concentrated testing in certain regions. Together, the analysis of clinical specimens collected pre-and post mortem capture the landscape of human prion diseases circulating in the Canadian population.

Poster #**31**

Regulation of neural progenitor cell proliferation by Dachshund family transcription factor 1 in the developing mouse neocortex

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Abstract

The neocortex is responsible for higher cognitive functions in mammals and consists of neurons generated from neural progenitor cells (NPCs) during development. The abundance and proliferative capacity of NPCs, which can be regulated by external signalling molecules, thus determines the number of cortical neurons that can be generated. Preliminary findings in our lab determined that the placental hormone growth differentiation factor 15 (GDF15) promotes NPC proliferation, and identified Dachshund family transcription factor 1 (*Dach1*) as a downstream effector of GDF15. This project aims to determine the role of *Dach1* in NPC behaviour in the developing mouse neocortex. We performed *in utero* electroporation to inject *Dach1* overexpression vector into the brain ventricles of wildtype and GDF15 knockout C57BL/6N mice at embryonic day (E) 13.5. The brains were dissected at E15.5 and E17.5 and examined using immunofluorescence and confocal microscopy. The abundance and proliferative capacity of NPCs and the abundance of cortical neurons in control and *Dach1*-overexpressed mouse neocortices were quantified to determine how *Dach1* affects NPC proliferation, and ultimately neocortex development. The findings from this study will contribute to an enhanced understanding of the regulatory effects that placental factors and their downstream targets have on cells of the developing brain. The knowledge obtained from this project can help reveal how proper brain structure and function is established and potentially be used to advance therapeutic approaches for neurodevelopmental disorders.

Poster #**32**

Understanding *SYNGAP1* Using *Drosophila melanogaster*

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Abstract

De novo SYNGAP1 variants cause neurodevelopmental conditions in children , including Intellectual Disability (ID), Developmental Delay (DD), Autism Spectrum Disorder (ASD), epilepsy, and behavioural difficulties. These phenotypes align with SYNGAP1's role in postsynaptic signaling. However, the impact of patient-specific variants remains unclear, and a *Drosophila* model has yet to be established. To address this, we generated transgenic flies expressing human *SYNGAP1* and 16 disease variants, including both truncating and missense changes. By crossing them with tissue-specific GAL4 drivers, we will overexpress *SYNGAP1* to analyze abnormal phenotypes and compare variant function. In parallel, we will study *SYNGAP1* using its *Drosophila* ortholog, raskol, in neurons and glia. We knocked down *raskol* using two independent RNAi lines and employed pan-neuronal (nSyb-GAL4) and pan-glial (Repo-GAL4) drivers to assess lethality, lifespan, climbing, and seizure behaviour. Finally, using a germline mutant, *raskol*^{TG4}, we will humanize with our UAS-SYNGAP1 transgenes to rescue phenotypes (still characterizing) and assess variant function. Our preliminary data show that two *raskol* RNAi lines cause minimal phenotypes but may induce heat-induced seizures. Expressing human *SYNGAP1* and patient variants in flies will help understand genotype-phenotype correlations and condition heterogeneity. If a model of *raskol* loss can be generated in flies, future studies can screen drugs that may translate to the clinic.

Poster #**33**

Cell-Type-Specific Analysis of the Genetic and Environmental Contributions to Autism Spectrum Disorder

Anha Afrin Shefa, Robert Beattie

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Abstract

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder which is heterogenous in nature. In context of Manitoba, Canada, almost 1% children are affected with ASD. ASD is associated with a number of developmental and behavioral abnormalities, including but not limited to-brain overgrowth, social communication impairment. So, improving the quality of ASD children became global priority. We hypothesize that 16p11.2 gene dosing and environmental factors can contribute to overcome 16p11.2 deletion's effect. This study measures the dosage-dependent requirements of 16p11.2 by applying Mosaic Analysis with Double Markers (MADM). MADM is a lineage tracing approach that allows cells with two different genotypes (wildtype/knockout) to be generated within the same tissue and for cells of each genotype to be identified with a different fluorescent marker. To test the hypothesis, MADM-based mouse models were combined with tissue-specific Cre drivers, Nestin (central nervous system), Nkx (interneurons in telencephalon and hypothalamus cells) and Emx1 (excitatory neurons of the neocortex), targeting the deletion of 16p11.2 in neural stem cell progenitors. 16p11.2-MADM mosaic mice were successfully generated, and analysis is ongoing. Brains were dissected on postnatal day 1 and 21 followed by immunohistochemistry to identify cortical structures and cell types. To identify populations of cells requiring 16p11.2, we will compare the ratio of green to red cells in defined brain regions. MADM technology permits the identification of key molecular changes in each brain cell type as ASD progresses. This understanding is vital for validating future therapies before clinical trials.

Poster #**34**

Immunohistochemical Analysis of Astrocyte-specific P2Y1 Receptor Knockout in Mice

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Abstract

Introduction

Alzheimer's disease (AD) is a leading cause of dementia and is an increasingly large burden on aging populations across the world. The hallmark features of the disease include neurofibrillary tangles and amyloid plaques. The underlying molecular mechanisms that could lead us to possible therapeutic targets are still not completely understood. PY2 purinoreceptor 1 (P2Y1) is a type of purinergic receptor linked to Alzheimer's pathogenesis. While P2Y1 has been studied in context to neurons and AD, astrocytes also possess these receptors. Genetic knockout of P2Y1 receptors from astrocytes can help us shed light on the relevance of this pathway in AD.

Methodology

Using CreERT2, the astrocytic P2Y1 gene was knocked out in 5 month-old mice and immunohistochemistry was preformed to detect a difference in P2Y1 fluorescence. Astrocytes were imaged using confocal microscopy and the images were analyzed precisely to avoid noise from neuronal P2Y1.

Results and Conclusions

It was observed that the effectiveness of the CreERT2 system can be immunohistochemically verified in the tissue of healthy mice by avoiding neurons. Precise mapping is a valuable method to analyze subtle differences in immunohistochemistry, especially when a protein of interest on nearby cells cannot be removed. Furthermore, characterization of P2Y1 knockout is the first step in understanding the role of this receptor in Alzheimer's Disease Pathology.

Poster #**35**

Laminar-Specific Roles of LRRTM Proteins in Hippocampal Synapse Organization

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Abstract

Neuronal communication relies on chemical synapses, whose precision is regulated by specific synapse organizers. Leucine-rich-repeat transmembrane neuronal proteins (LRRTMs) play a central role in the formation, maturation, and plasticity of excitatory synapses. In the hippocampal CA1 region, our investigations demonstrate that LRRTM1 and LRRTM2 exhibit distinct laminar expression: LRRTM1 is primarily localized in the stratum radiatum, while LRRTM2 is enriched in the stratum lacunosum moleculare. This differential distribution appears to underlie their selective roles in synapse development and input-specific circuit organization.

Building on our previous findings, we explored the functional consequences of selectively deleting LRRTM2 in the dorsal CA1. Mice lacking LRRTM2 in this region displayed anxiety-related behaviors in a gender-specific pattern, implicating LRRTM2 in the modulation of behavioral outcomes and suggesting its involvement in the pathophysiology of neuropsychiatric disorders.

Our ongoing research focuses on elucidating the molecular pathways by which LRRTM isoforms influence synaptic architecture and hippocampal plasticity.

Poster #**36**

Optimizing Diffusion Weighting Parameters for Axon Diameter Estimation in the Mouse Corpus Callosum Using Oscillating Gradient Spin Echo

Madison Chisholm[1], Bibek Dhakal[2], Sheryl Herrera[1], Morgan Mercredi[1], Melanie Martin[1]

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Abstract

Oscillating Gradient Spin Echo (OGSE) temporal diffusion spectroscopy (TDS) is a promising technique for non-invasively estimating axon diameter (AxD) in white matter tracts. This study compares two OGSE-TDS acquisition strategies: one with a higher signal-to noise ratio with reduced diffusion weighting (DW) and another using larger DW at the cost of lower SNR to assess how these strategies affect AxD measurements for a given imaging time.

A 1 mm image slice perpendicular to the genu substructure was imaged at 15.2T in two *ex vivo* 12-week-old male CDI mice in separate experiments .In experiment one, 9 diffusion-weighted images with OGSE were acquired per frequency (50 - 450 Hz) using apodised cosine waveforms (TE = 50 ms, TR = 800 ms, b-values up to 298 s/mm^2). In experiment two, 9 DW images were acquired per frequency (50 – 450 Hz) using apodised cosine waveforms (TE = 50 ms, TR = 1250 ms, b-values up to 1500 s/mm^2). AxD was inferred by fitting the image signals to a two-compartment ActiveAx model.

Experiment 1 yielded smaller AxD estimates with smaller uncertainty (1.9 ± 0.2 μm), compared to experiment 2 which produced larger AxD estimates with larger uncertainty (4 ± 2 μm).

Based on the uncertainties, these experiments demonstrate that for a given imaging time, it is optimal to prioritize higher SNR with reduced DW to achieve better precision in of axon diameter inferences using OGSE-TDS.

Acknowledgements: Funding from NSERC, and Vanderbilt Institute of Imaging Science for use of their 15 T magnet in the experiments.

