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17th Canadian Neuroscience Meeting Abstract Proceedings

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Back to top

Thank you to our exhibitors:

Table of Contents

PRESIDENTS WELCOME

Dear Colleagues and Friends

It is a great pleasure to welcome you to the 17th Annual Canadian Neuroscience Meeting in Vancouver. We once again have a great scientific program to offer, thanks to the hard work of Scientific Program Chair **Stephanie Fulton** and Co-Chair **Matt Hill**, who, together with the other members of the program committee, have brought together leading experts in neuroscience research from Canada and abroad, including: **Bill Newsome** (Stanford U), **Bita Moghaddam** (OHSU), **Michael Greenberg** (Harvard Medical School), **Baljit Khakh** (UCLA), **Isabelle Peretz** (U Montréal) & **Alon Chen** (Weizmann - Max Planck Laboratory).

Mark Cembrowski, Chair of the local organising committee, has organised an exciting series of **public lectures** on *Cannabis and Psychedelics: Hype or hope for addictions, brain diseases, and mental health*, featuring **Catharine Winstanley, Leah Mayo** and **Matt Hill.** This is a very timely subject with broad appeal and brings together a stellar team of speakers.

I am especially excited for the new **Trainee power pitch** sessions, which will take place at the beginning of each main poster session. This session, organised by trainees and for trainees, will be an opportunity for trainees at all levels to "pitch" their poster to the full CAN audience, in a series of very short (3 minute) talks. Congratulations to **J Quinn Lee** & **Gilberto Rojas Vite**, who proposed and organized these sessions.

Liisa Galea and **Tabrez Siddiqui,** co-chairs of the CAN Advocacy committee, have organized an **advocacy session** focused on the *power of storytelling* to strengthen our advocacy message. Stories are a powerful tool to engage the public, combat misinformation and increase research funding.

The CAN 2024 **EDI session**, organized by Co-Chairs **Derya Sargin** and **Jennifer Murray**, will feature a discussion on *Sex & Gender in the Neurosciences*, with panelists **Caroline Ménard, Bita Moghaddam, Liisa Galea** and **Justin Matheson**. Holding this session in the main meeting hall, right after the Keynote lecture, allows all our members to participate and engage in the conversation about this very important topic.

A special highlight of the CAN meeting is always the lectures by award-winning young neuroscientists. We are very pleased to feature a lecture by **CAN2024 New Investigator Award winner Caroline Ménard**, from Laval University. We will also host short talks by the top three Brain Star award winners, **Masayuki Hata, Adam Ramsaran** and **Hovy Ho-Wai Wong**. We are

also happy to announce **Nina Cluny**, Assistant Director of CIHR-INMHA, will be presenting the awards this year.

For the first time, the CAN meeting will host the **CIHR Canadian National Brain Bee Showdown**. This exciting event will take place on Tuesday, May 21 at lunch time. This is an opportunity to see the top three Canadian brain bee winners compete to determine the 1st, 2nd and 3rd place. The top place champion will advance to the next level of competition: the Internation Brain Bee.

I want to finish this letter by highlighting the importance of our **sponsors and exhibitors**, whose contributions allow us to present this great program of events at a reasonable cost to our attendees, the majority of which are trainees. We are grateful to all our sponsors for their very important support.

We look forward to welcoming you in Vancouver for this great event!

Adriana Di Polo, President of the Canadian Association for Neuroscience

KEYNOTE & PLENARY TALKS

Presidential Lecture: Detecting covert decision dynamics from neural population recordings in primate motor cortex

William Newsome, Stanford University

The neural mechanisms underlying decision-making are typically inferred from the average activity from sequentially recorded single neurons, which obscures important aspects of decision-making dynamics. I will show that covert decision variables (DV) can be tracked dynamically on single behavioral trials via simultaneous recording of large neural populations in primate motor cortex. The data reveal surprisingly large swings in DV during single trials, raising intriguing questions about the source(s) of this pronounced, rapid variability in internal decision states.

Featured Plenary Speaker 1: Challenges of designing animal models to test the therapeutic effects of psychedelics

Bita Moghaddam, Ohio Health and Science University

Psilocybin has the potential of enhancing the quality of life in individuals who are suffering from mood and substance use disorders, and whose symptoms have not responded positively to conventional drug therapy. Psilocybin also offers exciting new possibilities for enhancing our mechanistic understanding of the biological basis of these symptoms because it appears to be working on different cellular targets and brain pathways as most conventional pharmacological modes of treatment. All clinical data so far with psilocybin, which have reported positive clinical outcomes for mood and addictive disorders, have involved clinician-assisted intervention or psychological support. In this presentation I will focus on providing 1) a brief review of the literature showing that the context or the process of drug administration has been an integrative component of published work, 2) importance of future research to compare the efficacy of the drug (“pill”) as a stand-alone treatment vs drug in combination with clinician assisted psychological support or formal therapy (“process”), and 3) review of current work, and suggestions for future approaches, in animal models that take into account the role of systems and behavioral neuroscience in explaining a potential role for context, experience, and expectancy in drug effect.

Featured Plenary Speaker 2: Astrocyte-neuron interactions: critical for physiology and disease

Baljit Khakh, University of California, LA

The study of the brain, a highly complex multicellular organ, has witnessed spectacular advances in the last few decades. Despite this laudable progress, severe blind spots in our understanding of basic brain function and disease still remain. Many cell types that make up the brain are non-neuronal, and many aspects of these cells have been understudied or overlooked. The brain's non-neuronal cells include glia, which represent about half of all brain cells. I will describe a series of studies from my laboratory that provide new insights on predominant glial cells called astrocytes, named historically because of their alluring, yet deceptively simple, star-like shapes. I will address long-standing questions in neuroscience concerning the functions of astrocytes in the brain. After a period of intense exploration, it is now clear that signaling between astrocytes and neurons plays pivotal roles in both normal brain physiology and in disease conditions.

Featured Plenary Speaker 3: A paradigm shift in mouse phenotyping: The role of social context

Alon Chen, Weizmann-Max Planck Laboratory

Mental disorders are a significant cause of disability worldwide. They profoundly affect individuals' well-being and impose a substantial financial burden on societies. However, despite decades of extensive research, the effectiveness of current therapeutics for mental disorders is often not satisfactory or well tolerated. Moreover, most novel therapeutic candidates fail in clinical testing, which also brings into question the effectiveness of using animal models in preclinical studies. In this lecture I will present a paradigm shift in the methodologies used to measure animal behavior in laboratory settings. Behavioral readouts obtained from short, highly controlled tests in impoverished environments and social contexts as proxies for complex human behavioral disorders might be of limited face validity. Conversely, animal models that are monitored in more naturalistic environments over long periods display complex and ethologically relevant behaviors that reflect evolutionarily conserved endophenotypes of translational value. I will present how semi-natural setups in which groups of mice are individually tagged, and video recorded continuously can be attainable and affordable. Moreover, novel open-source machine-learning techniques for pose estimation enable continuous and automatic tracking of individual body parts in groups of rodents over long

periods. The trajectories of each individual animal can further be subjected to supervised machine learning algorithms for automatic detection of specific behaviors (or unsupervised automatic detection of behavioral motifs. Compared to studies of animals in the wild, semi-natural environments are more compatible with neural and genetic manipulation techniques. As such, they can be used to study the neurobiological mechanisms underlying naturalistic behavior.

Brain Prize Lecture: How Nature and Nurture Conspire to Regulate Brain Development and Plasticity

Michael Greenberg, Harvard Medical School

Experience-dependent neuronal activity plays a critical role in shaping the connectivity and function of the central nervous system. These actions are mediated in part by the action of a program of neuronal activity-driven gene expression. Investigation of these gene expression programs has uncovered important roles in dendritic growth, the development of excitatory and inhibitory synapses, the composition of protein complexes at pre- and post-synaptic sites, and the production of neuropeptides that control neural circuit development. Moreover, defects in the activity-dependent gene program contribute to disorders of human cognition. Thus, study of this transcriptional response promises new insights into neuronal plasticity and disease.

New Investigator Award: Neurovascular adaptations underlying mood disorders vs stress resilience

Caroline Ménard, Université Laval

Our research program aims to shed light on the biological mechanisms underlying stress vulnerability vs resilience, with help of state-of-the-art photonic technology, in order to develop innovative treatments and identify biomarkers of mood disorders. Our multidisciplinary approach combines behavioral experiments to functional, cellular, molecular, and imaging studies and validation of our rodent findings in human samples. We showed that chronic stress exposure promotes blood-brain barrier and gut barrier hyperpermeability leading to passage of circulating inflammatory mediators into the brain and the establishment of depressive behaviors. These changes occur in a sex-specific manner which may contribute to sex differences in depression prevalence, symptoms and treatment responses.

Keynote Lecturer: Singing in The Brain

Isabelle Peretz, Université de Montréal

Singing, a natural and widespread human behavior, is processed uniquely in the human brain. Strong evidence supports its universality, debunking the common misconception that singing is limited to a select few. Remarkably, even those considered poor singers can accurately improvise. Moreover, singing often yields a profoundly enjoyable experience, particularly when performed in groups. Melodies sung are notably more memorable than instrumental renditions. Furthermore, the cortical networks recruited by songs are distinct from those involved in speech or instrumental music. Hence, singing holds significant biological importance, underscoring its fundamental role in human communication and expression.

PLENARY SYMPOSIA

Plenary Symposium 1: Psychedelics as novel therapeutics: What do we know and where do we go from here?

Multi-Region Effects of Psychedelic drugs on Neuronal Activity and Threat Responding Behavior

Melissa A. Herman, University of North Carolina

Current research suggests that the psychedelic compound psilocybin, or it's active metabolite psilocin, may have therapeutic efficacy in the treatment of psychiatric conditions including anxiety, treatment-resistant depression, and substance use disorders. While clinical results are promising, the underlying neurobiology and brain region-specific effects of psilocin remain poorly understood. Preclinical studies of the divergent and sex-specific effects of psilocin in key brain regions including the prefrontal cortex (PFC) central amygdala (CeA), and paraventricular nucleus of the hypothalamus (PVN) are warranted. Using electrophysiology, we found that psilocin has variable effects on excitability in the mouse PFC, but consistently increases firing in 5-HT2A PFC neurons via actions at the 5-HT2A receptor and intracellular Gq signaling. Psilocin decreases excitability of individual CeA neurons in mice, potentially through population-specific actions on local CeA microcircuitry. A single psilocin injection in rats produces sex- and region-specific effects on reactivity to an aversive stimulus. Psilocin produces acute increases in CeA reactivity in females, potentially through projections from paraventricular thalamus. In contrast, psilocin produces prolonged decreases in CeA reactivity in males, effects that are largely driven

by rats displaying active baseline threat responding. Psilocin increases reactivity in the PVN of male rats and decreases threat response behavior, particularly in rats with an active threat response at baseline. Collectively, these findings identify complex and region-specific actions of psilocin that may provide key mechanistic insight into the potential therapeutic effects of psychedelic compounds like psilocybin.

In search of mechanism: exploring the therapeutic potential of psilocybin in preclinical models

Rosemary Bagot, McGill University

Promising data from clinical trials suggests that the psychedelic drug, psilocybin, has the potential to rapidly and enduringly alleviate symptoms of depression. Clinical and pre-clinical data point to the prefrontal cortex (PFC), a key brain region implicated in depression and resilience, as a potential locus of psilocybin's antidepressant effects. However, much remains unknown about the specific cell-types that are impacted by psilocybin and how a single dose can mediate enduring effects. Using a mouse model, we confirm that a single injection of psilocybin induces lasting behavioural effects, which, surprisingly, are more pronounced in female than male mice. This was observed despite similar acute modulation of head-twitch response in both sexes. To begin to understand the molecular mechanisms and specific cell-types that mediate the enduring effects of psilocybin, we used single-cell sequencing to probe cell-type specific changes in medial prefrontal cortex (mPFC) in female mice 24h after a single injection of drug. Our analyses reveal prominent cell type specific transcriptional modulation indicating opposing effects in intermingled cell types. Exploring the molecular identity of the most impacted cell-types indicates that 5HT2A receptor expression does not account for observed cell-type specificity and rather suggests a role for additional serotonin receptors in lasting effects of psilocybin in mPFC. Ongoing research is exploring the behavioural and functional consequences of this cell-type specific modulation by psilocybin. Ultimately, our findings demonstrate that understanding how psilocybin exerts enduring effects will require careful consideration of a range of factors including sex and cell-type specificity.

Deconstructing the psychedelic experience: insights across species

Boris Heifets, Stanford University

Rapid acting, profoundly psychoactive therapeutic drugs like ketamine, MDMA and psilocybin (broadly considered 'psychedelics') offer exciting new avenues for treating neuropsychiatric disorders. These therapies are also quite complex to deliver and understand from a mechanistic

perspective, requiring new approaches to clinical trial design and preclinical testing. Mental health benefits of psychedelic medicine have variously been attributed to biochemical actions of the drug, the psychedelic experience, and non-drug factors, like expectancy and 'hype', associated with conducting psychedelic trials. These factors reflect the current realities of psychedelic therapy, which involves many hours of psychological support in preparation for a psychedelic experience, the psychedelic experience itself, and integration of the experience into patients' lives with the goal of cementing changes in behavior and outlook. Human studies have emphasized extra-pharmacological factors that could modulate psychedelic-induced therapeutic responses including set, setting, and integration – factors that we are only beginning to incorporate into animal models. In this talk I will discuss progress in modeling the acute and persistent behavioral effects of psychedelics, and how this work aligns with what we know from human studies. I will also present some novel approaches to get at the question of “does the trip matter?”, including the development of non-hallucinogenic psychedelic analogues, masking the drug experience with anesthesia, and comparing the phenomenology, physiology and therapeutic effects of psychedelics with related altered states of consciousness.
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Plenary Symposium 2: Glial impact on neuronal function

Microglial remodelling of the extracellular matrix: outcomes on synaptic plasticity and behaviour

Marie-Eve Tremblay, University of Victoria

Microglia, the brain's resident immune cells, are essential regulators of synaptic plasticity and extracellular matrix (ECM) composition. Recently, it was demonstrated that microglial modulation by the fast-acting antidepressant ketamine promotes a marked reinstatement of juvenile-like plasticity in the adult brain through the remodelling of perineuronal nets (PNNs). PNNs are specialized ECM structures that were proposed to serve as the gates of synaptic plasticity in the adult brain. This microglial effect on PNNs was associated with ketamine's ability to entrain brain circuits in the spectrum of gamma oscillations (30–100 Hz), which are severely impaired in multiple neuropathology conditions, ranging from major depression to Alzheimer's disease. Light flickering stimulation (LFS) provides a non-invasive treatment strategy to entrain brain oscillations in the gamma spectrum, which has emerged as a promising approach to rescue neurodegeneration hallmarks (e.g., amyloid-beta pathology) and associated cognitive impairment. In my talk, I will present our recent findings describing the profile of LFS actions on

microglial density, distribution, morphology and ultrastructure, their interactions with markers of PNN assembly and remodelling, as well as their outcomes on synaptic plasticity and behaviour. Overall, this work provides novel evidence that will help pave the way to better understanding the roles of microglia in controlling synaptic plasticity and behaviour in the adult brain. Further, with these findings, we hope to propose novel strategies, notably LFS, that allow to rescue impaired synaptic plasticity associated with a range of neuropsychiatric and neurodegenerative disorders through microglial modulation.

Glia regulate network states to modulate processing of sensory information
Ed Ruthazer, McGill University

In vivo 2-photon calcium imaging of GCaMP-expressing cells in the optic tectum, the primary visual centre of the amphibian brain was performed in intact Xenopus laevis tadpoles. Neurons and astrocytes in the tectum both exhibited calcium transients with very different timecourses in response to visual stimulation: neurons presented rapid, brief responses, while astrocytes showed much slower, delayed calcium elevations. Perfusion of the tectum with the neuromodulator norepinephrine (NE), associated with states of alertness, resulted in the robust induction of calcium transients in the tectal astrocytes. We observed that this intense glial activation, which was mediated by alpha-1-adrenoceptors, was accompanied by a significant reduction in neuronal spontaneous and visually-evoked activity. Interestingly, the decrease in neuronal activity was specific to cells whose responses preferred innocuous stimuli such as small moving dots, while leaving unaltered the responses to more threatening stimuli like a rapidly looming dark spot. This transition between visual response states was mediated by glial activity, as it could be mimicked by direct chemogenetic activation of the astrocytes in the absence of NE. Behaviorally, glial activation also made tadpoles more likely to attempt to escape a dark looming stimulus. In electrophysiological whole cell recordings, tectal NE perfusion significantly reduced the frequency of miniature synaptic events, but had no measurable effect on the intrinsic excitability of tectal neurons. Taken together, our findings suggest that astrocytes act at tectal synapses to mediate profound changes in how the visual system responds to sensory input under different states of neuromodulation. Funded by the CIHR and Brain Canada

Microglia as modulators of neuro-glial interactions
Masha Prager-Khoutorsky, McGill University

Neuro-glial interactions play a central role in regulating synaptic transmission and neuronal excitability. Structural plasticity of astrocytes is associated with numerous physiological and pathological conditions, however the mechanism underlying this process remains unknown. To examine the basis for structural astrocyte plasticity we used the classic example of loss of astrocytic processes that takes place in the hypothalamic magnocellular system during chronic high salt intake. We discovered that a high salt diet triggers a local accumulation of reactive microglia around vasopressin-secreting neurons, but not in other brain areas. Microglia phagocytose astrocytic processes, reducing astrocytic coverage of vasopressin neurons. The pruning of astrocytic processes impairs synaptic glutamate clearance, enabling activation of extrasynaptic glutamate NMDA receptors and increasing the activity of vasopressin neurons. Inhibiting microglia-mediated astrocyte pruning attenuates vasopressin-dependent hypertensive phenotype of rats fed high dietary salt. Thus, structural astrocyte plasticity is mediated by microglia that orchestrate neuro-glial interactions via astrocyte pruning.

Plenary Symposium 3: Social Neuroscience

Olfactory bulb astrocytes determine the cognitive impact of social transmission of stress
Giovanni Marsicanom INSERM

Social odors transmit emotions and alter behavior. Indeed, olfactory information, transmitted from stressed individuals, induces stress-like physiological and synaptic changes in naïve partners. Direct stress experience alters cognition, but whether socially transmitted stress can also alter memory processes is currently unknown. Here we show that social investigation of a stressed individual, or exposure to specific olfactory signals from that individual, is sufficient to impair novel object recognition (NOR) in unstressed male mice. This requires mitochondria-associated cannabinoid type-1 (mtCB1) receptors and Mitochondrial Calcium Uniporter (MCU) activity. Targeted genetic manipulations, in vivo mitochondrial calcium imaging and behavioral analyses revealed that olfactory bulb astrocytic mitochondrial calcium regulation is necessary to specifically process odors coming from stressed partners and to define their cognitive consequences. Thus, olfactory bulb astrocytes provide a link between social odors and their behavioral meaning.

Neuropeptide nexus: Decoding oxytocin's evolutionarily conserved role on social contagion in zebrafish
Ibukun Akinrinade, University of Toronto

Survival in social environments is complex and requires information acquisition and processing for appropriate decision-making. It also demands the right balance between the interests of the self and the interests of others to make appropriate social decisions and generate relevant prosocial behaviours such as helping behaviour and altruism, the hallmark of empathy. Empathy is described as an individual's ability to understand and share the affective states of others. It is often considered one of the main factors driving prosocial behaviours. Conceptually, empathy ranges from simple emotional behaviours, such as mimicry and emotional contagion, to evolutionarily more complex behaviours, such as perspective-taking and helping.
We tested to what extent the proximate mechanisms of emotional contagion (the most ancestral form of empathy) are evolutionarily conserved by assessing the role of oxytocin, known to regulate empathic behaviours in mammals, in social fear contagion in zebrafish. Using oxytocin and oxytocin receptor mutants, we show that oxytocin is both necessary and sufficient for observer zebrafish to imitate the distressed behaviour of conspecific demonstrators. The brain regions associated with emotional contagion in zebrafish are homologous to those involved in the same process in rodents (striatum, lateral septum), receiving direct projections from oxytocinergic neurons in the preoptic area. Our results support an evolutionarily conserved role for oxytocin as a critical regulator of basic empathic behaviours across vertebrates.

Effects of Stress on Opioid Self-Administration and Subjective Experience in the Laboratory and the Operating Room

Marie Eikemo, University of Oslo

A mechanistic hypothesis for opioids' abuse liability rests on the belief that in addition to pain relief, acute opioid treatment relieves stress and anxiety and improves well-being. We tested how stress affects human opioid drug responses across one experimental and two clinical studies. In a double-blind placebo-controlled, randomized 4-session crossover study of 63 healthy volunteers (31 men) social stress or a control state was induced before injection of a moderate - but noticeable - dose of oxycodone (3.1mg/70kg) or saline. Shortly after, participants worked to obtain 0-125% of the first dose effect in a self-administration task. The resulting dose was given ~40 min later. In two cohorts of surgery patients, we recorded how good and how anxious patients felt before and after opioid injections on the operating table before anesthesia. In the first study (N=269), open-label remifentanil (effect site 5ng/ml) or

oxycodone (5 mg) was used. In the second, which was an RCT (N=210), morphine (10mg), oxycodone (5mg) and fentanyl (0.1mcg) was assessed. In the healthy volunteers, pre-drug stress increased oxycodone self-administration, an effect entirely driven by the men, who administered 15% more oxycodone following stress, although women showed higher stress responses across subjective, physiological and endocrine measures. In patients, opioids had modest anxiolytic effects (~d=.25), but there was robust evidence against group-level improvement in opioid-enhanced well-being. Notably, higher pre-surgery stress and prior opioid use predicted more positive opioid effects. In all, stress can impact the subjective opioid experience and shape drug motivation, also in individuals without an history of opioid misuse.

PARALLEL SYMPOSIA

Parallel Symposium 01: Bridging the Gap: Animal and Human Models in Neurodevelopmental Disorder Research

Genetic mutations perturbing nervous system development are a major cause of neurodevelopmental disorders (NDD). Despite recent advancements in gene discovery and NDD diagnosis, effective treatments are still scarce. Bridging the gap from gene to treatment requires an in-depth dissection of gene function, neurobiological pathways, and brain pathophysiology and necessitates integrating insights from complementary model systems, extending from human cells to various animal models. This panel features four speakers: Deborah Kurrasch (University of Calgary), Yun Li (The Hospital for Sick Children), Guang Yang (University of Calgary), and Yang Zhou (McGill University). They will discuss novel insights on the mechanistic basis of NDD, highlighting the power of phenotypic modeling and functional characterization in diverse genetically engineered model systems, including stem cell-derived human neurons, zebrafish, mice, and non-human primates. These collective talks will provide convergent evidence that advances our understanding of NDD, shedding light on relevant phenotypes and promising targets for therapeutic intervention.

PS.01.01 - Zebrafish recapitulate human phenotypes and enable the study of developmental epileptic encephalopathies

Deborah Kurrasch [1], **Deepika Dogra** [1]
[1] University of Calgary

BACKGROUND AND AIM: Children with developmental epileptic encephalopathies (DEEs) are largely pharmacoresistant to current anti-seizure medications, leading to unremitting seizures and concomitant developmental delays. A better understanding of the underlying etiology of these DEEs is necessary to reveal new druggable targets. Despite decades of research using mouse models of DEEs, many fail to recapitulate the full phenotypes observed in the children, raising concerns of the translatability from humans to rodents.

METHODS: Here, we used CRISPR/cas9 to create zebrafish lines of DEEs and characterized their epilepsy phenotypes using behavioral, bioenergetic, and electrophysiological assays.

RESULTS: We show that zebrafish genetic models display hallmark features of these epilepsies, including changes in excitatory and inhibitory neuronal populations. We also demonstrate a hyperexcitable phenotype and seizure-like activity that is reduced to baseline upon exposure to approved anti-seizure drugs. In this talk, I focus on two zebrafish models we created and our mechanistic findings into neuronal pathways that are changed. I will also compare our findings to data from corresponding mouse models, highlighting the utility of zebrafish in the study of epilepsy

CONCLUSIONS: Our studies here suggest that zebrafish are an especially useful model for the study of DEEs, displaying robust and high numbers of daily seizures not observed in mouse models. We propose zebrafish models of DEEs can be especially useful for studying the underlying biology of these disorders and also drug screening.

PS.01.02 - Investigating mTOR hyperactivation related neurodevelopmental disorders in human brain cells and organoids

Navroop Dhaliwal [1], **Octavia Weng** [1], **Yun Li** [2]
[1] The Hospital for Sick Children, [2] University of Toronto

Background and Aim:

The mechanistic target of rapamycin (mTOR) pathway is a crucial regulator of cellular function in the brain. Dysregulation of the mTOR pathway has been implicated in a wide spectrum of neurological conditions. In particular, mutations that hyperactivate the mTOR pathway result in similar clinical presentations including macrocephaly, intractable seizures, developmental delays and autism, and are now collectively known as â€œmTORopathiesâ€. Despite promising therapeutic developments in animal models, most drug candidates failed to show efficacies in patients.

Methods:

In the current study, we directly examined how mTOR hyperactivation impacts the development and function of human neural cells. We focused on the phosphatase and tensin homologue (PTEN) gene, which encodes a negative regulator of the mTOR pathway. Our study combines human pluripotent stem cells, CRISPR gene editing and brain organoid technologies.

Results:

We showed that pluripotent stem cells derived PTEN mutant human neurons, neural precursors and cortical organoids recapitulate disease-relevant phenotypes, including neuronal hypertrophy, electrical hyperactivity, enhanced proliferation, and structural overgrowth. We further dissected the contribution of mTORC1 and mTORC2 to the disease phenotypes, by generating double mutants of PTEN and RPTOR and RICTOR.

Conclusions:

Our study revealed that the synergistic hyperactivation of both mTORC1 and mTORC2 underlies the neural abnormalities of PTEN mutant human neural cells and organoids. Together, our findings provide new insights into the molecular mechanisms of mTORopathies and highlight novel therapeutic targets.

PS.01.03 - Deciphering rare neurodevelopmental disorders: insights from human cell and mouse models

Guang Yang [1]
[1] University of Calgary

Approximately 1 in 12 Canadians are impacted by rare genetic diseases, many of which affect brain development and function, resulting in long-lasting cognitive and behavioral defects. To develop effective treatments, it is crucial to understand the molecular and cellular mechanisms that mediate the effects of these genetic mutations on the brain. However, the roles of many genes linked to these rare conditions in the brain remain unknown, further posing significant challenges in determining the functional relevance of specific mutations. Here, we will focus on a group of rare neurodevelopmental disorders. Through insights gained from mouse models and patient-derived cells, we will discuss the novel roles of the disease-causing genes in the genesis and function of neurons, the impact of patient mutations, and potential therapeutic avenues.

PS.01.04 - A role of ASD-associated gene CHD8 in reactive gliosis in the adult mouse and marmoset brains

Yang Zhou [1]
[1] McGill University

Mutation of gene encoding chromodomain helicase DNA-binding protein 8 (CHD8) represents a high-confident genetic risk factor for ASD and neurodevelopmental disorders. Prior characterization of CHD8 concentrated on stem cells and germline-mutated model organisms, which shed light on its functions in development. However, little is known about the cellular function of CHD8 in the matured brain. Using mouse genetic approaches, we delineated the role of astrocytic CHD8 in orchestrating reactive gliosis and neuroinflammatory processes in the adult brain. Given that excessive reactive gliosis and neuroinflammation are hallmark features in neurodegeneration and injury. We engineered an AAV-mediated Chd8 gene editing strategy and revealed that injury-induced reactive gliosis can be directly mitigated in mouse and marmoset brains. I will introduce ongoing efforts and discuss the potential of targeting CHD8 for alleviating neuroinflammation and brain pathophysiology in experimental models of neurological disorders.

Parallel Symposium 02: New insights into immediate early genes (IEGs) in neural circuit plasticity during development and learning

Immediate-early genes (IEGs) are rapidly activated after sensory and behavioral experience, and they initiate cascades of gene expression that are believed to be crucial for converting experience into long-term circuit changes during development and learning. In this symposium, speakers will present results using newly-developed genetic tools, single-cell RNAseq, electrophysiology, and two-photon imaging to provide new insights into the function of IEGs in circuit plasticity during development and learning. Dr. Yingxi Lin will address how IEGs are involved in identifying cell-type and layer-specific maturation during development. Dr. Jason Shephard will reveal how Arc forms virus-like protein capsids to mediate a novel intercellular communication pathway. Dr. Simon Chen will show how NPAS4 is expressed in a specific subset of interneurons that is critical for motor memory formation. Lastly, Dr. Jung Ding will demonstrate the connections between motor cortex and striatum are strengthened during motor learning using the FOS-TRAP strategy.

PS.02.01 - Early cellular memories reveal the sequence and mechanisms of circuit maturation during early development

Yingxi Lin [1]
[1] University of Texas

Immediate-early gene (IEG) induction by experience marks the sites where cellular memories are created to shape future neural circuit functions. While they are believed to be components of the memory engram, the initial formation of these cellular memories during development

has not been systematically examined. In this study, we introduce a novel reporter system, DevATLAS (Developmental Activation Timing Longitudinal Acquisition System), designed to capture the initial events of cellular memory encoding during the early postnatal period. This developmental phase plays a pivotal role in the formation of neural circuits governing lifelong behavioral and cognitive functions, coinciding with the onset of symptoms in numerous neurodevelopmental disorders (NDDs).

DevATLAS captures neurons undergoing synaptic maturation, shedding light on the crucial roles of early cellular memories in the maturation of circuits. When combined with our newly developed whole brain imaging and analysis pipeline, DevATLAS enables the construction of a spatiotemporal map, unveiling the maturation and assembly of circuits across the brain during early development. Notably, DevATLAS elucidates the developmental sequence of hippocampus-dependent memory formation, a process occurring later than the development of circuits involved in primary sensory processing. We present evidence that early enriched environment accelerates the maturation of hippocampus-dependent learning by enlarging the synaptically mature neuron population in the dentate gyrus. Using single-cell RNA-sequencing, we have uncovered novel molecular pathways and critical genes promoting the maturation of the hippocampal learning circuit. Furthermore, employing the whole-brain imaging and analysis pipeline, DevATLAS proves to be effective in uncovering when and where neurodevelopment is perturbed in mouse models of NDDs, establishing it as a powerful tool for studying underlying disease mechanisms.

PS.02.02 - Virus-like intercellular synaptic plasticity

Jason Shepherd [1]

[1] University of Utah

BACKGROUND AND AIM: Current models of learning and memory have focused on cell-autonomous regulation of synaptic strength; however, intercellular signaling between cells in the brain is critical for normal cognition. The immediate early gene *Arc* is a repurposed retrotransposon critical for long-term forms of synaptic plasticity and memory. Arc protein forms virus-like capsids released in extracellular vesicles (EVs) that signal cell-to-cell but the function of this pathway is unknown.

METHODS: We used primary cultured neurons from mice and used various biochemical and cell biological assays to determine Arc release into EVs, EV purification, Arc-IRSp53 interactions, and AMPA receptor trafficking. We also used protein purification and electron microscopy to assess Arc capsid assembly.

RESULTS: We found that long-term potentiation (LTP) stimuli induce the biogenesis of Arc EVs by recruiting the I-BAR protein IRSp53 to dendrites, which facilitates Arc capsid assembly and release. Arc EVs transfer Arc protein and mRNA to neighboring neurons, where translation of transferred *Arc* mRNA induces a loss of surface AMPA-type glutamate receptors.

CONCLUSIONS: These results show that Arc EVs mediate non-cell autonomous long-term depression (LTD), revealing an intercellular form of synaptic plasticity that may be critical for memory consolidation.

PS.02.03 - Distinct NPAS4-expressing SST-IN inhibitory ensembles critical for different movement acquisition

Simon Chen [1], **Jungwoo Yang** [1], **Pablo Serrano** [1], **Xuming Yin** [1], **Jan Sidiangco** [1]
[1] University of Ottawa

Local GABAergic inhibitory neurons are known to play a critical role in memory formation and allocation by modulating the level of inhibition to downstream excitatory neuronal ensembles. During motor learning, dendritic spines on pyramidal neurons (PNs) in the motor cortex undergo reorganization, which coincides with axonal bouton remodeling in local somatostatin-expressing inhibitory neurons (SST-INs) and parvalbumin-expressing inhibitory neurons (PV-INs). Intriguingly, SST-mediated inhibition plays an important role in regulating the plasticity of downstream PNs and thus the acquisition of motor skills. However, the molecular mechanisms that underlie the selective engagement of particular SST-INs and modification of their inhibitory output during learning remain unclear. Here, we identified the activity-dependent transcription factor, NPAS4, is specifically expressed in SST-INs, but not in PV-INs or PNs, during motor learning. By combining in vivo two-photon imaging with a head-fixed pellet reaching motor learning task, we found that NPAS4-expressing SST-INs (NRAM+ SST-INs) activity throughout the entire motor learning process, and we found a reduction in the activity of NRAM+ SST-INs during reaching-related events compared to NRAM- SST-INs. Chemogenetically increasing the activity of NPAS4-expressing ensembles was sufficient to mimic the effects of Npa4 deletion in SST-INs. Preliminary data in the lab also reveals that learning two separate motor movements induces distinct NPAS4-expressing SST-IN ensembles in the motor cortex. Together, our results reveal an instructive role of NPAS4 within the motor circuits, in which it modulates the inhibition of a distinct subset of SST-INs during motor learning are critical for motor skill acquisition.