Poster #**37**

Exploring the Neuroprotective Effects of 17β-Estradiol in Alzheimer's Disease: Targeting Mitochondrial Dysfunction and Neuroinflammatory Pathways

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Abstract

Alzheimer's disease (AD), the most common form of dementia, is an age-related neurodegenerative disorder characterized by progressive cognitive decline, neuroinflammation, and mitochondrial dysfunction. A key pathological hallmark of AD is amyloid beta (Aβ), which activates proinflammatory NF-κB signaling and impairs mitochondrial function, thereby contributing to neuronal damage and neurotoxicity. The decline in estrogen levels during aging and menopause correlates with an increased risk of AD. Among estrogens, 17β-estradiol (E2) is the most potent form and exhibits neuroprotective effects by modulating mitochondrial function and suppressing inflammation. This study investigated whether E2 can counteract Aβ-induced neuroinflammation and mitochondrial dysfunction in primary neurons. Using primary cortical neurons isolated from C57BL/6 mouse embryos, we induced AD-like pathology by Aβ exposure, followed by E2 treatment. Western blotting was used to assess key proteins involved in inflammation and mitochondrial regulation. Mitochondrial oxygen consumption rate (OCR) in live cells was measured via Seahorse XF24 analyzer, and cytotoxicity and inflammatory responses were evaluated using MTT, LDH, and ELISA based assays. Aβ exposure decreased AMPK phosphorylation (pAMPK) and PGC-1α expression, impaired mitochondrial respiration, and activated NF-κB signaling, resulting in elevated inflammatory cytokines and neurotoxicity. E2 pretreatment restored pAMPK and PGC-1α levels, preserved ATP production, reduced proinflammatory NF-κB activity, and decreased inflammatory cytokine release. Cell viability assays confirmed E2's neuroprotective effects against Aβ toxicity. These findings demonstrate that E2 effectively counteracts Aβ-induced mitochondrial and inflammatory damage, highlighting its therapeutic potential in AD.

Poster #**38**

Milk Extracellular Vesicles Attenuate Intrinsic Apoptosis in human microglia

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Abstract

Early life stress leads to chronic proinflammation and increases rates of apoptosis in neonates, especially in the brain. As the central nervous system's resident immune cells, microglia play a crucial role in regulating immune responses, are sensitive to early-life stress, and may become polarized. Milk extracellular vesicles (MEVs) are a group of biological nanovesicles with immunomodulatory and anti-inflammatory properties. Our study aims to investigate if MEVs have the potential to mitigate neuroinflammation-induced apoptosis in homeostatic and polarized human microglia. IFN-gamma cytokine at a dosage of 10 ng/mL was used to polarize the homeostatic microglia. Using immortalized human microglia clone 3 (HMC3) cells, four treatment groups were tested across three time points: 6 hours, 12 hours, and 24 hours (n=5/treatment/timepoint). Group 1: Homeostatic, control microglia, homeostatic microglia that received 200 µg of MEVs, microglia that received IFN-γ, and microglia that received IFN-γ and MEVs. The transcript and protein abundance of candidate pro- and anti-apoptotic markers were measured to assess the impact of MEV treatment on microglial responses under homeostatic and polarized states. Our findings indicate that MEV treatment upregulates the expression of protective, anti-apoptotic targets, while downregulating pro-apoptotic targets in IFN-γ polarized microglia. This suggests that MEVs may induce pro-survival benefits to human microglia during polarization events. Our research highlights the therapeutic potential of MEVs as a novel approach that can counteract neuroinflammation-induced apoptosis in neonatal microglia, providing insight into potential interventions for addressing neurological disorders arising from early-life stress.

Poster #**39**

Determining the functional outcomes of PAK1 and PAK3 variants using Drosophila melanogaster

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Abstract

PAK1 and PAK3 are serine/threonine kinases essential for cytoskeletal dynamics, cell proliferation, and transcription regulation. Missense variants in these genes are implicated in neurodevelopmental disorders with overlapping phenotypes, including intellectual disability and speech delay. However, the genotype-phenotype relationship remains unclear due to limited sample sizes and in vitro-only studies. This project investigates the in vivo effects of PAK1 and PAK3 variants using Drosophila melanogaster as a model organism. Leveraging the UAS-GAL4 system, both ubiquitous and tissue-specific overexpression will assess variant impact on fly viability and tissue development. Preliminary data indicate that two missense variants in PAK1 act as gain-of-function variants, in line with the literature in vitro. This functional characterization aims to clarify variant pathogenicity, improve variant classification, and support genetic counselling for affected individuals, providing a foundation for targeted therapeutic development by modelling disease phenotypes and enabling future drug screening in Drosophila.

Poster #**40**

Milk-derived extracellular vesicles regulate microglial immunity

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Abstract

Microglia, the resident immune cells of the central nervous system (CNS) are potent immune effector cells. They initiate inflammatory processes, cytoprotective responses, synaptic pruning, circuit modulation, and contribute to neurodevelopment. Human milk also contributes to neurodevelopment and neuroimmunity and contains biologically active materials like milk-derived extracellular vesicles (MEVs). MEVs are nanovesicles that carry biological cargo from mother to infant. MEVs have been shown to exert anti-inflammatory outcomes after an immune stress/stimuli. However, the cellular dynamics of how MEVs may modulate microglial responses to an immune stress, if abundantly available prior to stress, remains to be explored. In this study, human microglia clone 3 (HMC3) cells were treated with 200 µg of MEVs prior to polarization with 10 ng/ml of IFN-γ cytokine. Candidate targets of chemokine secretion (CCL2, CCL5, CXCL10), cytokine signaling (IL-6, IL-1β, SOCS3, SAA), endosomal processes (CD68, IBA1, BIN1), and cytoprotective responses (RGS10, ARG1, CD200R1, TGF-β) were measured at the transcript and protein level. CD68, BIN1, and SAA transcript and IL-1β protein increased with MEV treatment. CD200R1 protein increased in polarized microglia and reduced to baseline levels in cells that received MEVs prior to polarization. Our findings indicate that MEV pre-treatment may enhance microglia's ability to initiate an acute immune response upon facing a stimulus, while minimizing microglia potentiation.

Poster #**41**

Asymmetric modulation of brain connectivity by anodal transcranial direct current stimulation in healthy individuals: A single-blind, randomized sham-controlled trial

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Abstract

Transcranial direct current stimulation (tDCS) applied to the dorsolateral prefrontal cortex (DLPFC) has shown asymmetric behavioral effects, though the underlying neurophysiological mechanisms remain unclear. In this preliminary study with 34 healthy individuals, tDCS was applied to either the left or right DLPFC or a sham group. Behavioral and neurophysiological changes were examined by the Stroop test and resting-state fMRI, respectively, which were measured before and after a 15-minute tDCS session. Seed-to-voxel connectivity analysis with seeds placed under the tDCS target regions (F3 and F4) showed no significant changes, but voxel-to-voxel whole brain intrinsic connectivity (IC) analysis revealed significant 3×2 interaction effects (stimulation site × time) in the right DLPFC (18mm off from the F4). Post-hoc analysis showed that only the right DLPFC stimulation led to an increase in IC from pre- to post-stimulation. Consistent with this finding, right DLPFC stimulation improved Stroop task performance measured by increased interference score, which represents better inhibition of irrelevant information. These findings provide further insights into the hemispheric difference of tDCS effects and its underlying neurophysiological mechanisms. However the small sample size limits the generalizability of the results and necessitates further research with a larger cohort for confirmation.

Poster #**42**

Development of the Precision Genomics Suite for Investigating Biological Pathways at the Single-Cell Level

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Abstract

Introduction: Advances in single-cell RNA sequencing (scRNA-seq) have opened new avenues to dissect biological pathways at a resolution not previously possible. However, the high cost of specialized instruments for single-cell experiments can be a barrier. Therefore, we established the Precision Genomics Suite, to allow for development of an integrated workflow for single-cell multi-omics (Chromium iX) and spatial transcriptomics (Visium/Xenium). Cisplatin treatment of pediatric cancer patients can cause sensorineural hearing loss. Here, we generated scRNA-seq data to examine the genetic basis of this adverse drug reaction, termed cisplatin-induced ototoxicity (CIO).

Methods: 6-day-old CBA/CaJ mice were treated with cisplatin (*n*=6, 3 mg/kg) or saline (*n*=6), administered intraperitoneally. Four-hours post treatment, whole cochlear ducts were dissected, nuclei were isolated, and libraries were prepared using the Chromium iX. Sequencing was performed (NovaSeq X Plus), and data was processed locally using CellRanger, Seurat and MiloR.

Results: After scRNA-seq libraries were successfully generated, 15,510 control nuclei and 12,194 treated nuclei were sequenced. Analysis revealed that treatment with cisplatin led to a reduction in proportion of specialized auditory cells, supporting cells, and bone cells in the inner ear and an increase in proportion of macrophages (MILO-R: LogFC<=-3, SpatialFDR<0.1).

Conclusion: Single-cell sequencing has identified novel insights into the biology of CIO. These cutting-edge technologies can be applied to an abundance of biological questions. Single-cell technologies show promise to produce data with high throughput and sensitivity. The Precision Genomics Suite offers opportunity for labs with an interest in single-cell assays to generate these state-of-the-art data.

Poster #**43**

Mild COVID-19 Infection and Increased Dementia Risk: A Longitudinal Analysis

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Abstract

Recent studies demonstrated brain alterations in those infected with COVID-19, even in mild disease. We examined whether mild COVID-19 infection accelerated aging-related brain changes.

Six males and 19 females (*n*=25) with pre-COVID MRI scans returned for follow-up assessment. We estimated dementia-related neuroimaging scores using our in-house, machine learning-based AD Designation (MAD), brain age gap scores (brain-PAD), whole-brain changes in grey matter volume (GMV), and intrinsic connectivity (IC) based on GMV regions of interest.

MAD score changes were insignificant pre- vs. post-COVID-19 in the entire group. However, males exhibited significantly greater increases in MAD scores compared to females ($t(23) = -2.437$, $p = 0.024$). Post-COVID-19 brain-PAD was reduced in the whole group ($z = -3.188$, $p = 0.001$) and in females ($z = -3.018$, $p = 0.003$). GMV increased along the cingulate gyrus in females ($p < 0.01$). Significant IC differences were found between sexes, $t(23) = 3.154$, $p = 0.004$, with males showing decreased IC in the cingulate gyrus ($p = 0.001$). Behavioural data changes were largely insignificant, with the Montreal Cognitive Assessment showing improvement post-COVID-19.