PS.02.04 - Motor learning induced transcriptomic changes in motor engram neurons

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Motor learning induces profound structural and activity changes in primary motor cortical (M1) neurons and striatal spiny projection neurons (SPNs). However, the molecular profile of motor engram cells and the underlying transcriptome regulation mechanism in motor learning remain unclear. Here, we combined activity-dependent genetic labeling tools (TRAP mice) and single cell RNA sequencing (scRNA-seq) to identify cell types and gene expression regulation mechanisms involved in motor learning and memory formation. TRAP mice were trained in a forelimb reaching task, and specifically activated cells in early and late training stages were TRAPed. Through scRNA-seq analysis, we identified TRAPed engram neurons in various neuronal populations, including corticostriatal projection neurons. By quantifying the percentage of TRAP cells in each cell type, we investigated preferential activation during different stages of motor learning. Notably, we observed significant changes in activation in interneuron populations, particularly in a specific M1 interneuron type expressing 5-Hydroxytryptamine receptor 3a (Htr3a-Int). Using 2-photon Ca imaging, we validated the correlation between Htr3a-Int activity and reaching behavior, suggesting their involvement in motor learning. Furthermore, we detected significant differential expression of genes associated with synaptic functions and movement disorders during early and late learning stages in M1 and striatum. Our scTRAP-seq findings, coupled with subsequent validations, identified novel cell types in corticostriatal circuits involved in motor learning, and provided mechanistic insights into pre- and postsynaptic genes regulating synaptic plasticity induced by motor learning.

Parallel Symposium 03: Sculpting Brain and Behaviour: The Profound Impact of Early Life Adversities

Chronic childhood stress profoundly affects brain development and increases the risk of anxiety and mood disorders, yet the underlying neurobiological mechanisms remain elusive. This session will delve into animal models of early life adversity (ELA) to better understand circuit dysfunction and inform potential treatments. Dr. Flores will highlight the effect of adolescent social stress on dopamine circuits and prosocial behaviour, demonstrating a novel approach to studying ELA in both sexes, with translational findings on epigenetic markers in adolescent depression. Dr. Opendak will focus on the enduring effects of stressors on brain structure, function, behaviour and peripheral factors including metabolome and microbiome, utilizing natural ELA models. Dr. Anacker will discuss the role of hippocampal circuits in shaping ELA-induced sex differences, and stimulation approaches to mitigate ELA-induced fear overgeneralization. Dr. Sargin will present her lab's latest research on the impact of postnatal stress affects brain-wide connectivity and stimulation treatments for stress-induced anxiety.

PS.03.01 - Benefits and costs: resilience to social stress in adolescence

Cecilia Flores [1]
[1] McGill University

BACKGROUND: Navigating adolescence involves confronting social stressors that can significantly impact mental health trajectory. Understanding how adolescent social stress impacts males and females differently and why certain individuals are more susceptible than others is becoming increasingly urgent. While existing models have effectively replicated physical and psychological aspects of social defeat stress on adolescent male mice, adapting these models for females has presented challenges.

METHODS: We recently introduced a tailored version of the accelerated social defeat stress (AcSD) paradigm specifically designed for adolescent female mice. We subjected early adolescent C57BL/6J female mice to the modified AcSD protocol, distinguishing between resilient and susceptible groups based on a social interaction test. In adulthood, we evaluated females in inhibitory control through the Go/No-Go task. To begin addressing the underlying molecular and cellular mechanisms, we measured changes in the expression of the Netrin-1/DCC guidance cue system and combined dual viral infection and quantitative neuroanatomy strategies to track dopamine axon growth and connectivity.

RESULTS: While most females displayed resilience against stress-induced social avoidance in adolescence, cognitive assessment in adulthood revealed a different outcome. Resilient females exhibited deficits in inhibitory control, contrasting with susceptible counterparts who showed protections against cognitive impairments. We found robust errors in dopamine axon targeting and growth in adolescence as well as alterations in dopamine synaptic connectivity in adulthood. Notably, social phenotype in adolescence dictated the constellation of dopamine changes. Unlike previous observations in males, AcSD did not influence Netrin-1/DCC expression in adolescent females, indicating that dysregulation of other guidance cues may be at play.

CONCLUSIONS: The preservation of prosocial behavior in adolescent females appears vital for survival, albeit accompanied by enduring cognitive and dopamine deficiencies. The female-specific AcSD paradigm produces findings comparable to those found in male mice, enabling deeper exploration of cellular and molecular mechanisms in both sexes.

PS.03.02 - Neurobehavioral signatures of early social adversity in the rat pup

Maya Opendak [1]
[1] Johns Hopkins University

Background and Aim: Flexible social behavior is critical during early life when environmental demands are in flux. Yet, heightened circuit plasticity during this period also renders the infant vulnerable to environmental influences that guide lifelong social behavior. The circuit mechanisms linking early adversity experience to lasting social behavior patterns are unclear.

Methods: We present neurobehavioral results using two complementary models of early adversity: a naturalistic model of low bedding and nesting, and a deconstructed paradigm in which rat pups are repeatedly stressed alone or in the presence of the mother rat. This approach permits us to identify specific effects of stress occurring in a social context. Rats underwent these procedures from postnatal days 8-12, a sensitive window for scaffolding lifelong socio-affective behavior.

Results: We will discuss immediate and lasting impacts of these stressors on structure and function of the basolateral amygdala (BLA) and lateral habenula (LHb). We present data on volume, gene expression, and baseline neuronal firing patterns across early development, as well as peripheral measures, including metabolomic profiles of infant and adolescent pups exposed to social and non-social stress. We also present data on social behavior across early development and results from optogenetic and chemogenetic manipulations identigying specific circuit impairments that are causal in these effects.

Conclusion: Together, these results describe specific early biomarkers of early adversity and targets for age-appropriate therapeutics and interventions for social behavior repair.

PS.03.03 - Early Life Adversity and Fear Generalization - A Role for Serotonin in the Dentate Gyrus

Christoph Anacker [1]
[1] Columbia University

BACKGROUND AND AIM: Understanding the neurobiological mechanisms underlying early life adversity (ELA) effects on fear generalization is important for the discovery of new preventions or treatments for psychiatric disorders. Our previous findings had shown that hyperactivity of the ventral dentate gyrus (vDG) region of the hippocampus confers susceptibility to stress in adulthood. Here, we investigated if early life adversity (ELA) - a major risk factor for psychiatric disorders - may predispose to vDG hyperactivity in adulthood, thereby promoting enhanced vulnerability to stress and fear overgeneralization in adulthood. Moreover, we tested if inhibitory neurotransmitter signaling via serotonin (5-HT) in the vDG can confer resilience to ELA effects on fear generalization.

METHODS: We exposed mice to the limited bedding and nesting (LBN) model of ELA from postnatal day (P) 3-10 and disinhibited 5-HT neurons using postnatal transgenic knockdown of 5-HT1A autoreceptors. Neural activity and 5-HT release were recorded in male and female vDG using *in vivo* fiberphotometry during a contextual fear discrimination task. Inhibitory hM_4D_i DREADD receptors were virally injected into the vDG to directly silence granule cells during contextual fear discrimination.

RESULTS: LBN-exposed and standard-reared females showed similar fear retrieval in the conditioned context A, and increased fear expression (freezing) in a novel context B (CTRL: 5.8Â±1.1sec,LBN: 17.7Â±2.9sec;***p=0.002, n=15-19). During generalization in the novel context B, vDG granule cell activity was increased (CTRL: 1.3Â±21.2 arbitrary units [AU],LBN: 108.7Â±52.8 AU; *p=0.04, n=6-9), and 5-HT decreased (CTRL: 44.2Â±5.4sec, LBN: 75.9Â±4.33sec; *p=0.03, n=5-6). Disinhibiting 5-HT neurons via knockdown of raphe 5-HT1A autoreceptors rescued LBN effects on vDG hyperactivity and generalization in females (Interaction F(1,49)=22.3; ***p<0.001, n=14-18). Direct vDG inhibition through hM_4D_i activation in context B recapitulated these effects of 5-HT neuron disinhibition and also rescued LBN effects on generalization in females (Interaction F(1,32)=5.3;*p=0.03; n=7-19). No effects of LBN, 5-HT, or direct vDG inhibition were observed in males (n=8-12).

CONCLUSIONS: Our findings indicate female-specific effects of ELA on fear generalization that are mediated by 5-HT regulation of vDG hyperactivity.

PS.03.04 - Unraveling the impact of early life stress on neural circuits

Raksha Ramkumar [1], **Moriah Edge-Partington** [1], **Dylan Terstege** [1], **Kabirat Adigun** [1], **Yi Ren** [1], **Nazmus Khan** [1], **Nahid Rouhi** [1], **Naila Jamani** [1], **Mio Tsutsui** [1], **Jonathan Epp** [1], **Derya Sargin** [1]
[1] University of Calgary

BACKGROUND AND AIM: Chronic childhood stress is a prominent risk factor for developing mood disorders, yet mechanisms underlying this association remain unclear. Serotonin plays a crucial role in neurodevelopment and vulnerability to mood disorders. Maintenance of optimal serotonin levels during early postnatal development is critical for the maturation of brain circuits. Developmental stress can alter the serotonin system, leading to chronic behavioural deficits. Yet, our understanding of the long-term impact of early life stress (ELS) on serotonin connectivity remains incomplete. Here, using a mouse model of chronic developmental stress, we sought to determine how ELS impacts brain-wide serotonin activity and behaviour in adulthood.

METHODS: Female and male mice were exposed to a limited bedding and nesting chronic stress paradigm during the first postnatal week. When mice reached adulthood, we performed behavioural analysis to determine anxiety-like behaviours, and stress-coping strategies. Using

control and ELS FosTRAP mice, we cross-correlated regional c-fos density across all mice within each group and used raphe nucleus as a seed region to determine its functional connectivity with numerous other brain regions. We next performed *in vivo* calcium imaging to reveal how serotonin neuron population responds to threat in control and ELS conditions. Following this, we determined the c-fos based activity levels on the downstream serotonin-modulated circuits and performed fiber photometry in selected brain regions to determine ELS induced changes in serotonin dynamics. Next, we optogenetically stimulated selected circuits to overcome ELS-induced deficits in anxiety-like behavior in mice exposed to ELS.

RESULTS: We first established that adult female and male mice exposed to ELS during the first postnatal week show increased anxiety-like behavior. We found that ELS enhances susceptibility to acute stress by disrupting the brain-wide functional connectivity of the raphe nucleus and the activity of the dorsal raphe serotonin neuron population, in conjunction with a profound increase in the orbitofrontal cortex (OFC) activity. We further identified that serotonin release in the medial OFC during environmental challenge is disrupted in mice exposed to ELS. Optogenetic stimulation of 5-HT terminals in the medial OFC elicited an anxiolytic effect in ELS mice in a sex-dependent manner.

CONCLUSIONS: Our findings highlight the potential of combining targeted stimulation and pharmacotherapies to improve serotonin neurotransmission as a promising approach for treating emotional dysregulation that arises from childhood stress.

Parallel Symposium 04: Brain circuits controlling the formation, modification, and prevention of memory

Memory function requires neural activity within brain regions including the neocortex and hippocampus. Distinct aspects of the memory process require dedicated cell-types and neural connections within and between these regions. This symposium will highlight recent advancements in dissecting the neuronal sub-types and connectivity motifs that enable the formation, modification, or prevention of memories. The speakers will present data showing how specific inhibitory and excitatory circuits in the hippocampal formation, neocortex, and brainstem uniquely participate in encoding, extinction, or temporal linking of specific episodic memories. Members of the panel will also describe how hypersynchronous activity in the cortex may underlie unconsciousness and the prevention of memory formation associated with general anesthesia. The talks will each highlight how diverse networks in the brain participate in aspects of memory function, using state-of-the-art approaches to measure and manipulate brain activity.

PS.04.01 - Subcortical modulation of memory-related hippocampus rhythms

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BACKGROUND AND AIM: Brain oscillations play a critical role in all aspects of memory, from facilitating or downscaling synaptic plasticity, to brain wide coordination of information transfer. The hippocampus is a key region in learning and memory and generates a wide diversity of rhythms. Those include theta rhythm, a 6-10 Hz oscillation present during active wake and REM sleep, and sharp-wave ripples, brief epochs of high frequency oscillations (50ms, 150-250Hz) present during quiet wake and slow-wave sleep. Both rhythms have been tightly linked to hippocampus-dependent memory since modulating or selectively disrupting theta rhythm or sharp-wave ripples impairs learning. METHODS: Combining fiber photometry, electrophysiology and optogenetics in freely behaving mice, we identify a novel subcortical pathway that exerts powerful control over hippocampal theta and sharp-wave ripples. RESULTS: We show that activity of raphe glutamatergic neurons (expressing the vesicular glutamate transporter VGLUT3) varies across sleep-wake stages and is maximal during theta rhythm epochs. During slow-wave sleep, VGLUT3 neurons are active in bursts and are tightly anticorrelated with sharp-wave ripples. Strikingly, optogenetic activation of VGLUT3 neurons abolishes sharp-wave ripples and disrupts theta rhythm. CONCLUSIONS: Taken together, raphe VGLUT3 inputs to the hippocampus are well positioned to profoundly influence learning and memory. Considering the dominant role of raphe nuclei in processing aversive or stress-related information, this raphe-hippocampus pathway could be a neural substrate to modulate learning as a function of stress.

PS.04.02 - Divergent forms of synaptic plasticity in cortical extinction circuits

Erik Bloss [1]

[1] The Jackson Laboratory

BACKGROUND AND AIM: We almost always use context rules to guide our behavior. Cessation of behaviors once they prove less useful in a particular context is colloquially referred to as context-dependent extinction. Work in rodent models have found that neural activity in the infralimbic cortex (IL) neurons leaves the acquisition extinction intact but impairs later extinction memory retrieval. Yet the precise mechanisms that occur in IL remain largely unknown.

METHODS: Here, we used transgenic cFosTRAP mice to show that IL neurons targeting the basolateral amygdala and the nucleus reuniens of the thalamus are recruited during extinction learning. Bidirectional manipulation of activity in these neurons during learning has asymmetric effects on extinction learning and memory retrieval. Using neuroanatomical reconstructions,

we have found these neurons show evidence for the formation of new synapses and the morphological remodeling of existing ones after learning. Interestingly, the magnitude of this plasticity differs between male and female mice. To determine if such a synaptic plasticity mechanism is causal for memory formation, we used viral strategies to delete Grin2b, a glutamate receptor subunit that is critical for plasticity, solely from these projection neurons. The results of these experiments revealed that males and females show opposite patterns of extinction memory retrieval when Grin2B was deleted from BLA-projecting neurons, yet no effect when deleted from reuniens-projecting neurons.

CONCLUSIONS: Collectively, these results suggest IL-to-BLA neurons as the major node of plasticity during extinction learning, and suggest male and female mice use divergent forms of plasticity to maintain long-term extinction memories.

PS.04.03 - The role of inhibition in shaping memory-encoding hippocampal spiking sequences

Jiannis Taxidis [1]

[1] University of Toronto

How does the brain keep track of events we need to remember as well as the intervals between them? This process involves the hippocampus. When a series of sensory cues is experienced by mice, hippocampal spiking sequences encode these cues and link them in memory by tiling the time gaps between them. At each timepoint, these sequences retain information on the identity of the most recent cue and the time elapsed since its presentation. They, therefore, form activity trajectories in â€˜memory spaceâ€™. But the role of inhibitory circuits in shaping these memory-encoding sequences remains unclear. I will present pioneering, longitudinal voltage imaging of cell-type-specific CA1 interneurons while mice perform a memory task. Combined with 2-photon calcium imaging and electrophysiological data, these recordings demonstrate that CA1 interneurons increase the signal-to-noise ratio of hippocampal sequences during cue presentation but not during time intervals between cues. Therefore, inhibition is crucial for efficient memory encoding but less so for memory linking across time.

PS.04.04 - The cortical circuits underlying unconsciousness

Arjun Bharioke [1], **Martin Munz** [1], **Botond Roska** [1]

[1] Institute of Molecular and Clinical Ophthalmology Basel

General anesthetics are associated with a loss of cognitive consciousness together with a concurrent loss of memory. What are the circuits underlying this unconscious state?

Using in vivo two-photon microscope in the adult mouse, we show that layer 5 pyramidal neurons rapidly transition into a synchronous activity state, globally across cortex, during anesthesia-induced unconsciousness. Because layer 5 pyramidal neurons serve as a major output channel of cortex, connecting different cortical areas and cortex to subcortex, this synchronous state drives a decrease in the information output from cortex. Therefore, the activity state across layer 5 pyramidal neurons may drive the loss of cognitive consciousness associated with general anesthesia.

Layer 5 pyramidal neurons form into a highly recurrent network, with most of their inputs coming from other layer 5 pyramidal neurons. The synchronous activity across layer 5 pyramidal neurons is generated internally within the cortical circuit and appears to be a property of the recurrent connectivity of this network. Through the use of a novel in vivo embryonic imaging method, we demonstrate that layer 5 pyramidal neurons first form recurrent circuits, with correlated activity, just one day after their migration into the neocortex. These circuits are transiently active, but layer 5 pyramidal neurons then organize into a second active circuit motif, just prior to birth, that demonstrates a transition to synchronous activity.

Hence, through the use of cell-type specific two photon imaging through the lifespan of the mouse, we have identified and characterized the development of the cortical circuits potentially underlying the cognitive state of unconsciousness.