Our study is one of the first to highlight sex differences while utilizing baseline data and machine learning to determine the impact of mild COVID-19 on age-related brain changes. Our findings highlight a penitential pattern of favourable brain changes in females. Utilizing machine learning to determine early dementia-like patterns may be advantageous in developing targeted interventions to slow down cognitive decline in identified at-risk subpopulations due to COVID-19 and future pandemics

Poster #**44**

Deciphering Ensheathing Pericyte Dysfunction and Cerebral Blood Flow Impairments in Alzheimer's Disease 5xFAD Mice

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Abstract

Alzheimer's Disease (AD) is characterized by cognitive decline and reduced cerebral blood flow (CBF), contributing to neuronal damage and disease progression. Pericytes, critical for regulating CBF and maintaining capillary integrity, remain poorly understood in AD, particularly regarding a pericyte subtype, ensheathing pericytes, located in the arteriole-capillary transition zone.

To address this knowledge gap, we crossed the 5xFAD mouse model of AD with mice that express the genetically encoded Ca^{2+} indicator RCaMP1.07 in cells with α-smooth muscle actin, which include ensheathing pericytes. A chronic cranial window over the whisker barrel cortex allowed long-term observation by two-photon microscopy of pericyte Ca^{2+} activity. Additionally, fluorescent dextran was injected intravenously before each imaging session to track hemodynamic changes during AD progression.

Preliminary findings reveal altered Ca^{2+} signaling in ensheathing pericytes, correlating with changes in blood vessel diameter and vasomotion. These effects are apparent early in disease and have important implications for blood delivery into downstream capillaries.

This study provides the first analysis of ensheathing pericytes in a model of AD, offering critical insights into vascular stress and dysfunction that may occur in the prodromal phase of disease. This paves the way for future innovative therapeutic strategies.

Poster #**45**

BET/BRD4 Bromodomain and ERBB RTK Inhibitors Synergize to Suppress Brain Metastases of Luminal-B Breast Cancer

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Abstract

Background: Brain metastases represent a lethal progression of HER2-positive breast cancer, occurring in up to 50% of patients with advanced disease. Although several clinically approved receptor tyrosine kinase inhibitors (RTKis) can cross the blood–brain barrier, their clinical efficacy in breast cancer brain metastases remains limited and is associated with substantial toxicity. We recently identified Poziotinib, a pan-ERBB RTKi, as a promising therapeutic candidate for brain metastases of Luminal-B breast cancer. This prompted a search for synergistic agents to enhance the efficacy and tolerability of RTKis.

Objectives:

1. To identify CNS-penetrant agents that synergize with Poziotinib and reduce systemic toxicity.
2. To validate these findings in preclinical models and elucidate the mechanisms of synergy.

Results: High-throughput screening identified the BET/BRD4 inhibitor AZD5153 as a synergistic partner to Poziotinib in a brain-metastatic Luminal-B BT474 breast cancer model. Western blotting confirmed BRD4 expression and acetylation of histones H3 and H4 at sites critical for BRD4 interaction. AZD5153 inhibited BT474 cell proliferation and reduced c-Myc expression in vitro. Combined treatment with Poziotinib further decreased cell viability and increased PARP cleavage in vitro. In vivo, dual therapy significantly reduced tumor cell proliferation (Ki67+) and tumor burden compared to Poziotinib monotherapy.

Conclusion: This study is the first to demonstrate the effective use of a BET/BRD4 bromodomain inhibitor in treating brain metastases of Luminal-B breast cancer. The combination of AZD5153 and Poziotinib allows for substantial Poziotinib dose reduction, improving treatment tolerability in vivo.

Poster #**46**

ERVK integrase within CD11b$^+$ myeloid & CD8$^+$ T cells as ALS blood biomarkers.

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Abstract

Endogenous retrovirus-K (ERVK) expression has been associated with Amyotrophic Lateral Sclerosis (ALS), and its viral proteins can be identified in affected brain and spinal cord tissues. Despite confirmation of ERVK load in the blood of patients with ALS, few studies have examined ERVK protein expression in immune cells. ERVK produces an enzyme called integrase (IN), which can cause DNA damage during the integration of viral DNA into the host genome. Given that genomic instability is a hallmark of ALS, we hypothesized that the ERVK IN enzyme may also be expressed in patient lymphoid and myeloid-derived immune cells. Human blood specimens were collected from consenting ALS patients and controls at the Saskatoon City Hospital. Peripheral blood mononuclear cells (PBMC) were isolated from blood specimens using Ficoll isolation and affixed onto slides using the cytospin technique for subsequent confocal microscopy analysis. Image analysis of confocal micrographs revealed that ERVK IN expression was significantly elevated in CD14$^+$CD11b$^+$HLA-DR$^+$ myeloid cells from patients with ALS, as compared to control (n=14 in each group, $p<0.0001$). Morphologically, CD14$^+$CD11b$^+$HLA-DR$^+$ERVK$^+$ myeloid cells exhibited notable membrane ruffling typical of immune cell activation. CD3$^+$CD8$^+$ T cells in ALS exhibit enhanced number and size of ERVK IN aggregates, and a strong correlation with DNA damage marker γH2AX in these cells. This work points to the use of ERVK IN in myeloid and CD8$^+$ T cells as a blood biomarker for ALS clinical trials, especially those focused on testing the efficacy of antivirals as a therapeutic strategy for ALS.

Poster #**47**

ERVK integrase-driven DNA damage leads to motor deficits.

Alex Vandenakker[1], Sasha Bird[1], Domenico Di Curzio[1], Ilena Benoit[2], Breanna Meek[2], Terry Enno[1], Alberto Civetta[2], Renée Douville[3,1]

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Abstract

Endogenous retrovirus-K (ERVK) is a genomic viral symbiont that is associated with motor neuron loss and the clinical course of Amyotrophic Lateral Sclerosis (ALS). ERVK encodes an integrase (IN) enzyme known to cause DNA damage during the integration of viral DNA into the host genome. Since genomic instability is a hallmark of ALS, we hypothesized that the ERVK IN enzyme contributes to neuropathology in ALS and that this could be modeled *in vivo* in *Drosophila* (fruit fly). Human brain and spinal cord tissues were obtained from the NIH Neurobiobank and the Veterans Affairs Brain Bank for immunohistological analyses. ERVK^{+} neurons in the motor cortex of individuals with ALS exhibit an accumulation of the DNA damage marker γH2AX in the nucleus ($p<0.0001$). To model this phenomenon, motor neuron-specific D42 driver flies were crossed with ERVK IN transgenic responders, resulting in transgenic progeny that displayed an early-phase hyperactive phenotype upon stimulation (Day 0-40) followed by a rapid decline of motor function and paralysis in late-phase (Day 40-60), as compared with littermate controls (n=3 independent trials). Reduced movement in ERVK IN transgenics was corrected by treatment with integrase inhibitors, demonstrating that ERVK IN enzyme activity is tied to motor disturbances in this model. Sex-specific differences in lifespan, behaviour, and drug responsiveness were apparent. Neuropathological examination of ERVK IN transgenic flies revealed accumulation of DNA damage (γH2AV) over time. Together, this invertebrate model system proves that ERVK IN activity *in vivo* can cause motor disturbances and molecular features of ALS neuropathology.

Poster #**48**

Investigating the role of *Fan1* and *Mecp2* in neurodevelopmental health and disease.

Mikaela Perron, Hailey Therrien, Robert Beattie

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Abstract

This research aims to understand changes occurring during the development of Rett Syndrome (RTT), a severe neurological condition that primarily affects girls and leads to the loss of previously acquired mental and physical skills. The brain is made of many pieces, each playing a specific role in development. Two genes *Fan1* and *Mecp2* are important for building and repairing the brain. It is known that changes to Mecp2 can harm development.

This research will explore if changes to *Fan1* can help rescue function after a loss of *Mecp2*. I will use advanced tools to study cells and genes closely. One key tool, called Mosaic Analysis with Double Markers (MADM), allows me to look at each cell one by one to see how changes to *Mecp2* and *Fan1* impacts the cells. Cells will be identified using red and green fluorescent dyes, which will allow me to compare how many cells have the normal (red) *Fan1* and how many cells have the mutant (green) *Fan1*. MADM technology allows me to trace cell lineage and analyze morphology and functional changes of individual cells. By comparing the number and location of red and green cells, I will be able to gain a better understanding of how these changes impact the brain as a whole.

This research will provide a better understanding of how different parts of the brain work together as it develops. By studying these genes, I hope to learn how *Fan1* and *Mecp2* interact and what happens when these interactions are disrupted

Poster # **49**

Smoke Inhalation During Pregnancy and Early Life: Effects on Neurodevelopment

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Abstract

Cigarette smoke contains harmful chemicals that can cross the placenta and interfere with fetal development, posing serious health risks to pregnant women and their unborn children. Prenatal tobacco exposure is linked to adverse birth outcomes and long-term cognitive and behavioral deficits. This study investigates how prenatal and postnatal cigarette smoke exposure affects cortical development in a mouse model. Pregnant dams were exposed to cigarette smoke, and offspring were divided into two groups: a prenatal-only exposure group and a prenatal/postnatal exposure group. Brain tissue was collected, fixed, sectioned, and stained using DAPI or Nissl stains to assess cortical thickness. Some samples were immunostained with GFAP to quantify astrocytes. Results show that prenatal/postnatal exposed mice display reduced cortical thickness in both the primary somatosensory and auditory cortices. Prenatal-only exposed mice show reduced thickness in the primary somatosensory cortex and display astrocyte mislocalization in the same region. Combining these findings with single-cell technologies such as Mosaic Analysis with Double Markers (MADM) may help us understand how neuropsychiatric disorders can be triggered by a combination of environmental exposures (such as cigarette smoke exposure) and genetics.