Parallel Symposium 05: Regenerating the injured nervous system: emerging cellular and molecular mechanisms

The mammalian central nervous system (CNS) has a limited capacity to regenerate, limiting recovery following traumatic injury and disease. Progress has been made that furthers our understanding of the cellular and molecular mechanisms controlling regeneration of the CNS. Microglia have emerged as an essential coordinator of cellular responses to injury that limit tissue damage. Neural stem cells can be transplanted into the injured or disease nervous system to regenerate lost neuron-glia networks and connections. Neurons lose the intrinsic capacity to regenerate axons as they mature, but intracellular processes underlying this loss have been identified that can be targeted pharmacologically. Genetic programs within neurons have also been identified that dictate their capacity to regenerate axons or to survive after injury and can be targeted using microRNAs. This session will focus on cellular and molecular mechanisms recently identified to contribute to regeneration and neural circuit repair in models of mammalian CNS injury.

PS.05.01 - New targets to optimize neural stem cell-mediated repair in spinal cord injury

Soheila Karimi [1]
[1] University of Manitoba

Permanent neurological impairments after spinal cord injury (SCI) are substantially attributed to degeneration of neurons and axons in the injured spinal cord. There is currently an unmet need for developing new targeted regenerative therapies for SCI aiming at promoting neuronal replacement and re-construction of neuron-glia network and circuit re-assembly. Multipotent adult neural precursor cells (NPCs) have the unique potential for brain and spinal cord repair as they show multilineage capacity to support neurogenesis and gliogenesis by forming new neurons and glia. However, extensive evidence has shown restricted ability of resident and transplanted NPCs for neurogenesis in SCI, where gliogenesis is predominant. Thus, strategies to promote multilineage capacity of NPCs for re-formation of neuron-glia network in the injured spinal cord is crucial to facilitate a meaningful functional recovery after SCI. Our evidence suggests neurogenesis is suppressed in the dysregulated milieu of SCI due to limited support for neuronal differentiation, maturation and activity, and an inhibitory niche that impedes synaptic connections. These challenges have also limited the functional benefits of NPC transplantation in clinical trials for people with SCI. This talk will highlight our new discoveries on the extrinsic mechanisms that regulate the activities of NPCs in the injured spinal cord by focusing on the role of CSPG/LAR/PTPs, and discuss new therapeutic approaches that hold promise to optimize NPC-based strategies for SCI repair.

PS.05.02 - Microglia coordinate cellular interactions during spinal cord repair

Faith Brennan [1]
[1] Queen's University

BACKGROUND AND AIM: Traumatic spinal cord injury (SCI) triggers a neuro-inflammatory response dominated by tissue-resident microglia and monocyte derived macrophages (MDMs). Since activated microglia and MDMs are morphologically identical and express similar phenotypic markers *in vivo*, efforts to identify the predominant roles that microglia play in SCI have historically been challenging. However, with the advent of new research tools, key roles for microglia in regulating diverse post-injury cellular responses are emerging.

METHODS: Here, we pharmacologically depleted microglia and used a comprehensive array of anatomical, histopathological, tract tracing, bulk and single cell RNA sequencing techniques to reveal the cellular and molecular responses to SCI that are controlled by microglia.

RESULTS: New data indicate that microglia are vital for SCI recovery and coordinate injury responses in CNS-resident glia and infiltrating leukocytes. Indeed, depleting microglia exacerbated tissue damage and worsened recovery of function. Conversely, restoring select microglia-dependent signaling axes, identified through bioinformatic analysis of sequencing

data, in microglia depleted mice prevents secondary damage and promotes endothelial-dependent vascular repair with improved functional recovery.

CONCLUSIONS: Optimal repair after SCI, and likely other forms of neurological disease, might be achieved by co-opting key ligand-receptor interactions between microglia, astrocytes and MDMs.

PS.05.03 - Promoting CNS axon regeneration through regulation of neuron intrinsic gene expression networks

Alyson Fournier [1]

[1] McGill University

Background and Aim: Numerous neurological conditions and traumatic injuries disrupt connections between neurons in the adult mammalian Central Nervous System (CNS). CNS neurons have a limited capacity to regenerate axons following injury and facilitating axon regeneration could support neurological recovery and serve as an adjunct for cellular transplantation approaches. There are currently no approved treatments to stimulate CNS axon regeneration. An exciting advance in the CNS regeneration field is the demonstration that global alterations in the cell-intrinsic growth state of the neuron can trigger extensive axon regeneration even in the inhibitory CNS milieu. Genetic manipulation of oncogenes and tumor suppressors promote the most significant axon regeneration ever reported and this can be further enhanced by enhancing neuronal activity. Unfortunately, therapeutic translation of these experiments is limited because of the likely off-target consequences of targeting tumor suppressors and oncogenes. Methods: An alternative strategy to positively regulate the intrinsic growth state of the neuron is through trans-acting gene regulatory molecules such as miRNAs. Epigenetic mechanisms such as miRNA regulation can affect broad programs of gene expression, and this is a promising strategy to promote axon repair. In this presentation, we will discuss multi-modal sequencing results from Retinal Ganglion Cell CNS neurons subjected to pro-regenerative inflammatory stimuli. Results: We have harnessed multi-modal sequencing information to identify pro-survival and pro-regenerative interventions based on miRNA regulation in in vitro cell culture and mouse optic nerve injury models. Conclusions: Regulating the intrinsic growth state of the neuron through trans-acting gene regulatory molecules such as miRNAs can positively influence neuronal cell survival and regeneration following CNS injury.

PS.05.04 - Targeting neuronal maturation to promote axon regeneration following spinal cord injury

Brett Hilton [1]

[1] The University of British Columbia

BACKGROUND AND AIM: The inability of neurons to regenerate their axons is a major contributor to sustained motor, sensory, and autonomic dysfunction following spinal cord injury. Neurons lose the ability to regenerate as they mature during embryonic development, but the mature nervous system also forms a fibrotic scar at the lesion site that is potently inhibitory to regrowth. Thus, a therapeutic strategy to promote regeneration needs to both stimulate the intrinsic growth capacity of the injured neuron and ameliorate growth inhibition by the lesion site. Our aim is to decipher the intracellular processes and extracellular inhibitors governing regenerative competence.
METHODS: To decipher new intracellular processes preventing axon regeneration, we compared gene expression changes in dorsal root ganglion (DRG) neurons across different paradigms in which their capacity to grow axons changes. We then performed genetic loss- and gain-of-function experiments combined with neuronal injury models and tissue clearing/3-dimensional imaging analyses to discern the role of core components of the presynaptic active zone in growth inhibition. Based on these results, we then evaluated the capacity of a GABAB receptor agonist, Baclofen, to promote regeneration and recovery following adult mouse spinal cord injury.
RESULTS: Transcriptomic analyses revealed that a major gene expression signature associated with axon growth competence is the downregulation of genes critical for synaptic transmission. Genetic loss and gain-of-function experiments revealed that a protein pivotal for neurotransmission called Munc13 suppresses regeneration. Growth-inhibition by Munc13 is driven by voltage-gated Ca^{2+} channel activity and can be targeted using the GABAB receptor agonist Baclofen. In turn, Baclofen promotes axon regeneration and forelimb recovery following spinal cord injury.
CONCLUSIONS: Genes pivotal for neurotransmission actively suppress regeneration. Baclofen can target this process by reducing voltage-gated Ca2+-influx. Current experiments in the lab are evaluating the role of Munc13 and voltage-gated $^{Ca2+}$ channel activity in growth cone dynamics and whether Baclofen can target growth inhibition at the lesion site following spinal cord injury.

Parallel Symposium 06: Lipids and the Nervous System

Lipids are enriched in the nervous system and are crucial components of cellular function. Recent studies have demonstrated that lipids play important roles in regulating the development and function of the nervous system. Modifications of proteins by lipids, such as palmitoylation, play critical roles in targeting proteins to specific locations in neurons. Furthermore, intracellular lipid storage organelles called lipid droplets are emerging as key regulators in alleviating cellular stress and maintaining energy homeostasis. In this parallel symposium, we will present largely unpublished data on mice and Drosophila; on the roles that lipids, lipid modifications, and lipid droplets play in synaptic plasticity, axonal transport, and

energy homeostasis. In addition, we will demonstrate sex-dependent effects of modulating lipids in neurons. Collectively, our findings will unravel the complex functions that lipids, lipid modifications, and lipid droplets play in proper nervous system development and function.

PS.06.01 - Activity-dependent changes in lipid content of presynaptic terminals regulates synaptic plasticity

Jeffrey Dason [1], **Amber Shaheen** [1], **Claire Richter Gorey** [2], **Adam Sghaier** [1]
[1] University of Windsor, [2] University of Toronto

BACKGROUND AND AIM: Synaptic plasticity is a fundamental property of neurons that allows their ability to transmit information to change with experience. Numerous studies have examined how synaptic plasticity is regulated by proteinâ€“protein interactions, cytoskeletal dynamics, and changes in the expression and activation of various proteins. In contrast, the roles of membrane lipids in synaptic plasticity have been studied far less. Here, we examined whether the cholesterol content of presynaptic terminals change in response to increased neuronal activity and whether these changes are a mechanism of activity-dependent synaptic growth. METHODS: The *Drosophila* larval neuromuscular junction is a well-established model system for studying synaptic growth that shares the basic molecular components found at most synapses across organisms. We used this model to determine the role of cholesterol in activity-dependent synaptic growth. RESULTS: We generated transgenic flies that express the cholesterol binding D4H domain of Perfringolysin O toxin and found increased levels of cholesterol in presynaptic terminals of *Drosophila* larval neuromuscular junctions following increased synaptic activity. Reduced cholesterol impaired synaptic growth and largely prevented activity-dependent synaptic growth. Presynaptic knockdown of adenylyl cyclase phenocopied the impaired synaptic growth caused by reducing cholesterol. Furthermore, the effects of knocking down adenylyl cyclase and reducing cholesterol were not additive, suggesting that they function in the same pathway. Increasing cAMP levels using a *dunce* mutant with reduced phosphodiesterase activity failed to rescue this impaired synaptic growth, suggesting that cholesterol functions downstream of cAMP. Finally, we used a PKA sensor to show that reducing cholesterol levels reduced presynaptic PKA activity. CONCLUSIONS: Collectively, our results demonstrate that enhanced synaptic activity increased cholesterol levels in presynaptic terminals and that these changes likely activate the cAMP-PKA pathway during activity-dependent growth. These findings point to a key role for membrane lipids, such as cholesterol, in activity-dependent synaptic plasticity and in development of the nervous system.

PS.06.02 - Regulation of axonal transport in neurons by S-acylation

Shaun Sanders [1], **Jordan Kogut** [1], **Arshia Leekha** [1], **Amelia Doerksen** [1], **Nisandi Herath** [1], **Charlotte Townsend** [1]

[1] University of Guelph

BACKGROUND & AIM: Neurons are large, complex cells requiring tightly regulated compartmentalization, a process where specific proteins and organelles are trafficked to and retained at distinct subcellular locations. Efficient axonal trafficking is critical for action potential initiation and propagation as well as neurotransmission and deficits in axonal trafficking underly many neurological disorders. One important mechanism governing neuronal protein trafficking and association with lipid membranes is the reversible modification of protein cysteine residues with 16-carbon palmitic acid, known as S-acylation. S-acylation is highly prevalent among neuronal proteins and well-studied in the context of the synapse. Comparatively, how S-acylation regulates trafficking and clustering of axonal proteins remains less understood. However, an overwhelming proportion of axonal proteins and axonal trafficking machinery are likely S-acylated. METHODS: We use biochemical and cell biological approaches in heterologous cells and primary rat neurons to characterize mechanisms of S-acylation of axonal proteins and trafficking machinery and to determine how S-acylation regulates their localization and function. These methods include mutagenesis and knockdown/replacement followed by biochemical subcellular fractionation, S-acylation assays, and high-resolution microscopy. RESULTS: S-acylation of axonal proteins and trafficking machinery proteins is critical for their axonal transport function and localization within the axon. CONCLUSIONS: Our findings provide insight into the molecular mechanisms that govern axonal transport with implications for neurodevelopment, synaptic function, and axon degeneration.

PS.06.03 - Regulation of neuronal lipid droplets influences sex differences in energy homeostasis

Colin Miller [1], **Jasper Fisher** [1], **Serena Hollman** [1], **Lianna Wat** [1], **Sanjana Prakash** [1], **Niyoosha Yoosefi** [1], **Yi Han Xia** [1], **Huaxu Yu** [1], **Romane Manceau** [2], **Danie Majeur** [2], **Tao Huan** [1], **Thierry Alquier** [3], **Elizabeth Rideout** [4]
[1] University of British Columbia, [2] Université de Montréal, [3] University of Montreal, [4] The University of British Columbia

BACKGROUND AND AIM: Triglyceride is the main form of stored fat in all animals, and is stored within specialized organelles called lipid droplets. While lipid droplet biology has been studied extensively in non-neuronal cell types, much remains to be discovered about the regulation and function of lipid droplets in neurons.

METHODS: We addressed this knowledge gap by expressing a lipid droplet-targeted GFP specifically in *Drosophila* neurons.

RESULTS: Visualizing neuronal lipid droplets in this way allowed us to uncover diet- and age-dependent regulation of lipid droplets, and to identify multiple genes that influence neuronal lipid droplet abundance. Our analysis of animals with changes to neuronal lipid droplets revealed defects in whole-body fat metabolism, suggesting the correct regulation of neuronal lipid droplets is essential to maintain energy homeostasis. In particular, we found that dysregulation of lipid droplet-associated genes in one group of ~18 metabolic neurons, called the adipokinetic hormone-producing cells (APCs), explained the defects in energy homeostasis.

CONCLUSIONS: Because phenotypes associated with changes to lipid droplet-associated genes in the APCs were only observed in males, and we found sex-specific changes to the brain lipidome caused by neuronal loss of lipid droplet-associated genes, our data suggest a model in which lipid droplets play a sex-biased role in supporting neuron function.

PS.06.04 - Neuronal lipid droplet breakdown regulates whole-body metabolism and feeding

Thierry Alquier [1]

[1] Université de Montréal

Background. Neuronal lipids are known to play key roles as structural and signaling molecules. Recent evidence suggest that glycerolipid (e.g. triglyceride) metabolism in hypothalamic neurons is regulated by metabolic signals and is involved in whole-body energy homeostasis. Adipose Triglyceride Lipase (ATGL) catalyzes the first step of triglyceride (TG) hydrolysis in lipid droplets in peripheral tissues and is crucial for lipid and energy homeostasis. Yet, the physiological role of neuronal ATGL in the hypothalamic control of energy balance is unknown.

Methods. We used multiple models and gene interventions to investigate the regulation of lipid droplets by neuronal ATGL and its role in the control of energy homeostasis.

Results: ATGL is expressed in different regions and cell types of the mouse brain, including hypothalamic POMC and AgRP neurons of the arcuate nucleus (ARC). In cultured hypothalamic neurons, ATGL promotes lipid droplets lipolysis, in line with its effects in peripheral tissues. In the ARC, ATGL expression is upregulated by cold exposure and fasting. Knockdown of neuronal ATGL in *C. elegans* inhibits peripheral fat breakdown as reported in male *D. melanogaster*. This suggests a conserved mechanism thereby neuronal ATGL promotes peripheral lipolysis. In mice, ATGL knockout specifically in ARC neurons or specifically in AgRP neurons affects body weight, energy expenditure, feeding behavior and thermoregulatory responses to cold in male mice. Such changes were not observed in female mice, highlighting a male-specific regulation of energy homeostasis by ATGL in AgRP neurons. At the cellular level, knockout of ATGL decreases the firing of AgRP neurons which is associated with altered mitochondria morphology. Lipidomic and metabolomic studies in cultured AgRP-like neurons demonstrate an ATGL-

dependent remodeling of TG and membrane phospholipids along with impaired mitochondrial oxidative function.

Conclusion: Taken together, our findings reveal a previously unrecognized role for ATGL in neuronal lipid droplet metabolism and regulation of whole-body energy homeostasis by AgRP neurons.

Parallel Symposium 07: Dopaminergic involvement in appetitive and aversive memories

That dopamine (DA) is linked to reward learning is well established. This symposium will integrate new research from the fruit fly, the mouse and the rat during a variety of learning tasks that go beyond the study of how dopamine regulates learning about rewards. Dr Mihaela Iordanova will present data from in vivo electrophysiological recordings and optogenetic interrogation studies showing that appetitive and aversive stimuli interact at the level of the DA signal in a valence dependant manner. Dr Erin Calipari will show data that link DA signaling to the perceived saliency of predicted aversive events. That DA sends different types of reinforcement signals via its effernt projections will be provided by Dr Vincent Breton Provencher. Lastly, an exciting and novel finding by Johannes Felsenberg links dopamine to the recovery of forgotten memory and its relationship to implanting false memories.

PS.07.01 - A role for VTA DA neurons in valence prediction error

Mihaela Iordanova [1]
[1] Concordia University

That the mesolimbic dopamine (DA) signal tracks prediction error in reward learning has been a pinnacle discovery in neuroscience. It has led to numerus investigations into the conditions that regulate this signal as well as studies that have used cutting-edge technologies to examine the causal role for DA in associative learning. Advances in understanding the role of dopamine in reward prediction error have not been matched by similar investigations into the role of dopamine in aversive learning. We sought to examine the role of dopamine neurons in aversive prediction error. We used in vivo electrophysiology to confirm that cues that predict reward lead to phasic excitation of VTA DA neurons at the start of the cue and inhibition at time of reward omission. In addition, we report that VTA DA neurons show a suppression shortly after onset of a cue that predicts shock and excitation to shock omission. Furthermore, we uncovered that neuronal firing of VTA DA neurons was in line with a valence prediction error. We use optogenetics in Th-cre rats to inhibit VTA DA neurons shortly after onset of a shock-predicting cue to determine if this modulation of the VTA DA signal would enhance the ability of an aversive cue to support aversive learning to another cue (second-order conditioning). Our data provide evidence that indeed VTA DA inhibition is critical for aversive learning.