Poster # **50**

Myelinated Axon Sizes in a Female Mouse at Multiple Angles

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Abstract

Electrical signals travel through the brain via axons in fibre tracts. *Postmortem* studies suggest that changes in axon diameter and density within fibre tracts are associated with disorders such as Alzheimer's disease, schizophrenia, and more. Comparing new diffusion magnetic resonance imaging (MRI) results to electron microscopy (EM), the gold standard, could validate *in vivo* MRI-based axon diameter as reliable biomarkers for disease-related microstructural changes. Our objective is to measure myelinated axons diameters at various orientations to determine if MRI measurement directions affect the axon's estimated size. Our dataset consisted of ten EM images from a female mouse that were uploaded to ImageJ. A 0.77 μm^2 grid, set horizontally and vertically or ±45o angles, was placed on the 9.4 µm x 6.6 µm EM images. If a grid line crossed a complete axon, a line was drawn along the grid and its recorded length represented the diameter. The weighted mean diameters, weighted by area, were 0.64±0.03 µm, 0.68±0.04 µm, 0.76±0.07 µm, and 0.61±0.03 µm for the horizontal, vertical, +45°, and -45° directions, respectively. No significant difference between various angled diameters was found using Analysis of Variance (ANOVA). Our findings suggest minimal variation in axon diameters at multiple angles, but further research is necessary to confirm these results. The authors thank Dr. Zou Yue for performing the perfusion fixation, the Vanderbilt Cell Imaging Shared Resource Core for performing the EM, and NSERC and The University of Winnipeg for funding.

Poster #**51**

Advancing MRI Technology: Evaluation of a Wireless RF Coil for Enhanced Imaging Performance in Phantom, Fruit, and Human Brain Studies

Yujie Ren

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Abstract

Introduction:
Magnetic resonance imaging (MRI) is essential in neuroscience for non-invasive visualization of brain structures and monitoring of neurological disorders [1] and subject comfort during imaging is desired. Image quality, particularly signal-to-noise ratio (SNR) and contrast-to-noise ratio (CNR), largely depends on radiofrequency (RF) coil performance [2]. This study evaluates a prototype wireless RF coil versus conventional wired coils for improving image quality and subject comfort while simplifying hardware.

Methods:
MRI was performed on a 1.5T Siemens Sempra system with three sets of samples: (1) a 5.3L Siemens water phantom with body, wired 12-channel, and wireless coils; (2) three fruits (watermelon, pineapple, banana) with wired and wireless coils; and (3) brains of two healthy volunteers using both coils. Identical imaging parameters were used within each image set. ROIs were manually defined (phantom: Siemens console; fruit/brain: MATLAB) to assess SNR and CNR in key regions, including white matter, grey matter, and CSF.

Results:
The wireless coil showed significantly higher SNR and lower CNR in phantom images ($p < 10^{-17}$), indicating better signal and uniformity. In fruits, higher SNR was observed in specific regions (e.g., banana flesh, watermelon), with no difference in pineapple. In brain imaging, the wireless coil achieved higher SNR in the caudate heads ($p < 0.01$) and splenium, and gyrus ($p < 0.004$).

Conclusion:
The wireless RF coil delivers improved or comparable image quality across models with enhanced SNR and simpler design, supporting its future application in neuroscience MRI.

Acknowledgements:
Supported by NSERC and Mitacs

Poster # **52**

Understanding the effect of grid spacing when mimicking MRI estimates of axon diameters using electron microscopy

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Abstract

Introduction: A new magnetic resonance imaging (MRI) method for inferring micron-sized axon diameters tends to overestimate axon sizes because it infers diameters based on the diffusion of water measured in a single direction. Previous studies have suggested that measuring axons in a single direction using electron microscopy (EM) yields results consistent with those from MRI.

Objectives: To assess whether the number of measurements in EM influences estimated axon size.

Methods: Using ImageJ, a grid was placed on a 9.4x6.6µm EM image of the corpus callosum from a female mouse. When a grid line intersected a myelinated axon, a line was drawn along the grid, across an axon. This was repeated across five grid sizes (0.15µm, 0.25µm, 0.5µm, 0.75µm, 1µm). The area-weighted mean axon length was calculated.

Results: The weighted mean axon diameters were 0.62±0.06µm, 0.62±0.06µm, 0.59±0.07µm, 0.60±0.08µm, and 0.55±0.06µm, for grid sizes 0.15µm, 0.2µm, 0.5µm, 0.75µm, and 1µm, respectively. Analysis of Variance (ANOVA) found no statistically significant differences between the measurements.

Conclusion: These results suggest that varying grid spacing does not significantly affect the measured axon diameters. This suggests that using a grid in which most axons are intersected at least once may be sufficient for reliable estimation. Further studies with a larger sample size and more EM images are needed to verify these results.

Acknowledgements: The authors thank Dr. Zou Yue for performing the perfusion fixation, the Vanderbilt Cell Imaging Shared Resource Core for performing the EM, and NSERC and The UWinnipeg for funding.

Poster #**53**

Back Projection of Image Reconstruction

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Abstract

Positron Emission Tomography (PET) uses radiotracers to visualize the function of the body. PET can be used to locate malignant tumours, and for understanding neurological disorders. An anatomical imaging method, traditionally CT, is needed in addition to PET so the functional maps can be overlaid on the anatomy. More recently, MRI has been proposed. Aspects of image processing need developing for PET-MR. Preliminary steps in image processing will be shown.
As a first step to understanding the field of image reconstruction, a Matlab program was used which generates CT projections from simulated data and then performs back projection to reconstruct the image. Parameters which were varied during the generation of CT projections include: the angle over which reconstruction is made, and the number of projections. Image matrix size was varied during the back projection.
Changes to the basic imaging and reconstruction parameters cause significant changes to the quality of the resulting image. Specifically, image resolution, image contrast, and presence of artifacts vary with image and reconstruction parameters. As expected, simple back projection does not provide high quality images.
With the understanding of simple back projection and the role imaging and reconstruction parameters play, study can continue into PET image reconstruction, and more complex reconstruction methods, such as OSEM. This will lead to the start of more complex image reconstruction for PET/MR data with the goal of increasing the use of clinical PET/MRI.
We acknowledge funding from Heart and Stroke Foundation, Brain Canada, CIHR, and NSERC.

Poster #**54**

Do cells other than myelinated axons contribute to MRI estimates of axon sizes?

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Abstract

Introduction: A new diffusion-MRI method inferring micron-sized axon diameters overestimates the intra-axonal volume fraction in the mouse corpus callosum (CC). MRI might be inferring the sizes of all cells, as cell walls and myelin restrict the diffusion of water within tissues.

Objective: To determine whether the average diameter of all cells within the EM images of the CC differs significantly from the average diameter of only myelinated axons.

Methods: A female mouse CC 9.4μmx6.6μm EM image was analyzed. The diameter of every fully visible cell was measured. Mean diameters were calculated for myelinated axons, all other cells, and all cells together.

Results: Mean diameter of the myelinated axons is 0.43±0.02μm, of all other visible cells is 0.146±0.005μm, of all visible cells is 0.24±0.03μm. All groups show significantly different axon diameters ($p<0.05$).

Conclusions: The cells that are not myelinated axons have diameters too small for MRI to resolve and are unlikely to influence axon diameter estimates. However, their abundance and the restricted diffusion of intracellular water within the cells likely affects the MRI-derived volume fraction. Further study of the volumes in EM is needed to compare with MRI results.

Acknowledgements: The authors thank Dr. Zou Yue for performing the perfusion fixation, the Vanderbilt Cell Imaging Shared Resource Core for performing the EM, and NSERC and The University of Winnipeg for funding.

Poster #**55**

Detecting Intraoperative Hemorrhage with MRI During Neurosurgical Procedures

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Abstract

Intraoperative magnetic resonance imaging (MRI) has become an important tool in neurosurgery, particularly for guiding tumour resections and biopsies, but its effectiveness in detecting acute hemorrhagic complications remains unclear. This study's objective is to determine whether intraoperative MRI can reliably detect hemorrhage within minutes of onset. Using an animal model, autologous blood was injected into the frontal lobe of 26 pigs to create intracerebral hematomas. The pigs were imaged with MRI 10 or 30 minutes post-injection. 8 pigs that weren't injected were imaged as the control group. Imaging sequences tested included T1-weighted, T2-weighted turbo spin echo (TSE), fluid-attenuated inversion recovery (FLAIR), and T2-weighted gradient echo (GE). The detectability of hematomas varied significantly across sequences: 88% were clearly visible on T2-weighted GE, 69% on T2-TSE, and 23% on FLAIR, while all of the T1-weighted images were ineffective. No false positives were seen in the control group. The results indicate that intraoperative MRI, particularly using T2-weighted GE sequences, enables rapid and reliable detection of bleeding complications shortly after they occur. These findings suggest that intraoperative MRI protocols should utilize GE imaging to enhance patient safety by facilitating immediate response to surgical hemorrhages and other imaging sequences, depending on the circumstance, to improve outcomes in neurosurgical procedures.

Acknowledgement: This work was supported in part by the Deutsche Forschungsgesellschaft (DFG) and the START Innovations program Forschung. Support from NSERC is also gratefully acknowledged.

Poster #**56**

Alterations in brain resting-state fractional amplitude of low frequency fluctuations in patients with trigeminal neuralgia are associated with severity of psychiatric comorbidities

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Abstract

Background:

Trigeminal neuralgia (TN) is a chronic neuropathic pain disorder which significantly impacts quality of life, with up to 50% of patients reporting comorbid depression and anxiety. Resting state functional magnetic resonance imaging (rs-fMRI) has previously revealed differences in intrinsic brain activity in persons with TN, however, the relationship between alterations in rs-fMRI and psychiatric comorbidity remain unclear.

Methods:

Thirty-one participants with TN (M_{age}= 58.21; 19 female) and 19 healthy controls (HC; M_{age} = 57.94; 14 women) underwent rs-fMRI and completed the Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI). Fractional amplitude of low frequency fluctuations (fALFF) were calculated and within-group voxel-wise general liner model (GLM) analyses tested the associations with psychological symptom severity. The association between fALFF and psychological symptoms was compared between groups. Results were thresholded using $p < 0.001$ (voxel-level) and $p\text{-}FDR < 0.05$ (cluster-level).