PS.07.03 - Dopamine encoding of reinforcement signals in time and space

Vincent Breton-Provencher [1]
[1] Université Laval

Dopamine signaling is involved in varied and at times contrasting functions with respect to reinforcement learning. Here, to understand the function of the dopaminergic system, we recorded the dynamics of dopamine release from several dopaminergic outputs in the context of reinforcement learning. We performed multi-site fiber photometry of an improved fluorescent dopamine sensor in various locations of the mesolimbic pathway (subregions of the nucleus accumbens, olfactory tubercle, and amygdala), the nigrostriatal pathway (dorsal and tail striatum), and the mesocortical pathway (medial prefrontal cortex). We found system-wide encoding of unexpected reward by dopamine, whereas the encoding of punishment was spatially heterogeneous, with parts of the ventral striatum showing an absence of punishment response. We next investigated the extent to which dopamine release signals reward prediction errors by training mice on a classical conditioning task where an auditory cue predicted the delivery of a reward. We found that the relative scaling of reward prediction error was similar in all recorded dopamine outputs. We are currently analyzing trial-to-trial dynamics to determine the extent to which these reinforcement signals correlate in time across the dopaminergic system. Together, our findings provide support for heterogeneous reinforcement signaling of dopamine across the brain.

PS.07.04 - Creating true and false memories from forgotten information in Drosophila

Johannes Felsenberg [1]
[1] Friedrich Miescher Institute

Recovering forgotten memories is a double-edged sword. While regaining access to forgotten information can be beneficial, targeted memory recovery carries the risk of generating false memories. The current understanding of neuronal circuits governing memory recovery or false memory implantation remains limited. We find in fruit flies that a reminder can recover forgotten aversive memories. This recovery requires a silent memory to recruit a specific pair of dopaminergic neurons. Modifying the reminder can mislead the recovery process and implant a false memory, switching avoidance to approach. Notably, the effectiveness of this false memory implantation depends on the contextual boundaries of the training event. False memory implantation and true memory recovery engage distinct dopamine pathways, underscoring the complexity of neuronal circuits governing these processes. Identifying the neuronal circuits governing true and false memories lays the groundwork for developing strategies to mitigate the acquisition and effects of misleading information.

Parallel Symposium 08: Mechanisms of Sensory Processing Disruptions Associated with Autism

Altered sensory perception and reactivity to sensory stimulation are a core symptom of neurodevelopmental disorders, including autism. Sensory signals are the only input into the brain, therefore aberrant sensory input during postnatal brain development has huge implications on synaptic connectivity and the formation of neuronal circuits. Sensory symptoms will also have cascading effects on other core autism symptoms, such as social function and other cognitive abilities.

PS.08.01 - Auditory processing and sensorimotor gating disruptions in Cntnap2 knockout rats are malleable during early postnatal development

Susanne Schmid [1], **Ella E Doornaert** [1], **Alaa El-Cheikh Mohamad** [1], **Brian Allman** [1], **Dorit Moehrle** [1]
[1] University of Western Ontario

BACKGROUND AND AIM: Autism spectrum disorder (ASD) is a neurodevelopmental condition affecting one in 160 children worldwide. The homozygous *Cntnap2*-knockout (*Cntnap2*$^{-/-}$) rat is a preclinical genetic model for studying ASD-related phenotypes. Previous work has demonstrated that there are greater ASD-like deficits in the *Cntnap2*$^{-/-}$ rat when bred and reared by a *Cntnap2*$^{-/-}$ compared to a heterozygous (*Cntnap2*$^{+/-}$) dam. Considering that these *Cntnap2*$^{-/-}$ offspring have the same genetic mutation, it suggests that environmental factors are influencing their development. This confirmatory project investigated if these environmental effects occur pre- or postnatally.

METHODS: We conducted a cross-fostering paradigm in which *Cntnap2*$^{-/-}$ offspring were bred from a *Cntnap2*$^{-/-}$ dam and transferred to be reared by a *Cntnap2*$^{+/-}$ dam. These cross-fostered animals were compared to *Cntnap2*$^{-/-}$ animals bred and reared by a *Cntnap2*$^{-/-}$ dam as well as *Cntnap2*$^{-/-}$ and wildtype animals bred and reared by a *Cntnap2*$^{+/-}$ dam. All animal groups contained both sexes and met adequate sample sizes. Throughout development, we examined ASD-like deficits in auditory processing and sensorimotor gating.

RESULTS: All *Cntnap2*$^{-/-}$ regardless of parental genotype and cross-fostering showed impaired neural responsiveness in the auditory brainstem response (the neural activity in the brainstem in response to auditory input), the acoustic startle response (the whole-body contraction reflex elicited by the sudden presentation of a loud auditory stimulus), and prepulse inhibition (the reduction in the startle response if the startling stimulus is preceded by a low-intensity

prepulse). However, cross-fostering restored a deficit in the maturation of hearing sensitivity for *Cntnap2*$^{-/-}$ rats bred from a *Cntnap2*$^{-/-}$ dam.

CONCLUSIONS: Together, this research provides evidence that some ASD characteristics observed in the *Cntnap2*$^{-/-}$ are not fixed by genetic predisposition but can be malleable by early postnatal environmental conditions. Furthermore, the results have implications for how all researchers conduct breeding when using genetic animal models to study neurodevelopmental conditions.

PS.08.02 - Neural alterations in the neocortex underlie tactile perception changes in a mouse model of autism

Andreas Frick [1], **Ourania Semelidou** [1], **Alexandre Cornier** [2], **Théo Gauvrit** [1], **Melanie Ginger** [1]
[1] INSERM, [2] CHU Bordeaux

BACKGROUND AND AIM:

Altered sensory experience is one of the core features of autism, affecting approximately 90% of autistic individuals, exerting a strong negative influence on day-to-day life and contributing to the development of other core symptoms and medical conditions that co-occur with autism. Touch is particularly crucial during development and tactile alterations in autistic individuals include differences in stimulus perception and sensory-related neural excitability. Better understanding and synthetizing these features is crucial in characterizing altered tactile experience in autism and can provide insight into the development of other core features. In this study, we characterized tactile alterations in Fmr1 mice, a genetic mouse model of autism.

METHODS:

To study perception at the behavioral and neuronal level, we developed a novel vibrotactile perceptual decision-making task that can be combined with measurements of neuronal activity and translated in human studies.

RESULTS:

Our findings recapitulate the tactile alterations observed in autistic individuals, with task acquisition deficits, higher detection thresholds leading to perceptual hyposensitivity, and increased inter-individual and intra-individual variability in the Fmr1 mice. Parallel in vivo calcium imaging recordings of neuronal activity of excitatory and inhibitory neurons in the primary somatosensory cortex revealed that a decreased signal-to-noise ratio phenotype in the Fmr1 mice underlies perceptual alterations. To examine whether these alterations are

malleable to pharmacological treatment, we targeted the voltage- and calcium-sensitive K+ channel BKCa, an approach previously shown to correct cellular hyperexcitability.

CONCLUSIONS:

Altogether, our results expand our knowledge of tactile alterations in autism and suggest objective biomarkers that can be used for developing mechanism-based treatment.

PS.08.03 - The effect of odor novelty in odor identification in mouse models of autism

Gonzalo Otazu [1]

[1] New York Institute of Technology

BACKGROUND AND AIM: Novel stimuli can cause distress to individuals with autism. To understand how novel stimuli are processed in mouse models of autism, we have been studying the behavioral responses to novel odors in mouse models of autism. We previously demonstrated deficits in target odor recognition in novel background odors in the Cntnap2 knockout mouse as well as in Shank3B$^{-/+}$ mouse, despite similar performance to wild-type (WT) mice in familiar background odors (Li et al., 2023, Ryndych et al., 2023). Although olfactory bulb representations in the output cells of the olfactory bulb can predict behavioral discrimination performance in WT mice (Gschwend et al., 2015), it is not clear what aspect of the neural representation of novel background odors resulted in this behavioral deficit in mouse models of autism.

METHODS: To test whether target odor recognition in mouse models of autism was affected by olfactory bulb output representations of novel background odors affect, we crossed GP5.11 mice, which express GCaMP6f in mitral and tufted cells in the olfactory bulb with Shank3B$^{-/+}$ mice. We performed widefield calcium imaging to record olfactory bulb output responses.

RESULTS: Background odors produced similar levels of average glomerular activation when they were novel to the animal compared to the activation produced after prolonged exposure. However, the trial-to-trial variability was higher when an odor was novel and odor representations became more stable after prolonged exposure. To determine whether the increased variability produced by novel odors affected target recognition, we recorded glomerular responses as Shank3B$^{-/+}$ mice were behaviorally tested in odor recognition in novel background odors. Distortions in the odor representation in the olfactory bulb output resulted in incorrect classification in both the Shank3B$^{-/+}$ mice behavior, as well as by a linear classifier trained using glomerular images in response to familiar background odors.
CONCLUSIONS: These findings indicate that the activity of mitral and tufted cells reflects the

performance of Shank3B$^{-/+}$ mice, and trials with altered glomerular responses are associated with errors in target recognition in mouse model of autism.

PS.08.04 - Bistable visual perception in autism: mouse models

Ganna Palagina [1]

[1] Harvard Medical School

To understand autistic brain, one must identify the common circuit ground for a diverse set of core traits of autism: altered social communication, cognitive rigidity, and atypical sensory perception. Studies in human subjects with autism have identified common anatomical substrates between restricted behavioral repertoire, cognitive rigidity, and over-stability of visual percepts during visual rivalry. To be able to study these processes with single-cell precision and exhaustive neuronal population coverage, we developed the visual rivalry paradigm for mice. This paradigm is based on plaid patterns consisting of two transparent gratings drifting at an angle of 120Â° relative to each other. Viewing this stimulus causes the perception to spontaneously reverse between plaid seen as local component motion (two gratings drifting on top of each other, â€˜transparentâ€™ percept) and integrated global pattern motion (fused moving texture, â€˜coherentâ€™ percept). This paradigm does not depend on the explicit report of the mouse, as the direction of the optokinetic nystagmus (OKN, rapid eye movements driven by either pattern or component motion) is used to infer the currently dominant percept. We combined this paradigm with mesoscale 2-photon imaging to dissect the neuronal circuit of bi-stable visual perception in regular mice and mouse models of autism â€“ MECP2 duplication syndrome and Shank-3B[-] mice.

We discovered distinct subnetworks of cortical neurons underlying bi-stable perception. Percept-supporting neurons in the visual cortex showed tuning for either local motion and orientation (transparent percept-supporting), or, alternatively, global scene motion (coherent percept-supporting). In addition, ~ 25% of cells across the visual hierarchy and secondary motor areas of the frontal lobe were reversal-sensitive, activating as early as 10 seconds before the reversal and firing until the reversal occurred. Reversal neurons are high-level integration units showing broad responses to complex motion, pupil dilations and exploratory whisking and walking.

In both MECP2 duplication and Shank-3B[-] mice the rate of perceptual reversals was reduced, recapitulating the human autism phenotype. This effect is apparently carried by two distinct mechanisms: impaired integration of visual motion and reduced firing rate variability of the reversal-locked neurons. First, in autistic brains, local motion-selective neurons supporting transparent percept produce unusually reliable responses; thus the transparent percept subnetwork can remain active longer. This is reflected in increased stability of transparent percepts at the expense of coherent percepts in autistic mice. Second, perceptual reversals

mostly occur during exploration states and carry a specific pupillary dilation signature mirrored in the activity profile of reversal-locked neuronal population. This suggests that the operation of reversal subnetwork depends on noradrenergic and cholinergic circuits associated with exploration and surprise. In both human and mouse autism syndromes these systems are impacted. In autistic mice the reversals are associated with smaller pupillary dilation amplitude compared to littermates, and the neurons display less firing variability under ongoing visual stimulation. It is thus likely that only the strongest exploratory states drive the reversal subnetwork enough to cause reversals in autistic brains.

Parallel Symposium 09: Myelin in neurodevelopment and neurodegeneration

Myelin, which is produced by oligodendrocytes, regulates neuronal signalling, axonal health, cognition and behaviour. Perturbations in myelin have been proposed to be drivers of pathobiological mechanisms in classically defined neuron-centric disorders like neurodevelopmental disorders (e.g. autism) and autoimmunity-centric disorders (e.g. multiple sclerosis). The development of therapies to replace damaged or lost myelin has been identified as one of the primary research foci for neurodegenerative disorders.

PS.09.01 - DEAD box protein Ddx20-mediated regulation of oligodendrocyte development, differentiation, and homeostasis

Norihisa Bizen [1]
[1] Niigata University

BACKGROUND AND AIM:
Oligodendrocytes form the myelin sheaths around axons and regulate the axonal conduction and metabolism in the central nervous system (CNS). Dysmyelination and demyelination lead to the disruption of neuronal networks and, thereby, severe neurological symptoms. Therefore, elucidation of the molecular basis underlying oligodendrocyte differentiation and maturation provides clues to identifying potential therapeutic targets for intractable neurological disorders. We identified DEAD box protein 20 (Ddx20) as a binding factor to Olig2, a bHLH transcription factor essential for oligodendrocyte development. Ddx20, a putative ATP-dependent RNA helicase, is a multifunctional regulator of transcription, RNA metabolism, and translation. In this study, we aimed to elucidate the role of Ddx20 in CNS development and oligodendrocyte differentiation.

METHODS:
In this study, we generated *Ddx20*-deficient mice in either neural precursor cells or oligodendrocyte lineage cells (*Nestin-Cre; Ddx20* cKO, *Cnp-Cre; Ddx20* cKO, *Mbp-Cre; Ddx20*

cKO). We investigated the phenotypes of these mice using histological methods, molecular biological and biochemical methods, and transcriptome analysis.

RESULTS:
Analysis of mice with *Ddx20* deletion in neural precursor cells and oligodendrocyte precursor cells revealed that drastic apoptosis and cell cycle arrest of these cells due to the activation of the p53 pathway, triggered by abnormal splicing of *Mdm2* mRNA. Furthermore, we found that Olig2 suppresses the p53 activation by stabilizing Ddx20 and ensuring accurate *Mdm2* splicing, suggesting that the transcription factor Olig2 regulates RNA metabolism through Ddx20. Oligodendrocyte-specific *Ddx20*-deficient mice, generated to elucidate the role of Ddx20 in oligodendrocyte differentiation and maturation, showed a gradual decrease in the number of mature oligodendrocytes, increased DNA damage, and hypomyelination. Transcriptome analysis also demonstrated a significant decrease in the expression of many myelination-related genes in *Ddx20*-deficient mice. Accompanying these phenotypes, neuronal loss, axonal degeneration, and activation of microglia and astrocytes were also observed.

CONCLUSIONS:
These results highlight the multifaceted contribution of Ddx20 to the development, differentiation, and homeostasis of oligodendrocytes. Our study provides significant insights into the role of Ddx20 in the biology and pathology of oligodendrocytes, contributing to the understanding of CNS pathologies mediated by oligodendrocyte dysfunction.

PS.09.02 - Novel imaging approaches untangling axonal selectivity of myelination by single oligodendrocytes

Nobuhiko Ohno [1]

[1] National Institute for Physiological Sciences

BACKGROUND AND AIM: Oligodendrocytes form myelin sheaths and regulate conduction velocity and functional integrity of axons in the central nervous system. Although individual oligodendrocytes myelinate multiple axons and control the functions of neural circuits involving these axons, it was unclear whether they selectively myelinate specific types of axons. In this talk, I will introduce our recent studies developing novel imaging approaches to visualize the axonal preference of myelination by individual oligodendrocytes in different areas of brains.

METHODS: The fixed brain tissue from an adult mouse was stained with heavy metals en bloc, embedded into carbon-based conductive resins and imaged with serial block-face scanning electron microscopy (SBF-SEM). From the acquired serial images, individual oligodendrocytes were identified. Their processes, myelin and axons ensheathed by each of them were

reconstructed to measure morphological parameters. In addition, we developed an approach for the detailed observation of myelination in relation to specific types of axons in mouse cerebellar white matter. This was achieved by combining immunohistochemistry of markers along with sparse fluorescent labeling of oligodendrocytes with an attenuated rabies virus vector.

RESULTS: The 3-dimensional ultrastructural analyses conducted with SBF-SEM revealed that single oligodendrocytes in mouse corpus callosum extended processes and showed preference for myelinating distant axons within specific diameter ranges with discrete myelin thicknesses. In addition, the sparse fluorescent labeling of oligodendrocytes in cerebellar white matter revealed that approximately half of oligodendrocytes preferentially myelinated Purkinje cell axons, and the preference was more prominent when the myelination was initiated. Consistently, the transgenic mice that label early born oligodendrocytes showed that their myelin sheaths were predominantly formed around Purkinje cell axons in the adult cerebellar white matter.