Results:

Unlike in HC, BDI scores in the TN group were positively correlated with fALFF in a cluster within the left post-central gyrus, corresponding to the facial region of the primary somatosensory cortex. Between-group analyses revealed stronger associations between fALFF and BDI in the frontal medial and left frontal orbital cortices in TN compared with HC. There was also a stronger association between fALFF and BAI in the left cerebellum (region 6) in TN compared to HC.

Conclusions:

Persons with TN exhibit distinct correlates between psychological symptom severity and spontaneous resting activity in brain regions involved in facial somatosensory processing. Somatosensory networks may contribute to emotional dysregulation in TN.

Poster #**57**

The Role of CLAUDIN1 in Poziotinib-resistant HER2+ Breast Cancer Brain Metastasis (BCBM) Cells

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Abstract

Background:
Brain metastases in HER2-positive (HER2+/ErbB2+) breast cancer carry a poor prognosis due to the blood-brain barrier and rapid resistance to treatment. Poziotinib (Poz), a HER1/2/4 tyrosine kinase inhibitor, is effective in BCBM94 cells, a patient-derived HER2+ breast cancer brain metastasis (BCBM) model, but resistance develops quickly. The tight junction protein Claudin-1 (CLDN1), particularly its phosphorylated form at tyrosine 210 (pCLDN1^{Y210}), is significantly upregulated in resistant cells, suggesting a role in adaptation to Poz.

Methods:
We assessed protein and mRNA levels, and subcellular localization using immunoblotting, qPCR, and immunofluorescence, respectively. CLDN1 stability was analyzed with cycloheximide (CHX), and kinase inhibitors (PKCδ, Src) were used to determine upstream regulators of pCLDN1^{Y210}. Time-course Poz exposure (0–48h) was used to monitor early adaptive signaling.

Results:
CLDN1 and pCLDN1^{Y210} were elevated 17- and 60-fold in PozR vs PozS cells. pCLDN1^{Y210} localized more to the membrane in resistant cells. CHX assays showed higher stability in PozR (t½ > 8h) versus PozS (t½ ~4h), suggesting phosphorylation enhances stability. Src inhibition reduced pCLDN1^{Y210}, while PKCδ inhibition did not, implicating Src as a regulator. Early Poz exposure rapidly increased p-Src and p-CLDN1^{Y210}, while HER signaling was downregulated, indicating a compensatory pathway.

Discussion:
These findings suggest pCLDN1^{Y210} may mediate resistance by promoting membrane localization and stability via Src. The rapid increase post-drug exposure indicates a protective role. Ongoing studies using CRISPRi-mediated CLDN1 knockdown and functional assays in HER2+ models (BT474, HCC-1954) will assess the therapeutic relevance of targeting the Src-CLDN1 axis.

Poster #**58**

Distinct glioma pathology induced by Relaxin family peptide receptor-1 (RXFP1) in mice

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Abstract

Introduction

Glioblastoma, the most aggressive brain tumor, remains incurable due to its complex biology and lack of biomarkers. The adiponectin-like complement 1q tumor necrosis factor-related protein (CTRP) 1–15 family has garnered interest in various cancers. We identified CTRP8, the least studied member and a pseudogene in mice, as a novel ligand for the G protein-coupled relaxin family peptide receptor 1 (RXFP1) in patient brain tumor cells. The CTRP8-RXFP1 axis enhances glioma cell migration and temozolomide resistance. Mice xenografted with U251 cells expressing CTRP8 and RXFP1 show rapid demise with highly proliferative gliomas. In contrast, U251-RXFP1 xenografts developed slow-growing, collagen- and elastin-rich gliomas, more pronounced in males.

Methods

U251-RXFP1 cells were orthotopically xenografted into immunodeficient RAG2 mice. Tumor and non-tumor brain hemispheres were analyzed using histology, immunohistochemistry, microCT, MRI, and Kaplan-Meier survival curves. We identified distinct human and mouse proteomic signatures in U251-RXFP1 tumors.

Results

U251-RXFP1 mice survived >90 days but developed extracranial osteolytic tumors around day 70. MicroCT revealed calvarial bone loss, and MRI identified a hyperdense tumor mass. Histology confirmed dense collagen and elastin deposition, absent in U251 and normal brains. Male mice exhibited the highest collagen/elastin levels and PDGFRβ staining. The human tumor proteome showed increased ubiquitination, cytoskeletal remodeling, migration, and PI3K signaling. In mice, TBC1D5 was >50-fold upregulated, suggesting roles in endosomal trafficking, autophagy, and tumor microenvironment (TME) adaptation.

Conclusion

RXFP1 overexpression in U251 cells generated a slow-growing, osteolytic glioma with strong stromal remodeling, especially in males, revealing an aggressive and fibrotic glioma phenotype.

Poster #**59**

Exposure to milk-derived extracellular vesicles promotes fat breakdown in offspring exposed to maternal obesity

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Abstract

Perinatal-exposure-to-maternal-obesity (PEMO) is associated with hypothalamic inflammation, which dysregulates signalling of key metabolic hormones (*e.g.,* insulin) leading to increased adiposity. Peroxisome-proliferator-activated-receptors (PPAR) modulate cellular fuel usage, and their activation may mitigate this dysregulation. PPARs regulate the pyruvate-dehydrogenase-complex (PDC), a mediator between glucose and lipid metabolism in the cell, by upregulating pyruvate-dehydrogenase-kinases (PDK). Milk-extracellular-vesicles (MEV) are shown to attenuate metabolic dysfunction in children. In this study, we investigated interactions between PEMO and MEVs, and their impact on PPAR abundance and activity in the liver-brain axis in neonatal rats. Adult female rats (n=6/diet) were fed a high saturated fat (60%-kcal-fat) or control (10%-kcal-fat) diet for four weeks prior to mating, during gestation and lactation. Beginning postnatal day (PND) 4, offspring (n=2M;2F) received human MEVs (1.78e11 particles/mL) at a dose of 50 µL/g of body weight via oral gavage twice daily until PND11. Liver and hypothalamus were used for analyses. Transcript abundances of candidate PPARs and PDKs were measured using RT-qPCR. Protein abundances of the regulatory PDC-E1a subunit and its phosphorylation sites were assessed with western immunoblotting. PEMO decreased transcript abundances of PPARs (PPARa,PPARg) and increased phosphorylation of the PDC in the liver. MEV supplementation increased transcript abundance of select PPARs, PGC1a, and decreased phosphorylation of the PDC in both the liver and hypothalamus. Elevated PPAR expression and increased phosphorylation of the PDC are adaptive responses to overnutrition and associated with decreased glucose and increased lipid metabolism. Our findings illustrate the potential ability of MEVs to modulate cellular fuel utilization.

Poster #**60**

Development and neural stem cell dynamics of the zebrafish Rostral Migratory Stream

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Abstract

Cellular migration is a fundamental mechanism for brain development, function and potential recovery after injury. The rostral migratory stream (RMS) is characterized by the continuous migration of neuroblasts from neural stem cells (NSCs) of the forebrain to the olfactory bulbs (OB), where they differentiate into neurons. The RMS was first described in mammals, though less is known in the highly neurogenic zebrafish model where a similar RMS-like structure persists. Currently, we lack knowledge on the formation and dynamics of the RMS as a distinct migratory route from larval to adult stages, and how it contributes to the organization of OB neuron population.

To characterize the zebrafish RMS across development, I analyzed its features from their niche of origin to the OB during larval, juvenile, adult and senescent stages. These features include NSC phenotypes, migrating progenitors, blood vessels supporting migration, proliferative and migratory dynamics, and differentiation rates. Using transgenic lines, electron microscopy and thymidine analogue (EdU), results revealed a progressive establishment of RMS structures marked by a shift in the NSC phenotypes and proliferative dynamics over time. By adulthood, migrating progenitors shift to migrating specifically to the OB where they differentiate into interneurons, but this output significantly decreases in senescent fish. Findings from this study offer novel insights in understanding the signals, NSC phenotypes, and structures supporting neurogenic migration along the RMS.

Poster #**61**

Development of a trifunctional boron-based pyrazole (B-Pyr) that delays disease onset, extended life span, prevented weight loss, and demonstrated target engagement in a hSOD1 mouse model of Amyotrophic Lateral Sclerosis

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Abstract

Introduction

Amyotrophic lateral sclerosis (ALS) is a highly complex and multifactorial disease. Further, the endpoint of ALS is always death. However, mutations in the SOD1 gene result in a toxic gain-of-function through its oxidative modification under pathological oxidative stress. Since the discovery of ALS in 1869 by Charcot, only two fully FDA-approved drugs are available: Edaravone (EDR), an antioxidant, and Riluzole (RZ), an anti-glutamatergic agent, with minimal effects on the disease course. Therefore, our overall goal is to develop novel boron-based pyrazole drug candidates that could slow down the progression of ALS.

Methodology

We have conducted randomized, longitudinal studies using age and sex-matched mice (both male and female) to evaluate the toxicity and efficacy of a lead B-Pyr candidate in-vivo, utilizing the humanized mutant SOD1-G37R mouse model (line 42). All animal experiments for this study followed protocol Ref# 21-014 (AC11693) approved by CACS, UFM.

Results

Single and 120 daily doses of **a** B-Pyr analog (10 mg/kg body weight) demonstrated no signs of treatment-associated toxicity. Further, presymptomatic treatment of a B-Pyr analogue (10mg/kg body weight) in mutant SOD1-G37R mice demonstrated statistically significant efficacy in modifying clinical disease phenotypes in terms of extension of life span, delaying the onset of the disease, and preventing ALS-induced weight loss.