CONCLUSIONS: These results suggest that a substantial number of oligodendrocytes selectively myelinate structurally and/or functionally distinct axons. The selectivity is established during development and would contribute to physiological roles of oligodendrocytes as well as to axonal pathology under oligodendrocyte dysfunction.

PS.09.03 - Posttranslational palmitoylation in oligodendrocyte development, myelination and disease

Shernaz Bamji [1]
[1] University of British Columbia

BACKGROUND: Palmitoylation is the most common post-translational lipid modification in the brain. ZDHHC9 is one of 23 palmitoyl acyl-transferases that mediates palmitoylation and has been strongly implicated in the etiology of X-linked intellectual disability (XLID). Indeed, 2% of all patients with XLID have loss-of-function mutations in ZDHHC9. Patients with ZDHHC9 loss-of-function mutations exhibit reduced corpus callosum volume and impaired white matter integrity.

METHODS: We have used Zdhhc9 knockout mice to understand the role of ZDHHC9 in oligodendrocyte development and function.

RESULTS: Our work demonstrates that ablation of Zdhhc9 in mice substantially impairs the maturation of oligodendrocytes and results in a concomitant decrease in the density of myelinated axons. Ultrastructural analysis of the remaining myelinated axons in the corpus callosum revealed further disruptions in myelin integrity. Transcriptomic and proteomic

analyses reveal decreased expression of genes and proteins crucial for lipid metabolism, cholesterol synthesis, and myelin compaction.

CONCLUSIONS: These findings unveil a previously overlooked role for ZDHHC9 in governing oligodendrocyte maturation and myelinogenesis, and offers mechanistic insights into white matter volume deficits in patients with ZDHHC9 mutations.

PS.09.04 - Mechanisms of myelin formation in the developing and regenerating brain: lessons from CX3CR1 signalling

Sana Bibi [1], **Adrianne Watson** [1], **Yauheniya Tkalich** [1], **Monique De Almeida** [1], **Yana Kibalnyk** [1], **Kara Goodkey** [1], **Nicole Dittmann** [1], **Astrid Cardona** [2], **Anastassia Voronova** [1]
[1] University of Alberta, [2] The University of Texas at San Antonio

Regeneration of the demyelinated central nervous system (CNS) requires renewal of oligodendrocytes. We showed exogenous chemokine fractalkine enhances oligodendrocyte and myelin regeneration from resident murine oligodendrocyte precursor and neural stem cells in the developing and demyelinated CNS (Watson et al. 2021; Almeida et al. 2023 Stem Cell Rep). Notably, polymorphisms in the fractalkine receptor (CX3CR1) are associated with multiple sclerosis (MS) severity. Interestingly, gene ontology analysis shows that MS severity risk genes cluster into CNS development category. Yet, we do not understand whether and how the severity risk gene variants affect brain development, and if these developmental perturbations lead to an altered response to a demyelinating injury later in life. We show that in comparison to WT, developing but not adult CNS in mice that express human pathogenic *CX3CR1* variant displayed i) a delay in OPC differentiation and myelination; ii) increased activation of microglia; and iii) aberrant levels of cytokines. Rescue of deficient developmental myelination improved poor remyelination upon a demyelinating injury in adulthood. Our work suggests that mechanisms of CX3CR1-mediated myelin formation in the developing and demyelinated CNS are shared. Our results also suggest that MS severity in genetic variant carriers may manifest via two hits. The first insult may occur due to the detrimental effects of variants on OPCs and microglia leading to delayed developmental myelination. A second demyelinating hit later in life may further disrupt the affected cells leading to exacerbated neurodegeneration and poor recovery.

Parallel Symposium 10: Reframing astrocytes in brain circuits and behaviour, a quest for new questions

The question “what do astrocytes do?” still has no simple answer. Could agreeing on a broad definition of astrocytes function help unify the field and propel future inquiries in new directions? In this symposium, we will delve into the new ‘conceptual guidance’ framework that describes astrocytes as decoders of organismal and environmental contexts that configure neural circuitry in an adaptive, state-dependent fashion. We will highlight the broad applicability of this framework through a diverse set of talks bringing together researchers from fields spanning astrocytic control of arousal, learning and memory, emotion, and pain. The symposium will cover all scales of investigation, from molecular and cell mechanisms to synaptic physiology, circuit activity and behavior. Additionally, we will present the latest astrocyte-specific tools available, engineered by several of the symposium speakers, and how they are being used to reshape our understanding of these complex glial cells.

PS.10.01 - Astrocyte-neuronal metabolic coupling in the cingulate cortex promotes chronic pain development in a sex-specific manner.

Paige Reid [1], **Kaitlin Scherer** [1], **Danielle Halasz** [1], **James Tang** [1], **Fariya Zaheer** [1], **Ana Leticia Simal Dourado** [1], **Jana Michaud** [1], **Andrea Clark** [1], **Giannina Descalzi** [1]
[1] University of Guelph

Background and Aim: One in four Canadians over the age of 15 suffers from chronic pain. Despite much progress on understanding the neuronal mechanisms involved, research has largely ignored the impact glial cells have on the processes of chronic pain development. Human and rodent neuroimaging studies indicate that chronic pain corresponds with reorganization of an emotion-pain brain circuit, and evidence indicates that neuroplasticity of the anterior cingulate cortex (ACC) is a critical step in this reorganization. Previously, we found that chronic pain and fear learning are both associated with enhanced neuronal excitability and cause similar neuroplasticity-related gene expression changes in the ACC of male mice. Neuroplasticity however imposes large metabolic demands. In the brain, neurons have the highest energy needs and interact with astrocytes, which extract glucose from blood, mobilize glycogen, and release lactate in response to neuronal activity. We recently used a rat model of *memory-related neuroplasticity* to demonstrate that lactate, provided to neurons through astrocyte-neuronal metabolic coupling, provides neurons with energy for neuroplasticity-related changes. Beyond mechanisms classically associated with memory however, neuroplasticity represents fundamental processes that promote lasting changes in behaviour, such as nociceptive hypersensitivity. In this talk, we present data from our group showing that astrocyte-neuronal lactate shuttling is also critical for the development of chronic inflammatory pain, but in a sex-dependent manner.

Methods: Male and female adult mice were exposed to the CFA and formalin models of inflammatory pain, and were assessed for expression of pain hypersensitivity. Using a combination of lactate colorimetric assays, western blot, antisense oligonucleotides, and behavioural paradigms, we assessed the involvement of astrocyte-neuronal lactate shuttling in the development of chronic inflammatory pain.

Results: We found that astrocyte-neuronal lactate shuttling in the mouse ACC promotes the development of chronic inflammatory pain, in a sex specific manner. Specifically, whereas both male and female mice show similar levels of chronic pain hypersensitivity, only male mice show sustained increases in lactate levels. Accordingly, chronic pain alters the expression levels of proteins involved in lactate metabolism and shuttling in a sexually dimorphic manner, and disrupting astrocyte-neuronal lactate shuttling in the ACC reduces pain in male, but not female mice. Furthermore, using a transgenic mouse model (*itga1*-null mice) that displays a naturally occurring form of spontaneous osteoarthritis (OA), a painful inflammatory pain condition, we found that once again, whereas both female and male mice develop OA, only male mice show increases in mechanisms involved in astrocyte-neuronal lactate shuttling.

Conclusions: Our data thus indicates that there are sex differences in astrocyte-neuronal coupling in the mouse ACC during chronic pain development.

PS.10.02 - Mapping the Contribution of Astrocytes to Brain Computation and Outputs

Thomas Papouin [1]

[1] Washington University School of Medicine, St Louis

The discovery that astrocytes are responsive to neuromodulators has advanced our understanding of the determinants of astrocyte activity and the roles its plays in state-dependent network regulation. But a more agnostic and integrative understanding of how much astrocytes contribute to the known circuit and behavioral effects of neuromodulators is still lacking. Functional alterations of circuit connectivity by locus coeruleus (LC)-derived norepinephrine (NE) are central in driving network-level and behavioral adaptations to environmental saliencies, but how the LC-NE system achieves such concerted synaptic remodeling is loosely defined. Short of a precise mechanism, it is broadly accepted that NE acts directly on neurons to alter synaptic strength. Here, using widely available methods and cell-specific approaches, we show that the canonical effect of NE on synaptic activity is in fact completely independent from its binding on neuronal receptors. Instead, electrophysiology and 2-photon imaging data show that astrocytic adrenergic receptors and Gq-dependent astrocytic Ca^{2+} dynamics are engaged by NE and gate synaptic modifications in response to NE occurrence. The astrocyte-specific deletion of adrenergic receptors and three independent astrocyte-silencing approaches all render synapses insensitive to NE, demonstrating that LC-NE activity remodels synaptic circuits by engaging astrocytes. Furthermore, pharmacological and

genetic examinations indicate that the modification of synaptic strength elicited by NE relies entirely on an ATP-derived and adenosine A1 receptor-dependent control of presynaptic efficacy. Together, this fuels a new model wherein astrocytes are a core component of monoaminergic systems and the circuit effector through which they produce network adaptations, challenging an 80-year-old status quo.

PS.10.03 - Astrocytic modulation of prefrontal neural circuits and anxiety-like behavior

Xinzhu Yu [1]

[1] University of Illinois Urbana-Champaign

Anxiety is an emotional state triggered by potentially dangerous conditions that is essential for our survival. However, excessive and uncontrolled anxiety may lead to anxiety disorders, which are the most common psychiatric disorders worldwide affecting more than 30% population during their lifetime. Both clinical and basic research studies have reported that defective prefrontal neural circuits result in aberrant processing of potentially aversive stimuli and impaired socioemotional behavior. Yet, the precise role of astrocytes in modulating the prefrontal neuronal function to mediate anxiety-like behavior remains inadequately understood. In this study, we employed genetic manipulation of astrocytes in the medial prefrontal cortex in combination with *in vivo* Ca^{2+} imaging of astrocytic and neuronal activity dynamics in freely behaving mice to reveal the relationship between cellular Ca^{2+} signaling and anxiety-like behavior. Moreover, we utilized a cell-specific and proximity-dependent proteomic approach to profile molecular changes associated with anxiety-like behavior. Our data reveal astrocytes as a crucial modulator of prefrontal neuronal function and anxiety-related behavior that could be targeted to develop therapeutic strategies in mental disorders.

Parallel Symposium 11: Vision Unveiled: Navigating the Intricacies from Eye to Brain to Behavior

Comprehending brain function necessitates a detailed understanding of neural circuits, the computations they execute, and, ultimately, their influence on specific behaviors. The visual system stands out as a crucial domain for integrating these fundamental aspects. In this context, we present recent advancements in our endeavor to unravel the circuit computations that underlie visual feature preferences in both the retina and the brain. Furthermore, we strive to establish connections between these visual computations and behaviors such as visual discrimination and spatial navigation.

PS.11.01 - Neural circuits underlying multi-feature extraction in the retina

Gautam Awatramani [1]

[1] University of Victoria

The landmark study by Hubel and Wiesel (1959) elucidated that neurons within the cat visual cortex exhibit selectivity for various stimulus features, including direction and orientation. Despite the clarity regarding the algorithms involved in multi-feature extraction, the specific neural circuits responsible for such computations have yet to be identified.

In our investigation, we reveal that direction-selective ganglion cells (DSGCs) in the mouse retina also exhibit selectivity for stimulus orientation. This finding suggests that intricate circuits facilitating multi-feature extraction exist earlier in the visual system than previously assumed. Through a combination of patch-clamp techniques, cell-specific genetic knockout experiments, and optogenetic methodologies, we demonstrate that the mechanisms supporting multi-feature coding differ between the nasal/temporal and dorsal/ventral coding DSGCs

PS.11.02 - The feature landscape of mouse visual cortex

Stuart Trenholm [1]

[1] McGill University

BACKGROUND AND AIM: Understanding visual system computations requires knowledge of feature preferences of neurons in different visual cortical areas. In mice, while many visual cortical areas have been identified, relatively little is known about the specific feature preferences and tuning properties of neurons across these different areas.
METHODS: To address this issue, we used in vivo 2-photon calcium imaging to record the responses of thousands of neurons from six different visual cortical areas while mice were presented with thousands of natural images. We then used deep neural networks to model the responses of each neuron and generated an optimal stimulus for each neuron.
RESULTS: We find significant differences in the preferred visual features of neurons in different visual cortical areas and are developing data-driven methods to measure tuning curves of these neurons.
CONCLUSIONS: These results provide novel insights into the functional organization of mouse visual cortex.

PS.11.03 - The role of visual cortex in perceptual decisions

Arbora Resulaj [1]

[1] University of Toronto Mississauga

BACKGROUND AND AIM: The visual world changes quickly and continuously. Thus, the rapid detection of relevant features in the visual world is essential for an organism to cope with such ongoing changes. For example, humans are quick at identifying specific features, like an animal

or a face, in the visual scene. The extraction of such features, however, is thought to require the sequential activation of many brain areas. This implies that each involved brain area must process information over a brief period of time. In this talk, I will address two fundamental questions. First, how long does it take a visual area in the mammalian cortex to process enough information for the organism to make a decision about the visual scene? Second, how is the visual scene represented in the visual cortex during this time period?

METHODS: I will address these questions in the mouse using optogenetics, electrophysiology, lesions and behavior.

RESULTS: I will show that the earliest activity in the visual cortex is sufficient to extract key features of the visual scene for the organism to make an appropriate decision.

CONCLUSIONS: Next, we are determining which brain areas read out this essential early activity.

PS.11.04 - The dynamic role of visual inputs in generating spatial representations

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[1] University of British Columbia, [2] Johns Hopkins University, [3] University of Texas at San Antonio

BACKGROUND AND AIM: The cognitive map in the mammalian hippocampal formation combines visual and non-visual sensory inputs into a coherent representation of place. This process of spatial localization involves triangulation using landmarks and integration of self-motion inputs. Stable landmarks are recognized through salience, coherence and relevance. Self-motion inputs provide velocity and acceleration signals which have to be integrated, scaled and continuously calibrated against stable landmarks to maintain a stable allocentric frame of reference. Through a series of experiments, we seek to understand the interaction between landmarks and path integration, and the how the cognitive map deals with conflicting reference frames using task relevance.

METHODS: We developed a virtual-reality Dome apparatus where rats freely running on a circular track can be exposed to controlled visual and auditory cues, including visual landmarks, optic flow cues and tones of different frequencies. These cues can be changed as ratios of the ratsâ€™ own movement, termed experimental gains. Using implanted hyperdrives containing 16-32 tetrodes, we record multi-unit activity from hippocampal CA1. From the recorded neural population, we can decode spatial information scores in different moving reference frames, and the movement of the coherent internal frame of reference termed the hippocampal gain. The hippocampal gain decoded during an experiment can be used to manipulate visual cues in real time. In one experiment, rats can be trained to request reward at specific locations in a moving reference frame.

RESULTS: We show that visual landmarks can robustly control place fields, and predictably recalibrate path integration. However, the representation can decouple from less reliable visual landmarks. Optic flow, a visual self-motion cue, can by itself influence place fields and recalibrate non-visual path integration. Optic flow cues can be used to directly control hippocampal gain through a neurally closed-loop controller. Place cells encode conflicting reference frames introduced by visual and auditory cues, but retain more spatial information in task-relevant frames.

CONCLUSIONS: Visual inputs are highly influential in forming and updating the hippocampal representation of place in rats. Using a virtual reality apparatus that retains intact self-motion inputs, we find that the influence of visual cues can be highly dynamic and variable, depending on sensory, perceptual and cognitive factors such as salience, reliability and task-relevance.

Parallel Symposium 12: Network alterations in psychiatric disorders and novel treatment strategies

Advances in genetic and human neuron models of psychiatric disorders implicate altered neuron network assembly and function. We discuss how defining network alterations improves our understanding of the underlying biology of psychiatric conditions, and guides new treatment strategies. James Ellis will explain how modelling Rett syndrome in human neurons alters network activity and Ca2+ handling, and how it can be reversed. Evelyn Lambe will show adult mice can be rescued from persistent synaptic and behavioural deficits induced by developmental NMDAR-deficiency linked to autism spectrum disorders. Jasmin Lalonde describes how micro-RNAs contribute to altered Ca2+ handling in neural progenitor cells, neurons, and organoids of Bipolar Disorder (BD) patients. Austen Milnerwood will discuss how comparisons of neurons from lithium-responsive and lithium non-responsive BD patients guides small molecule interventions that reverse BD neuron hyperactivity in all BD patient neurons, regardless of lithium responsiveness.

PS.12.01 - Hyperexcitability & lithium response signatures provide novel phenotype rescue of bipolar disorder patient neurons via AKT & AMPK

Austen Milnerwood [1]
[1] McGill University

BACKGROUND AND AIM:

Bipolar disorder (BD) is a multifactorial psychiatric illness affecting ~1% of the global adult population. Lithium (Li), is the most effective mood stabilizer for BD, but works only for a subset of patients and its mechanism of action remains largely elusive. BD and treatment response to Li run in the family, suggesting a tractable (if multifaceted) genetic contribution. We sought to use neurons from patients with a known clinical treatment response to tease out the mode of action of Li, and mimic the effect in Li non-responsive patient neurons.