Conclusion

A longitudinal preclinical study with a novel B-Pyr drug candidate **demonstrated a satisfactory safety profile and statistically significant preclinical, proof-of-concept efficacy in a humanized SOD1** mouse model of ALS. The lead molecule is under patent with strong ISR report for novelty PCT/CA2023/051352 (WO/2024/103151).

Poster #**62**

Determining the Role of IRF2BPL in Neurological Disease

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Abstract

De novo mutations in the gene *IRF2BPL* cause a severe neurodevelopmental disorder in children known as NEDAMSS (Neurodevelopmental Disorder with Abnormal Movements, Loss of Speech, and Seizures). Affected children exhibit typical early development, but around age five, they begin experiencing motor skill regression, stumbling, and progressive movement impairments. By adolescence, patients may become immobile. Some *IRF2BPL* mutations have also been linked to autism spectrum disorder and early-onset Parkinsonism, highlighting the gene's broader neurological significance. Given the heterogeneity of patient symptoms, our study aims to investigate specific *IRF2BPL* variants, particularly truncating mutations, using Drosophila melanogaster as a model to explore genotype-phenotype correlations. Additionally, we will screen drugs targeting different potential disease mechanisms. To complement these studies, we have generated Irf2bpl knockout (KO) mice, which are runted and display motor impairments. We hypothesize that heterozygous mice may also develop neurological and motor symptoms with age. To assess this, we will evaluate their motor and social behaviors at two and eleven months and analyze brain tissue at three and twelve months. Furthermore, we will use single-nucleus RNA sequencing to examine transcriptional changes associated with *IRF2BPL* mutations. By using these models, our research aims to deepen the understanding of NEDAMSS and its connection to broader neurological conditions such as autism and Parkinson's disease, potentially guiding the development of novel therapeutic strategies.

Poster #**63**

Exploring Commissural Connectivity Between the Cerebellar Nuclei in Mice

Azam Asemi Rad, Farshid Ghiyamihoor, hassan marzban

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Abstract

- **Introduction:** Cerebellum is essential for motor control, ensuring smooth and accurate movement, maintaining balance and posture, emotion and cognition. The primary output of the cerebellum is mediated by the cerebellar nuclei (CN), which are categorized into four major groups in mice and receiving and integrating information from both limbs, suggest a connectivity between the right and left cerebellar nuclei to coordinate movement and balance. However, the potential for direct or indirect communication between the right and left CN remains largely unexplored.
- **Method:** To assess the existence of a connectivity between right and left lateral CN, we stereotactically injected the AAV tracer (AAV2/1.pSynI.EGFP.WPRE.bGH) into the lateral CN of adult C57BL/6J mice (P60) in right side. We coronally sectioned them (serial section, 20 µm) to trace axonal projection to the contralateral CN.
- **Result:** Immunolabeling with NAA showed axonal bridges between right and left lateral CN. The analysis of cerebellar section after injection of AAV tracer confirmed the presence of labeled fibers crossing the midline and terminating in the contralateral lateral CN. Together, these findings provide novel anatomical evidence for a commissural structure connecting the right and left lateral cerebellar nuclei.
- **Conclusion:** Understanding whether a cerebellar analog to the corpus callosum exists, especially within the lateral CN, has significant implications for our knowledge of interhemispheric coordination in cerebellar function. Such connectivity may play a role in bilateral motor control, compensation after injury, or cerebellar contributions to cognition.

Poster #**64**

Novel extracellular interaction of mGluR5 and neurexin-1

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Abstract

Metabotropic glutamate receptor 5 (mGluR5) stands out as a particularly promising pharmacological target for neurological conditions; however, complications from mGluR5-targeting drugs in clinical trials for the childhood neurodevelopmental disorders fragile X syndrome and autism spectrum disorder demonstrate our incomplete understanding of mGluR5 biology. In recent years, the pharmacological principles of group III mGluRs have been altered by the discovery of trans-synaptic complexes involving synaptic adhesion molecules altering their pharmacological properties. Given the high level of ectodomain sequence homology across all eight mGluRs, we hypothesize that there are more trans-synaptic interactions with other mGluRs that remain to be discovered. Here, we present evidence of a novel trans-cellular interaction between mGluR5 and the prominent autism-associated synaptic adhesion molecule neurexin-1. Interestingly, immunoprecipitation followed by mass spectrometry data reveal that postsynaptic mGluR5 may bind presynaptic neurexin-1. Subsequent trans-cellular co-immunoprecipitation experiments demonstrate that not only does neurexin-1 bind mGluR5, but the physiologically critical neurexin-1β (SS4-) preferentially binds mGluR5 as compared to neurexin-1β (SS4+). Furthermore, the ectodomain of neurexin-1β (SS4-) is sufficient to bind mGluR5, suggesting that this interaction occurs extracellularly in trans. Finally, the recently developed ONE-GO GPCR biosensors are being used to visualize Gαq and Gαi1 activity upon mGluR5 stimulation, and trans-cellular assays are currently underway to determine if neurexin-1 modulates mGluR5 pharmacology in trans. This research demonstrates a novel mGluR-synaptic adhesion molecule complex, with future directions focusing on characterizing the functional consequence of this interaction and its contribution to synaptic biology

Poster: #65

Investigating atypical communication mechanisms between ELFN1 and mGluR4 in rare neurodevelopmental disorders

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Abstract

Glutamatergic neurotransmission is involved in different neurodevelopmental diseases (NDDs) such as attention deficit hyperactive disorder (ADHD), autism spectrum disorder (ASD), learning and intellectual disorders, obsessive-compulsive disorders (OCD). Metabotropic glutamate receptors (mGluRs) are a type of G-protein coupled receptor (GPCR), critical for modulating excitatory glutamatergic neurotransmission and considered as highly viable drug design avenues for NDDs. Among mGluRs, group III mGluRs are known to be trans-synaptically regulated by synaptic adhesion molecules (SAMs). Extracellular leucine rich fibronectin type III domain 1 (ELFN1) is a type of SAM which engages group III mGluRs trans-synaptically and alters the accepted pharmacological principles. In recent years, disease-causing human ELFN1 gene variants have been identified in diverse NDDs. In this study, we aim to evaluate the consequences of ELFN1 genetic variants on mGluR4 pharmacology. To accurately assess the impact, ELFN1 variants and mGluR4 were transfected into the separate populations of HEK293 cells. After harvesting cells, we performed expression analysis via Western blotting and co-immunoprecipitation experiments to assess their transcellular interactions with mGluR4. The refence ELFN1 and the extracellular ELFN1 variant express similarly. Co-immunoprecipitation demonstrated that reference ELFN1 interacts transcellularly with the mGluR4; however, an extracellular ELFN1 variant demonstrated significantly disrupted binding with mGluR4. Transcellular signaling assay elucidated that mGlu4 is down regulated by reference ELFN1 but not ELFN1 variant. This research aims to provide a characterization of the molecular effects of pathogenic ELFN1 variants which would help in understanding the mechanistic etiology to guide the future therapeutics targeted at mGluR4.

Poster: #66

Systemic administration of nimodipine influences pericyte calcium signaling, baseline hemodynamics, and neurovascular coupling in the healthy mouse brain.

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Abstract

Nimodipine, a L-type voltage-gated calcium channel (VGCC) blocker commonly used in subarachnoid hemorrhage management, has significant effects on brain pericytes, which express L-type VGCCs and play a critical role in regulating cerebral blood flow (CBF) through vasomotion and neurovascular coupling. Our work demonstrates that systemic administration of nimodipine (1 mg/kg; i.p.) reduces calcium transients in all pericyte types, including ensheathing and thin-strand pericytes, across various cerebrovascular regions, as revealed by two-photon microscopy. This reduction leads to local vasodilation near penetrating arterioles but decreases red blood cell (RBC) velocity, suggesting complex hemodynamic consequences. In contrast, topical application of nimodipine increases RBC velocity, highlighting differential effects based on the administration route. Furthermore, we show that L-type VGCCs in both pericyte types mediate functional hyperemia by facilitating vasodilation. By blocking these channels, Nimodipine impairs neurovascular coupling at all points in the vascular network. These findings underscore nimodipine's ability to alter cerebrovascular dynamics and pericyte physiology, with distinct outcomes depending on systemic versus topical application, offering critical insights for its clinical use.

Poster: #67

Milk-derived extracellular vesicles attenuate maternal obesity induced liver disease in offspring by modulating neurological and hepatic pro-inflammation

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Abstract

Perinatal (pre and postnatal) exposure to maternal obesity (MO) increases the risk of metabolic dysfunction in offspring. Children with MO exposure have a higher risk for metabolic-associated liver disease (MALD), characterized by increased lipid uptake/lipogenesis and reduced lipid oxidation/export. MALD leads to chronic pro-inflammation, liver damage, is linked to impaired neurodevelopment and disrupts the liver-brain axis. However, a biologically active component of human milk, milk-derived extracellular vesicles (MEVs), may attenuate MALD. MEVs are lipid-coated nanovesicles that transport microRNA, fats and lipids. MEVs are anti-inflammatory and readily cross biological barriers (intestinal epithelium, blood-brain-barrier). Our study investigates if MEV treatment of neonates with MO exposure mitigates MALD onset or progression.

MO was induced in six female Long Evans rats using a 60% saturated fat diet, while six females consumed a control (10% saturated fat) diet, matched for sugar. Diets were maintained for four weeks pre-mating, throughout mating, gestation and lactation. Offspring (n=1/litter/sex) were randomly assigned to handling, vehicle gavage, or MEV gavage (1.54 x 1011 particles/mL in 50 µl/g body weight) treatments. MEVs were isolated from pooled human donor milk using serial ultracentrifugation and filtration. Offspring liver and hypothalamus were collected at postnatal day 11 for analysis.

Results indicate MO-exposed offspring exhibit MALD markers, including reduced antioxidant (NRF1, MnSOD) transcript abundance and increased pro-inflammatory markers (TNFα and NFκB). MEV treatment may relieve MO-induced MALD by reducing TNFα abundance in the liver and NFκB liver and hypothalamus activation. These results highlight MEVs therapeutic potential to treat pro-inflammatory conditions.

Poster: #68

Adaptive Tumour Microenvironment (TME) Responses in HER2+ Breast Cancer Brain Metastasis (BCBM)

Emily Fedora[1], Danyyl Ippolitov[1], Thatchawan Thanasupawat[1], Victor Spicer[2], Rene Zahedi[1,2], Thomas Klonisch[1], Sabine Hombach-Klonisch[1]

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Abstract

Nearly 50% of HER2+ breast cancer patients experience breast cancer brain metastasis (BCBM). BCBM treatment options are limited due to blood-brain-barrier impermeability and drug resistance in recurrent tumours. Spatial TME responses must be understood for more efficacious HER2+ BCBM treatments to improve prognosis.

Patient-derived BCBM mouse models explore a HER2-targeted treatment which reduces metastatic HER2+ brain tumour burden. Mice with BCBM tumours were treated for 2 weeks with lapatinib, poziotinib, and solvent control. Immunofluorescence was performed on mouse brain sections to detect reactive astrocytes (GFAP) and microglia (IBA1). Nanostring GeoMX Digital Spatial Profiler analyzed tissues for spatial transcriptomics and bulk RNA sequencing. Transcriptional responses in GFAP+ and IBA1+ segments were compared across tumour core, edge, and adjacent regions of mouse brain sections.

Reactive mouse microglia and astrocytes surrounded BCBM tumours +/-HER2 inhibitor treatment, suggesting TME-tumour cell crosstalk. Bioinformatics analysis of spatial transcriptomics data revealed elevated ubiquitin-proteasome protein turnover at core-edge and edge-adjacent boundaries. The core-edge region showed innate immune/inflammatory markers and phagocytic genes, and the edge-adjacent region showed immune cell infiltration. The TME adapts protein synthesis/degradation cycles to address metabolic demands, attenuates pro-inflammatory mechanisms, and engages in immune cell co-adaptation at tumour-TME boundaries. Multiplexed immunofluorescence and in-situ hybridization will validate TME responses to HER2+ BCBM tumours and assess TME signalling pathways.

HER2+ BCBM tumour progression and drug resistance rely on mouse brain co-adaptation. The TME undergoes dynamic proteostasis, immunoregulation, and anti-inflammatory responses. Identifying TME molecular pathways may inform novel treatment strategies to improve outcomes for HER2+ BCBM patients.

Poster: #69

Mental Imagery and Emotion Regulation: Positively Valenced Distraction in the Mind's Eye

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Abstract

Research suggests that distraction, the goal-directed shifting of one's attention away from a threatening stimulus, can regulate negative emotion. However, no research has evaluated whether positively valenced mental imagery can distract from a threatening stimulus, or if differences in trait negative affect may influence positive imagery in emotion regulation. To address these gaps in the literature, 104 participants were recruited. A differential fear association was first created, followed by a distraction manipulation where participants either attended to the stimulus presented or visualized a neutral or positively valenced distractor. We primarily hypothesized that imagining a distracting stimulus would downregulate one's fear response when presented with a threat cue, as measured through the skin conductance response (SCR) and self-reported fear. Additionally, positively valenced imagery was predicted to regulate fear to a greater extent than neutral imagery. Exploratory analyses evaluated potential group differences in emotion regulation between individuals who scored high in trait negative affect and controls. Self-reported fear and SCR measures revealed the successful acquisition and persistence of differential fear. Whereas the self-reported fear results supported only positively valenced imagery in the regulation of fear, SCR results revealed that both positively valenced and neutral imagined distraction had downregulated the differential threat response. No direct evidence for the increased efficacy of positively valenced distraction was found. Exploratory group effects proved non-significant, suggesting that trait negative affect did not significantly affect emotion regulation efficacy. Together, the present investigation informs the use of an effective emotion regulation strategy for those who struggle with emotion dysregulation.

Poster: #70

Investigating the Neurodevelopmental Effects of Cigarette Exposure in Pregnancy and Early Life

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Abstract

Despite the negative implications of cigarette smoke (CS) exposure being well understood, the effect of CS exposure on neural stem cells during development and post-mitotic cells within the infant brain remains an enigma. The objective of this study is to examine the effects of CS exposure on the developing neocortex during pregnancy and after birth.

We used two exposure groups of mice to gather data during pregnancy (prenatal) and early life (prenatal/postnatal). In the prenatal exposure group (n=3), the pregnant mother (dam) was exposed to CS 2 times a day for 2-hour intervals using the machine. The pups were born and sacrificed on postnatal day 3. For the prenatal/postnatal exposure group, the pregnant dam was exposed to CS 2 times a day for 2-hour intervals. The pups were born and exposed to CS in a manner simulating second-hand exposure and sacrificed at 8 weeks. For each group, the brain was dissected, fixed in paraformaldehyde, frozen, and sectioned at 0.25 mm for downstream analysis.

The combined prenatal/postnatal exposure group saw an overall decrease in cortical thickness in the primary somatosensory region, specifically in the lower layers (IV-VI) (P=0.098), as well as thinning in the auditory area (P=0.089). The prenatal exposure group also saw an overall thinning of the primary somatosensory area.

The results display thinning of specific cortical regions, indicating that CS exposure during pregnancy and early life may have negative implications on both neural stem cells during development and post-mitotic cells after birth within the infant brain.

Poster: #71

Characterization of Ursus Maritimus Fibroblasts to Generate Induced Pluripotent Stem Cells

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Abstract

Polar Bears (Ursus maritimus) are keystone arctic species that are currently facing rapid population decline, posing severe implications for the stability of their ecosystems. Recent findings have suggested accelerated epigenetic aging among polar bears, furthering population decline. This study aims to characterize primary fibroblasts derived from skin biopsies to create a foundation for the generation of induced pluripotent stem cells(iPSCs). This offers promising applications in conservation through reintroduction of genetic material into populations, as well as understanding species specific developmental processes with the use of cerebral organoids to model brain development. Fibroblasts from primary skin biopsies from old and young bears were cultured and assessed for proliferative ability, reactive oxygen species (ROS) and mitochondrial oxidative stress. Younger fibroblast cultures exhibited significantly faster doubling times as compared to older bears (22.5 hours vs 44 hours). Trends of increasing cellular ROS were noticed among increase age of fibroblast cultures although mitochondrial differences were less pronounced. These results suggest age associated changes in cellular health, that could reflect the various environmental stressors. These fibroblasts demonstrated sufficient viability and genomic stability to continue with iPSC reprogramming. This research represents the first documented doubling times and ROS characterization of polar beat fibroblasts and lays the foundation for future protocols on iPSC generation. It offers a novel and comprehensive approach to study aging, neurodevelopment and conservation of species undergoing climate stress.

Poster: #72

The Effect of Acute Exercise on Emotion Regulation

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Abstract

Background: Physical Exercise supports aspects of mental health through factors involving autonomic and emotion regulation. Together, these factors influence our ability to effortfully up- or down- regulate emotional reactions. This two-day experiment evaluated the acute effects of moderate-intensity continuous exercise on emotion regulation and skin conductance response (SCR).

Methods: On day one, 60 participants either exercised or sat (control) on an ergometer while watching a 30-minute video. Participants then completed an emotion regulation task involving instructions to passively view or reappraise emotional pictures while physiological responses were recorded. Participants returned 24 hours later to provide valence ratings on previously seen and new pictures. A 2x2 mixed design was used with condition (passively viewing & reappraisal) and group (Exercise & Control) as the independent variables.

Results: SCRs were significantly lower during reappraisal compared to passive viewing across regardless of group. Negative affect ratings showed a significant Group × Condition interaction, with reappraisal reducing negative affect more in the exercise group than in controls. In contrast, positive affect ratings showed a main effect of Condition, with reappraisal enhancing positive affect across both groups.
Discussion: These findings suggest that while reappraisal reduces the autonomic response, acute exercise does not upregulate this effect. However, exercising showed greater reductions in negative affect 24-hours later when reappraising previously seen images, suggesting that exercise may strengthen the lasting emotional benefits of reappraisal.

Conclusion: Exercise may not improve all aspects of emotion regulation but may strengthen the delayed effects of reappraising negative emotions, warranting further investigation.

Poster: #73

Fearless Extinction: Extinguishing Fear Without a Fear Response Using Semantically Related Generalization Stimuli

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Abstract

Pavlovian extinction learning mechanisms are implicated in exposure-based therapies for anxiety and related disorders. The present study evaluated the efficacy of generalized fear extinction through exposure to high- or low-related semantic associates of a conditioned stimulus (CS+) relative to traditional CS extinction. Participants underwent fear conditioning to a neutral word and were then exposed to semantically related words (generalized conditioned stimuli; GCSs) to assess fear generalization. In an extinction phase, participants were randomly assigned to one of three groups: a CS exposure group, a high-related GCS group, or a low-related GCS group. Here, participants were exposed to the original CSs, the high-related GCSs, or the low-related GCSs, respectively. Approximately 24 hours later, participants completed an extinction recall test in which only the original CSs were presented, allowing for a direct comparison of fear responding across exposure groups. All groups demonstrated a return of fear responding to the CS+, as evidenced by both skin conductance responses (SCRs) and self-reported fear ratings. However, both the CS and high-related GCS exposure groups exhibited diminished fear responding in the latter half of the extinction recall phase, perhaps reflecting the successful retrieval of an extinction memory. Conversely, fear responding persisted in the low-related GCS exposure group, perhaps reflecting a failure to retrieve an extinction memory. The results suggest that conducting extinction with high-related GCSs may promote the reduction of conditioned fear responses.