METHODS:

iPSC-derived neurons from patients with BD who are responsive (LR) or not (LNR) were subject to combined electrophysiology, calcium imaging, biochemistry, transcriptomics, and phosphoproteomics.

RESULTS:

We found a selective rescue of neuronal hyperactivity phenotype in BD LR neurons by Li, correlated with changes to Na^+ conductance. Whole transcriptome sequencing of BD neurons revealed altered gene expression pathways related to glutamate transmission, and selective alterations to those involved in cell signalling and ion transport/channel activity by Li. Akt signaling was identified as a potential therapeutic effect of Li in BD LR patient neurons, and Akt activation mimiced Li effect in BD LR neurons. Furthermore, increased neural network activity observed in both LR & LNR patient neurons was reversed by AMP-activated protein kinase (AMPK) activation.

CONCLUSIONS:

Testing BD patient neurons for these defined Li responses soon after clinical presentation could shave years off treatment selection and reduce quality and loss of life. The results also suggest potential for novel treatment strategies in BD, such as Akt activation in BD LR cases, and AMPK activation for LNR patients with BD.

PS.12.02 - Multi-scale perspective to decipher and treat NMDA receptor dysfunction in GRIN disorder

Sridevi Venkatesan [1], **Daria Nazarkina** [1], **Megan Sullivan** [1], **Yao Fang Tan** [1], **Sarah Qu** [1], **Amy Ramsey** [1], **Evelyn Lambe** [1]
[1] University of Toronto

BACKGROUND AND AIM: Missense genetic variants in NMDA receptors (NMDARs) cause GRIN disorders: a spectrum of severe neurological symptoms that include epilepsy and intellectual disability. However, the impact of patient genetic variants in NMDARs has mainly been explored

with cell systems; their electrophysiological impact in a native neuronal environment is largely unknown. Since NMDARs exert complex, integrative roles in neurons, we probe the consequences of a patient-specific NMDAR mutation in a mouse model to gain mechanistic insight and identify candidate treatments.

METHODS: We evaluate NMDARs at multiple levels (isolated, cellular, circuit) in transgenic mice heterozygous for a human patient variant in the obligate NMDAR subunit (Grin1 Y647S+/-). This mutation occurs in the highly conserved pore. Heterologous expression studies report reduced surface expression of mutant NMDARs, but variable changes in receptor properties. It is unclear whether this will result in loss or gain-of-function of the receptor population. To identify the impact of this variant within a native neural environment, we pursue electrophysiological recording with electrical stimulation and wide-field calcium imaging in acute brain slices. To test efficacy of an electrophysiologically-identified candidate treatment, we assessed seizure frequency and severity in vivo.

RESULTS: Multi-scale assessment in the GluN1 Y647S variant reveals different consequences for isolated receptors versus integrated cellular and circuit signalling. Isolated NMDARs exhibit clear loss-of-function characteristics, but an intriguing paradox emerges for integrative responses. We discover that impaired patient-variant receptors cause seizure-promoting dendritic excitation by loss of NMDAR negative feedback within neurons. This mechanism enables targeted treatment of seizures in the Grin Y647S+/- mouse using oral magnesium threonate supplementation. These results emphasize the importance of understanding the impact of NMDAR variants on integrated signalling in order to identify effective treatments.

CONCLUSIONS: Functional context is essential to decipher and treat NMDAR dysfunction in GRIN disorder. Seizure-promoting excitation arises from loss-of-function NMDAR through impaired negative feedback within neurons. Successfully targeting this mechanism is sufficient to treat seizures.

PS.12.03 - Bipolar disorder-iPSC derived neural progenitor cells exhibit dysregulation of store-operated calcium entry and accelerated differentiation

Jasmin Lalonde [1], **Tristen Hewitt** [1], **Begum Alural** [1]
[1] University of Guelph

BACKGROUND AND AIM: Bipolar disorder (BD) is a chronic mood disorder characterized by severe symptomatic cycling between periods of depression and mania/hypomania. The discovery of disease mechanisms responsible for BD has progressed slowly due to the heterogeneity of clinical symptoms in patients, the complexity of the underlying genetics, and the lack of comprehensive animal models. However, the growing availability of patient-derived induced pluripotent stem cells (iPSCs) and culture methods, such as differentiated neurons in a

monolayer fashion or as three-dimensional (3D) brain organoids, offers new options to face this challenge. While most of the efforts to uncover mechanisms contributing to BD focused on phenotypes at the mature neuron stage, little research has considered events that may occur during earlier time points of neurodevelopment. Further, although aberrant calcium signaling has been implicated in the etiology of this condition, the possible contribution of store-operated calcium entry (SOCE) is not well understood. Here, we report calcium and developmental dysregulations related to SOCE in BD patient iPSC-derived neural progenitor cells (BD-NPCs) and cortical-like glutamatergic neurons. METHODS: We used live-cell calcium imaging to characterize the difference between iPSC-derived models from BD patients and healthy control individuals in calcium mobilization through SOCE. We also combined biochemical assays (RNA-sequencing and western blotting) with live-cell imaging to relate differences in SOCE activity between the two groups to function. RESULTS: Using a calcium re-addition assay, we found that BD-NPCs and neurons had attenuated SOCE. Intrigued by this finding, we performed RNA-sequencing and uncovered a unique transcriptome profile in BD-NPCs suggesting accelerated neurodifferentiation. Consistent with these results, we measured a slower rate of proliferation, increased neurite outgrowth, and decreased size in neurosphere formations with BD-NPCs. Also, we observed decreased subventricular areas in developing BD cerebral organoids. Finally, BD NPCs demonstrated high expression of the *let-7* family while BD neurons had increased *miR-34a*, both being microRNAs previously implicated in neurodevelopmental deviations and BD etiology. CONCLUSION: In summary, we present evidence supporting an accelerated transition toward the neuronal stage in BD-NPCs that may indicate early pathophysiological features of the disorder.

PS.12.04 - Calcium-dependent hyperexcitability in Rett syndrome neuronal networks

James Ellis [1]

[1] University of Toronto

BACKGROUND AND AIM: Rett syndrome (RTT) is predominantly caused by mutations in *MECP2* and can be modelled in vitro using human induced pluripotent stem cell-derived neurons. We produced *MECP2* null excitatory neurons using Ngn2 over-expression and phenotyped cell morphology, activity and circuitry (Mok et al, Translational Psychiatry 2022). RTT neurons had smaller soma size and dendrite lengths, reduced synapse density and deficits in synaptic connectivity. However, they were also hyperexcitable with increased evoked action potentials at low current injection. In contrast, multi-electrode array (MEA) experiments demonstrated that *MECP2* null neurons had reduced frequency of network bursts consistent with synaptic hypoconnectivity and increased duration of network bursts. A computational model explained the changes in network burst frequency based on intrinsic Na+ and K+ currents in individual neurons. We examine how increased hyperexcitability in single neurons can lead to decreased network bursts in circuits.

METHODS: We re-analysed MEA data from isogenic MECP2 cell line pairs recorded over 6 weeks. A custom burst detection algorithm analysed network events and isolated a phenomenon that we termed reverberating super bursts (RSBs). RSBs are composed of an initial large amplitude network burst followed by high frequency repetitive low amplitude mini-bursts. Standard network burst detection algorithms do not consistently separate RSBs into their mini-burst components and therefore under-estimate network burst frequency and inflate network burst duration. To probe potential mechanisms of RSBs, we conducted pharmacological manipulations using bicuculline, EGTA-AM and DMSO on *MECP2* null neurons.

RESULTS: *MECP2* null excitatory neurons have increased frequencies of RSBs and their mini-bursts consistent with hyperexcitable networks. Application of the Ca++ chelator EGTA-AM selectively eliminated RSBs and rescued the network burst phenotype, but bicuculline had no effect consistent with the absence of inhibitory neurons in our cultures.

CONCLUSIONS: Our results indicate that during early development, human excitatory neurons transiently produce RSBs as circuits form and networks begin to mature. When taking the increased minibursts in the RSBs into account, RTT neuronal networks are hyperexcitable in agreement with the earlier single neuron findings. RSBs may predispose RTT neurons to the emergence of hyper-synchronic states that potentially translate into seizures. Network hyperexcitability is dependent on asynchronous neurotransmitter release driven by pre-synaptic Ca++ and can be rescued by EGTA-AM to restore typical network dynamics (Pradeepan, McCready et al, Biological Psychiatry: Global Open Science 2024).

Poster Sessions

Poster Key

A - Development
B - Neural Excitability, Synapses, and Glia: Cellular Mechanisms
C - Disorders of the Nervous System
D - Sensory and Motor Systems
E - Homeostatic and Neuroendocrine Systems
F - Cognition, emotion and motivation
G - Novel Methods and Technology Development
H - History, Teaching , Public Awareness and Societal Impacts in Neuroscience

Poster Session 01

P1-A-1 - The effect of Glyphosate and RoundUp on neurogenesis and neural activity in Zebrafish

Rachel Lacroix [1], **Valentina Almanza** [1], **Sophie Mckenzie** [1], **Mataea Armstrong** [1], **Deborah Kurrasch** [1]

[1] University of Calgary

N-(phosphonomethyl)glycine (glyphosate) is the top agricultural chemical in the world to date, with an annual estimated spray rate of ~1.6 billion pounds. Glyphosate acts by inhibiting an enzymatic reaction in the shikimate pathway, which is exclusive to plants and microorganisms. Although no molecular targets are known in higher animals and humans, recent evidence of off-target glyphosate toxicity has prompted a new demand for the investigation of effects in animal models. Here, using zebrafish as a model, we investigate how glyphosate and a commercial glyphosate formulation, RoundUp, affect neurogenesis and neural activity. Glyphosate or RoundUp was exposed to zebrafish from 10-48 hpf, a developmental stage coinciding with neurogenesis. A 5-ethynyl-2-deoxyuridine (EdU) birth-dating assay was used to quantify and compare neurogenesis, immunohistochemistry was performed on telecephalic brain slices for cell cycle markers, and whole-mount immunohistochemistry was performed to investigate markers of neuron activity.we detected newborn neurons to form a neurogenic curve across timepoints 10, 24, 48, 72, 96 and 120 hpf. We detected a significant change in the proportion of newborn neurons born to RoundUp-exposed fish at 48 hpf and 72 hpf. Next, to understand whether neurogenic changes are a result of altered cell-cycle dynamics, we performed staining of 5 dpf zebrafish telencephalic brain slices for PHH3 and PCNA, which showed no significant changes in proportion of positive cells. Tangentially, we performed whole-mount immunochemistry on fish exposed to vehicle or treatment for pERK and ERK, and whole-brain activity mapping we uncovered significant changes in primarily telencephalic regions of 5 dpf brains. Overall, this research starts to shed light on whether neurogenic exposure to glyphosate or RoundUp affects neurogenesis and neural activity of young zebrafish.

P1-A-2 - Characterising neuropathy and retinopathy in a newly developed mouse model of abusive head trauma

Sydney Harris [1], **Richelle Mychasiuk** [1]

[1] Monash University

Abusive head trauma (AHT) is the leading cause of childhood abusive mortality and morbidity. Colloquially known as â€œshaken baby syndromeâ€, AHT is difficult to diagnose as symptomology is largely non-specific. Moreover, as caregiver transparency is often non-existent, AHTs tend to occur repetitively. AHTs can lead to lasting behavioural deficits; manifesting in the weeks to years post-injury. Additionally, AHTs may lead to subdural and retinal haemorrhaging, visual deficits, and retinoschisis. Despite the high burden AHTs, there is a scarcity of animal models regarding this condition. Given this, we aimed to develop a clinically relevant mouse model of AHT whereby repeat injuries lead to the development of brain, retinal, and behavioural deficits that worsen with time post-injury and with each

successive injury. Our mouse model was developed by exposing young mice (postnatal days (P) 8-12) to 0, 1,3, or 5 shaking injuries for either 15 or 60s in length. Mice were behaviourally tested acutely and chronically prior to euthanising on P14, P21, or P45. Their eyes and brains collected for analysis. Mice showed differences in genes related to brain water content and growth; the changes in gene expression preceded behavioural deficits. Mice also exhibited differences in astrocyte and oligodendrocyte expression in the hippocampus. Mice did not have altered visual function; however, they did show immune cell infiltration into the eyes. Overall, we have developed a mouse model of AHT that can be used for the examination of biomarkers and treatments for children with an AHT.

P1-A-3 - Memory specificity is determined by distinct CA1 input populations on parvalbumin interneurons

Cory Mckenzie [1], **Adam Ramsaran** [2], **Matteo Saderi** [2], **Sheena Josselyn** [2], **Paul Frankland** [2]

[1] University of Toronto, [2] The Hospital for Sick Children

The specificity of childrenâ€™s episodic memory tends to increase with age, with early memory lacking in detail specificity. While this early bias toward generalization might help children adapt to being faced with novel experiences, the neurobiological mechanisms responsible for this shift in memory specificity have only recently begun to be investigated. Recently, the maturation of parvalbumin interneurons (PV+ INs) in the CA1 of the hippocampus were identified as critical for the development of memory specificity. One possibility is that there are also circuit-level changes in the hippocampus that may contribute to PV+ IN development in the CA1. We used optogenetic and neural circuit tracing methods to characterize the structural and functional development of inputs from CA3 and the medial entorhinal cortex (MEC) to pyramidal neurons and PV+ INs in the CA1. We find that CA1 PV+ INs frequently receive inputs from the CA3 but rarely from the MEC. These CA3 projections activate CA1 PV+ INs in postnatal day (P)24 and adult mice, but not at P20, and accelerating the development of CA3 projections leads to the early development of memory specificity and CA1 PV+ INs. These findings suggest that activity from CA3 inputs to CA1 are necessary for the development of PV+ INs which promote memory specificity, while medial entorhinal inputs to CA1 develop earlier, do not activate PV+ INs, and promote memory generalization. This also provides evidence for how the brain can simultaneously support both specific and general memory representations of the same event with parallel pathways.

P1-A-4 - Investigating the role of Baf53b in mouse neuronal gene expression and autism behaviours

Megan Rowland [1], **Annemarie De Vries** [1], **Fortune Rantuana** [1], **Kenza Zobaidi** [1], **Annie Ciernia** [1]

[1] University of British Columbia

Autism spectrum disorder (ASD) is a prevalent neurodevelopmental disorder. It is well established that ASD is heritable, however, there are hundreds of genes that have been implicated. BAF53B is one of the neuronal BAF (nBAF, BRG1/BRM associated factor) nucleosome remodelling complex subunits and was recently found to be mutated in ASD. Baf53b is expressed in all neuronal subtypes, but it has not been studied in interneurons. Parvalbumin (PV) interneurons are depleted in ASD and mice with reduced levels of PV expression display ASD phenotypes. I hypothesize that deletion of Baf53b in PV neurons will alter gene expression required for PV neuronal function, resulting in ASD-relevant behaviours. To begin parsing the molecular impacts of Baf53b on neuronal gene expression, we performed RNA-sequencing in primary cortical neuron cultures. Baf53b deletion produced significant increases in several markers of inhibitory interneurons indicating a potential shift in differentiation or misregulation of interneurons. To examine potential impacts on neuronal structure, we measured dendritic branch complexity and found a significant blunting of dendritic outgrowth in both excitatory and inhibitory neurons either lacking Baf53b or with a Baf53b variant found in ASD. Reintroduction of wild type Baf53b prevented branching deficits, suggesting a causative role for Baf53b in dendritic branching. Conditional deletion of Baf53b specifically in PV neurons results in increased ultra-sonic vocalizations in females and impaired spatial memory in both sexes, validating a key role for Baf53b in PV neuron regulation of ASD-relevant behaviours. This work expands our understanding of the nBAF complex in neuronal function and gene expression, providing insight into the etiology of ASD.

P1-A-5 - Development of a unique and sparse neuronal type in the neocortex

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[1] University of British Columbia

The neocortex is crucial for higher-order cognitive functions like socialization, learning, and memory. Deep within the neocortex lies a sparse and often-ignored excitatory cell type within layer 6b. In contrast to all other excitatory neurons in the neocortex, layer 6b neurons are derived from a precursor population during embryonic development known as the subplate, and are the first neuronal population to differentiate during cortical development. Perturbations during the development of layer 6b have been associated with neurodevelopmental disorders, with these disorders often exhibiting a sex bias. As such, we were motivated to examine the developmental properties and sex specificity of layer 6b development. We investigated sex-dependent layer 6b development using multiplexed fluorescent *in situ* hybridization targeting marker genes unique to layer 6b. There were no sex-specific differences in layer 6b marker gene expression between neonatal and adolescent stages. To further examine if the developmental window of layer 6b occurred prior to the neonatal stage, we conducted single-cell RNA

sequencing analysis at various embryonic stages using published data. The analysis provided evidence that layer 6b cells have differential marker gene expression across development and may be derived from specific ventricular and subventricular zone subpopulations. This study provides insight into the developmental window of layer 6b in males and females, which can contribute to a better understanding of the etiology of neurodevelopmental disorders.