Poster: #74

Evaluating the Modulatory Activity and Binding Capacity of ELFN1 on mGluR8 In Trans

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Abstract

G protein-coupled receptors (GPCRs) are essential in the proper regulation of synaptic transmission; therefore, their pathways act as promising targets for pharmacological intervention with GPCRs already being targeted by 35% of FDA approved pharmaceuticals. Recent studies on GPCR extracellular binding partners highlight the concerningly limited understanding of GPCR synaptic neurobiology. Interestingly, one of these extracellular binding partners is a synaptic adhesion molecule called extracellular leucine-rich repeat and fibronectin type-III domain-containing protein 1 (ELFN1), which can modulate a subset of GPCRs- group 3 metabotropic glutamate receptors (mGluRs). Mutations within ELFN1 have been linked to various neurodevelopmental diseases (NDDs) including the general etiology of epilepsy, ADHD, and autism. This study aims to determine the trans-synaptic consequence of ELFN1 on mGluR8 binding in trans. Co-immunoprecipitation experiments were conducted by co-culturing HEK293 cells transfected with mGluR8-HA and wildtype ELFN1 or a clinically pathogenic ELFN1 variant. Binding capacity was analysed via western blotting and densitometry. In comparison to reference ELFN1, the extracellular ELFN1 variant exhibited significant reduction in its binding capacity with mGluR8 suggesting an mGluR8-dependent pathogenic mechanism. Furthermore, we are evaluating ELFN1's modulatory ability using the Transcellular GPCR Signalling Assay Platform where mGluR8-HA expressing cells are co-cultured with control or ELFN1 expressing cells to assess mGluR8 activity using various biosensor readouts. The disruption of mGluR8-ELFN1 complex sheds light on potential therapeutic strategies for patients harboring this variant.

Poster: #75

Patterns of Brain Glucose Metabolism Predict Cerebellar Symptoms in Multiple System Atrophy

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Abstract

Multiple system atrophy (MSA) is the second most common neurodegenerative movement disorder next to Parkinson's disease (PD). MSA symptoms are traditionally categorized as reflecting either Parkinsonian (MSA-P) or cerebellar symptoms (MSA-C). Using FDG-PET, Carther-Krone et al. (under review) have recently identified an MSA-P-Related Pattern of glucose metabolism (MSA-P-RP) in the brains of MSA-P patients, characterized by hypermetabolism in the pons, pallidum, and sensorimotor cortex, as well as hypometabolism in the putamen, premotor and parieto-occipital regions. A separate MSA-C-Related Pattern (MSA-C-RP) was identified in MSA-C patients, characterized by hypometabolism in the cerebellum and putamen. Using a recently acquired comprehensive and independent dataset, we have explored if patients' MSA-Related Pattern scores predict their clinical symptomatology.

FDG-PET and clinical assessment data from 39 (30 MSA-P, 9 MSA-C) MSA patients obtained from an ongoing phase 2 clinical trial (clinicaltrials.gov ID: NCT05695378) was analyzed. Multiple linear regression was used to test the predictive relationship between patients' MSA-Related Pattern scores and their outcomes on the Scale for the Assessment and Rating of Ataxia (SARA), the Unified Parkinson's Disease Rating Scale part III (UPDRS3), and the Unified Multiple System Atrophy Rating Scale (UMSARS I+II).

Increased MSA-C-RP scores significantly predicted an increase in SARA scores. Patients' MSA-P-RP scores did not significantly predict participants' clinical scores.

The hypometabolism of glucose in the cerebellum and putamen indicated by patients' MSA-C-RP scores likely contribute to the symptoms of cerebellar ataxia (reduced motor coordination, balance, and gait). MSA-C-RP scores may serve as a predictive biomarker for ataxia symptoms associated with MSA.

Poster: #76

Role of $Na_v1.7$ in Electrical Excitability of Rat Subfornical Organ Neurons

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Abstract

The subfornical organ (SFO) is a sensory circumventricular organ that plays a key role in regulation of homeostasis. Its roles in homeostatic regulation is supported by the SFO's lack of an intact blood-brain barrier, which enables it to come into direct contact with circulating signaling molecules. Previous studies demonstrate that SFO neurons show heterogeneity in current expression and spiking behavior, leading to two predominant spiking phenotypes: tonic and burst firing. Electrophysiology studies, supported by Hodgkin-Huxley single compartment models support the notion that unique properties of voltage gated Na+ current play a key role in determining electrical phenotype. Interestingly, transcriptomic studies show that SFO neurons express a unique complement of voltage gated Na+ channels, including Nav 1.3 and Nav 1.7, in addition to the commonly expressed Nav 1.1, Nav 1.2 and Nav 1.6 isoforms. The specific role of Nav 1.7 in SFO neurons is presently unknown.

Poster: #77

L1 syndrome-causing L1CAM in complex with mGluR5 *in trans*

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Abstract

Introduction: In the brain, synaptic interactions occur between billions of neurons to facilitate optimal functioning. Interruptions in these interactions can lead to neurodevelopmental and neuropsychiatric disorders. Recently, these trans-synaptic interactions were found to involve a subgroup of G protein-coupled receptors (GPCRs) known as the metabotropic glutamate receptors (mGluRs) and synaptic adhesion molecules. L1 syndrome is a neurodevelopmental disorder caused by disruptions in the synaptic adhesion molecule L1CAM, and this molecule was recently found to interact with mGluR5. As a well-established drug target for neurological disorders, mGluR5 in complex with L1CAM may have major implications in the etiology of L1 syndrome and possibly other neurological disorders.

Objective: The objective of this study is to determine whether there is a transcellular molecular interaction between L1CAM and mGluR5.

Methods: Using public mass spectrometry data, we highlighted L1CAM as a potential binding partner of mGluR5. Co-immunoprecipitation experiments were conducted to detect binding of mGluR5 with full-length L1CAM and the ectodomain of L1CAM.

Results: Co-immunoprecipitation experiments showed that mGluR5 binds to full-length L1CAM as well as the ectodomain of L1CAM, verifying that the interaction occurs transcellularly.

Conclusion: Verification of L1CAM-mGluR5 transcellular complexes leads to new hypotheses, including whether L1CAM modulates mGluR5 pharmacology, and whether pathogenic mutations for L1 syndrome in L1CAM disrupt interactions with mGluR5. This research will further our understanding of L1 syndrome, and possibly other neurological disorders associated with mGluR5.

Poster: #78

Developmental Trajectory of a Neural Circuit Organizer

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Abstract

Leucine-rich repeat transmembrane neuronal proteins (LRRTMs) are postsynaptic adhesion molecules critical for excitatory synapse development. LRRTM3 is essential for synapse formation and implicated in neurodevelopmental disorders, including autism spectrum disorder. While previous studies explored *LRRTM3 mRNA*expression and function in vitro, its protein expression and function in vivo remain less unexplored. This study investigates LRRTM3 at the protein level in mouse brain to elucidate its role in synapse development. Using CRISPR-Cas9, we generated HA-tagged LRRTM3 and LRRTM3 knock-out (KO) mouse models. We performed Immunohistochemistry with various antibodies, followed by widefield and confocal microscopy. LRRTM3 protein is predominantly expressed in the cerebellar cortex, striatum, hippocampus, and cerebellum of adult mice, colocalizing with excitatory postsynaptic markers. In the hippocampus, LRRTM3 expression begins at P12 and becomes confined to the outer molecular layer of the dentate gyrus by P21. In the cerebellum, expression starts at P08 in the granule layer. Super-resolution imaging showed LRRTM3 redistributes within cerebellar glomeruli, shifting from a diffuse pattern at P16 to enriched postsynaptic sites by P30. In LRRTM3 KO mice, expression of LRRTM1, LRRTM2, and LRRTM4 remains unchanged. Our findings establish LRRTM3 as a postsynaptic excitatory protein expressed in brain regions important for cognition and motor control. It exhibits spatiotemporal dynamics during postnatal synaptogenesis in the hippocampus and cerebellum. Additionally, LRRTM3 stabilizes in mature cerebellar glomeruli. The lack of compensatory changes by other LRRTMs in KO mice highlights its unique role. Our future research will use expansion microscopy, electrophysiology, and behavioral assays to explore LRRTM3's functional role.

Poster: #79

Structure-function analysis of ELFN1 on mGluR7-dependent and -independent mechanisms

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Abstract

Background:

Targeted by over 35% of FDA-approved drugs, G protein-coupled receptors (GPCRs) have become key therapeutic targets for neurodevelopmental disorders (NDDs). Unfortunately, failures in translating therapeutic strategies from bench-to-bedside emphasizes our limited understanding of GPCR synaptic function and neurotransmission. Excitingly, our previous work illustrates that metabotropic glutamate receptors (mGluRs), a subset of GPCRs, are trans-synaptically modulated by the synaptic adhesion molecule extracellular leucine rich fibronectin type III domain 1 (ELFN1) – once thought to be simply structural. Interestingly, recent studies identified human ELFN1 variants with pathogenic and correlative roles in NDDs across the entirety of the protein.

Objectives:

This study aims to provide structure function analysis of ELFN1 on mGluR7-dependent and -independent functions in hopes of better predicting pathogenic consequences of ELFN1 variation.

Methods:

Structure-function analyses are performed using intracellular and extracellular truncations and clinical ELFN1 variants (ELFN1ΔCT, NTΔELFN1) to explore the molecular consequences on membrane-localization, dimerization, and transcellular mGluR7 interactions in co-culture. Statistical significance was determined using a students t-test or one-way ANOVA at a significance level of 0.05%.

Results:

We establish diverse autoregulatory roles, pinpointing an intracellular 30aa membrane-trafficking domain and an extracellular dimerization site distinct from its mGluR7-binding region. We highlight NTΔELFN1 differentially reduces mGluR7 interaction by 96% (p-value=0.0006), while ELFN1ΔCT has no impact (p-value=0.3720) compared to reference ELFN1.

Conclusions:

GPCRs are key players in brain cell communication and a major therapeutic target. Excitingly, we found ELFN1 variation differentially effects mGluR7-dependent and -independent functions, highlighting potential pathological mechanisms as targets in NDD treatment for individuals harbouring ELFN1 variants.