P1-A-6 - Identifying metabolic alterations in astrocytes from a 16p11.2 deletion mouse model

Nicole Blakeley [1], **Baptiste Lacoste** [1], **Julie Ouellette** [1], **Shama Naz** [1]

[1] University of Ottawa

Maintaining homeostatic metabolism is essential for brain function, but also critical during development. Dysfunction in the neurovascular unit (NVU) during critical periods of development is linked to neurodevelopmental disorders (NDDs), specifically in the 16p11.2 deletion syndrome (16pDel). As astrocytes represent central hubs within the NVU, it is imperative to ask if, and how, their metabolic function is altered in 16pDel. **Our hypothesis is that 16p11.2 haploinsufficiency leads to metabolic imbalance in astrocytes.**

Using an established 16pDel mouse model, we extracted intracellular metabolites from primary astrocytes and quantified metabolite abundance using liquid chromatography-mass spectrometry (LC-MS). Astrocytes from 16pDel males showed significantly reduced Adenosine ($P<0.037$) and significantly increased ribose-5 phosphate ($P<0.043$). Female 16pDel astrocytes showed significantly reduced alpha-ketoglutaric acid ($P<0.015$). As a measure of astrocyte mitochondrial function, a Seahorse Cell Mito Stress test will compare the oxygen consumption rate and extracellular acidification rate. Changes in gene expression will also be assessed using bulk RNA sequencing. Each assay will be done from at least n=5 biological replicates per genotype and per sex.

This project is identifying functional alterations in astrocytes from a mouse model of in 16pDel, unveiling mechanisms of astrocyte dysfunction in NDDs. Our preliminary results support the existence of metabolic alterations in 16pDel astrocytes, including sex differences in their metabolic signature.

P1-A-7 - Regulation of the developmental programmed cell death of hippocampal Cajal-Retzius neurons

Zain Patel [1], **Rebekah Van Bruggen** [1], **Mi Wang** [1], **Qiumin Tan** [1]

[1] University of Alberta

Cajal-Retzius (CR) neurons are crucial in brain development, particularly in the hippocampus. Shortly after birth in mice, the vast majority of CR cells undergo apoptosis. This process must be tightly regulated as inappropriate CR cell death has been linked to neurological disorders such as epilepsy.

The mechanisms that regulate CR cell death remain elusive. To address this, we have generated a mouse model where capicua (Cic), a gene crucial for brain development, is selectively deleted from CR cells. Loss of Cic from CR cells impairs their programmed cell death, leading to their persistence in the adult mouse brain. However, abnormal CR cell persistence due to Cic loss did not affect hippocampal-dependent behaviors such as anxiety and memory, as well as seizure susceptibility.

Some hippocampal CR cells undergo apoptosis during early postnatal development. We thus analyzed expression of the anti-apoptotic protein Bcl2 and found it to be expressed by more CR cells in Cic knockout mice. To determine the signals that regulate developmental CR cell death, we performed single-cell RNA sequencing of Cic-deficient CR cells at postnatal day 14 and identified Fgf1/Fgfr1 signaling pathway as a promising target.

This project will deepen our understanding of the regulatory mechanisms underlying programmed cell death during brain development. We will better understand the roles of CR cells in normal development and in diseases such as epilepsy, and identify strategies to overcome abnormal CR cell death regulation.

P1-A-8 - Cannabis vapour exposure in adolescence leads to sex-dependent changes in behaviour and brain connectivity in adulthood

Pedro Marinho [1], **Jaiden Smith** [2], **Hakan Kayir** [2], **Patrick Mccunn** [1], **Jibran Khokhar** [1]

[1] Western University, [2] University of Guelph

Cannabis use is common in adolescence and there is evidence for sex differences regarding the long-term effect of cannabis use. We aimed to investigate how exposure to 3 types of cannabis vapour in adolescent rats impacts brain development through behavioural tests and magnetic resonance imaging. Male and female Sprague Dawley rats were divided into four groups and exposed to high-CBD, high-THC, balanced CBD + THC, or air at post-natal days 28-42 using a vaporizer. On days 0 and 14 of exposure, rats underwent cannabinoid tetrad test. In adulthood, rats underwent Pavlovian autoshaping, active avoidance, prepulse inhibition and diffusion and functional MRI. Results indicated sex-dependent differences in the physiological effects of cannabis exposure in the cannabinoid tetrad test. Pavlovian autoshaping showed that male rats exposed to high-THC and high-CBD had less lever-directed behaviour

compared to controls and balanced groups, while all female rats showed no significant differences between the exposure groups. All three male cannabis-exposed groups showed impaired avoidance learning. The female active avoidance data showed significant differences between the THC and CBD-exposed groups only. Both males and females showed no group differences in PPI. Regarding MRI data in male rats, we found a single network with altered functional connectivity amongst the four groups and two networks with altered structural connectivity amongst the four groups. Females showed no altered networks. Adolescent cannabis vapour exposure can lead to long-lasting effects in adulthood in both sexes.

P1-A-9 - Long-term impacts of bupropion exposure during critical developmental periods on reward learning

Shannon Smith [1], **Hanna Issaqzai** [1], **Matthew Holahan** [1]

[1] Carleton University

Throughout post-natal development, the reward system undergoes critical changes for the establishment of connections that underline the emergence of adaptive behaviours. This can be disrupted by psychoactive drugs which alter connectivity and neurotransmission resulting in maladaptive behavioural output. Bupropion (BUP) is a dopamine and norepinephrine reuptake inhibitor used off-label to treat ADHD in children and adolescents. The current study examined short- (on drug) and long- (off drug) term impacts of BUP during development. Male and female rats were administered 10 or 20 mg/kg BUP or saline (s.c.) for one week during preadolescence (p18-24) or peri-adolescence (p34-40). On the second injection day, both BUP doses elicited increased locomotion in peri-adolescent but not preadolescent rats. On the last injection day, both BUP doses elicited a significant increase in locomotion in preadolescent rats, with a more pronounced effect in peri-adolescent rats. Seventeen days later, operant training and extinction, progressive ratio and locomotor tests were conducted. No long-term locomotor effects were seen. During extinction and progressive ratio tests, peri-adolescent rats in the high-dose condition exhibited increased inactive lever pressing over time. No significant differences were found in the preadolescent group. On-drug effects of BUP seem to be more effective during peri-adolescence due to the observed hyperactivity. Exposure to BUP during peri-adolescence also had long-term effects evidenced by reduced stimulus discrimination when reward delivery was inconsistent.

P1-A-10 - Molecular cartography of the human and mouse down syndrome brain

Anna Feng [1], **Wuxinhao Cao** [2], **Nareh Tahmasian** [2], **Bharti Kukreja** [2], **Keon Arbabi** [3], **Bianca Rusu** [2], **Shreejoy Tripathy** [4], **Brian Kalish** [5]

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Down syndrome (DS) is a common genetic cause of intellectual disability and early-onset neurodegeneration. Triplication of chromosome 21 triggers a cascade of transcriptional changes that disrupt fundamental aspects of neurodevelopment, yet the precise underlying mechanisms remain elusive. To bridge this gap, we performed single-nucleus sequencing (n=5 DS, n=5 CTL), and profiled > 120,000 cells from prenatal human brain tissue, capturing gene expression changes and dysregulated ligand-receptor (LR) mediated interactions present during the second trimester of human gestation. Notably, neural progenitors in the human DS brain exhibited altered expression dynamics of lineage commitment and senescence-associated signatures, indicating potential shifts in the fate and functionality of resulting neuronal and glial cells. Given the importance of linking human and model system pathobiology, we also performed multiplexed error-robust fluorescent *in-situ* hybridization on a well-established trisomic mouse model (Ts65Dn) to study the cellular landscape of the DS brain during early life (postnatal day 0; n=3 DS, n=3 CTL), and aging (6 months; n=3 DS, n=3 CTL). We profiled > 1 million cells and revealed region-specific gene expression patterns and spatially-informed LR changes that provide insights into the molecular control of neurogenesis, gliogenesis, and myelination. Together, our research provides a comprehensive cross-species understanding of the complex multicellular processes underlying DS neurodevelopment, highlighting potential molecular targets for therapeutic interventions.

P1-A-11 - Functional dissection of early life stress-sensitive hippocampal neurons in the developing brain

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High synaptic plasticity in the developing brain heightens susceptibility to early life experience, with increasing evidence indicating that early life stress (ELS) permanently affects learning and memory by disrupting CA1 hippocampal circuit organization. During early postnatal development, neuronal networks and classical cell types are known to be heterogeneous both spatially and temporally at baseline. However, most studies examine global alterations in synaptic function in randomly sampled neurons, failing to detect heterogeneous responses to ELS in subpopulations of neurons. To address this flaw, we use the c-Fos- green fluorescent protein (GFP) activity-dependent tagging system to demonstrate that limited bedding and nesting (LBN)-induced ELS from P2-9 induces selective activation in a subpopulation of CA1 hippocampal neurons in the immature mouse. Furthermore, using whole-cell patch-clamp recordings, we highlight ELS-induced heterogeneity in CA1 cell function as selectively

activated, GFP+, neurons show significant enhancement in excitability after examining Î±-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) function compared to non-activated cells. Importantly, we show that precise chemogenetic suppression of selectively activated neurons reversed ELS-induced AMPAR enhancement, long-term cognitive behavioral deficits, in addition to plasticity impairments at the hippocampal CA1 circuit. Our results strongly support a critical role for stress sensitive neurons in early-life-induced neuronal circuit reorganization and long-term cognitive deficits

P1-A-12 - A role of Arid1b in cerebellar Purkinje cell development and function in mice

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The cerebellum is a brain structure that is critical to balance and movement. Pathological changes of cerebellum, including reduction of Purkinje cell (PC) density, cerebellar vermis underdevelopment, reduced connectivity have been frequently reported in patients with neurodevelopmental disorders (NDD). Mutation of *ARID1B* gene has been frequently identified from NDD, it has been shown to play roles in proliferation, dendritic innervation, and synapse formation of neurons. *ARID1B* is enriched in cerebellar neurons, but very little is known about its function in the cerebellum. In this project, we use mice with *Arid1b* conditional knockout (cKO) in PC as a model to examine the alteration of cerebellum development. We identified an increase of PC number in the anterior lobules of cerebellum in cKO mice from postnatal to young adult stage. However, we did not detect a difference in PC number in the posterior lobules of cerebellum. We found dendritic atrophy of PC in the cKO mice throughout lobules. We detected altered electrophysiological features, including increased input resistance and altered firing rate. Our preliminary results suggested a developmental stage-dependent and regional-specific role of *Arid1b* in cerebellum. More experiments involving transcriptional analysis and behavior studies are currently ongoing. Through mouse genetics and neurobiological approaches, we aim to provide in-depth understanding of molecular and cellular mechanisms underlying cerebellum abnormality in NDD resulting from *ARID1B* mutation.

P1-A-14 - Neurological and Biomolecular Changes in Adult Rat Offspring Hippocampus Identified by Fourier-Transform Infrared Spectroscopy Following Gestational THC Exposure

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Fetal development is sensitive to phytocannabinoids as clinical findings suggest that children exposed to *Cannabis* in utero are more susceptible to neurodevelopmental disorders later in life. Preclinical investigations demonstrate that prenatal Î”9-tetrahydrocannabinol (THC) exposure produces sex-specific changes in rodent behaviour. The present study aims to investigate potential neurological and biomolecular alterations in male and female adult rat brains of offspring exposed daily to 3 mg/kg THC i.p. between gestational day 6-20. Fourier-transform infrared spectromicroscopy (FTIR) was used to examine changes in distribution of endogenous biomolecules in prefrontal cortex and hippocampus of offspring. FTIR characterizes regions rich in proteins, protein aggregates, lipids, and other essential biomarkers associated with oxidative stress such as demyelination, lipid oxidation, and alterations in lipid and protein ratios. Preliminary analysis indicates lipid oxidation and changes in lipid/protein ratio associated with THC exposure, indicating an increase in lipid content within the hippocampus as well as the surrounding grey and white matter. These data also show an increase in hippocampal lipid peroxidation. Complementary follow up analysis using X-ray fluorescence Imaging (XFI) will highlight the presence of metal dysregulation, focusing on endogenous metals including Cu, Fe and Zn and metal ions Cl- and K+ which are essential to brain development and function. These data will aid in pinpointing the biochemical changes caused by gestational phytocannabinoid exposure.

P1-A-15 - Changes in multi-scale entropy during aging. A large sample size replication study

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As we age, the complexity of the organization of our brain changes. Multiscale entropy has been used to evaluate changes in brain complexity over the lifespan, whereby complexity increases rapidly during development and is followed by a shift in the scale of peak entropy as we age (McIntosh, Kovacevic & Itier, 2008, McIntosh et al., 2014, Wang et al. 2016). However, these studies are limited by their small

sample sizes (n<100). In this study we attempted to replicate findings of the previous literature (McIntosh et al., 2014, Wang et al. 2016) by applying a pipeline developed by Xifra-Porxas et al., 2021 to extract time series for the Schaefer 400 atlas from resting state magnetoencephalography data recorded in 585 participants from the Cam-CAN dataset (Shafto et al. 2014, Taylor et al. 2017). Investigating the relationship between age, sex, fluid intelligence and MSE in each region of interest revealed two significant latent variables. The first described an effect of age that replicated the effects found in the literature (McIntosh et al., 2014, Wang et al. 2016) that was more prominent in females and was negatively correlated with fluid intelligence scores. The second latent variable described a relationship where females with better visual short-term memory have increased fine scale entropy and decreased mid-scale entropy. These findings validate previous interpretations of multiscale entropy related to aging and highlight potential sex differences in network dynamics that lend to its potential as a personalized measure for cognitive decline in our aging population.

P1-A-16 - Beyond homogeneity: Transcriptomic insights into the diverse development of Retinal Pigment Epithelium (RPE) in Zebrafish

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Vision is crucial for human physiology and cognition, with the retina playing a key role in processing visual information. Retinal Pigment Epithelium (RPE), a specialised set of cells in the retina performs multifaceted functions, predominantly maintaining photoreceptor (PR) health. Disruption of RPE is implicated in various retinal degenerative diseases (RDDs). We focus here on RPE development, exploring transcriptional differences in embryonic zebrafish retina to illustrate RPE heterogeneity. This study also addresses the knowledge gap of molecular mechanisms regulating RPE proliferation during developmental stages. We tested the hypothesis that transcriptionally distinct RPE cell subtypes exist and exhibit different developmental trajectories for that we used zebrafish embryos at various developmental stages to analyse RPE-specific gene expression by in situ hybridization (ISH), immunohistochemistry, and EdU labeling, coupled with confocal imaging. Integral to my methodology is the analysis of both publicly available and in-lab scRNA-seq datasets. Results indicate that RPE cells post-optic cup morphogenesis are not transcriptionally homogenous, with different subtypes showing distinct spatiotemporal expression over development. Our data argue that the transcriptionally distinct RPE subtypes represent RPE progenitors and RPE cells at different developmental stages. These data shed light on RPE cell development and subtype identification both embryonically and through to the mature larval providing valuable insight into RPE biology that may aid future treatments of RDDs.

P1-A-17 - Single cell approaches define two groups of mammalian oligodendrocyte precursor cells and their evolution over developmental time

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Developmental myelination occurs postnatally via genesis of oligodendrocyte precursor cells (OPCs) that make oligodendrocytes. Myelination also occurs in the adult brain, in part via adult parenchymal OPCs that are generated neonatally but persist in an undifferentiated state. Little is known about how this adult OPC pool is established. Using single cell transcriptomic and epigenetic approaches, we demonstrate that the early postnatal murine cortex contains two transcriptionally and epigenetically distinct populations of OPCs. One population appears metabolically active and primed to differentiate (active or actOPCs), whereas the second is less active, enriched for genes involved in synaptic function, and is predicted to derive from actOPCs (homeostatic or hOPCs). Spatial transcriptomic and immunohistochemical analyses reveal that actOPCs are enriched within white matter, whereas hOPCs are more abundant in grey matter. Single cell sequencing indicates that transcriptional OPC heterogeneity persists into adulthood, but that adult OPCs become epigenetically indistinguishable and undergo a widespread shutting down of their chromatin. Furthermore, this epigenetic state is unchanged by cuprizone-induced demyelination, suggesting that injury does not change the potential of adult OPCs at the chromatin level. These data support a model where two OPC populations serve distinct postnatal functions, and that neonatal and adult OPC-mediated oligodendrogenesis are fundamentally different.

P1-A-18 - Purinergic regulation of the zebrafish spinal-cord-injury response

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In contrast to mammals, adult zebrafish (*Danio rerio*) undergo successful neural regeneration following spinal cord injury (SCI). Radial glia lining the zebrafish central canal function as neural progenitors that undergo a massive injury-induced proliferative response before differentiating into both neurons and glial cells. However, the molecular mechanisms that underlie these processes remain elusive. Among the signaling pathways that are dysregulated following mammalian SCI is the purinergic signaling system. While purines such as ATP and its metabolites mediate diverse cellular processes within the mammalian central nervous system (CNS), their roles have not been explored within the zebrafish CNS. Given that

the purinergic system is evolutionarily conserved among vertebrates, we sought to characterize potential roles for P2X7 and P2Y2 receptor signaling in neurogenesis following SCI in adult zebrafish. Our findings demonstrated that expression of P2X7 and P2Y2 receptors were both upregulated following injury, and activation of P2X7 signaling in particular enhanced injury-induced neurogenesis in this species. Further work will elucidate the roles of both receptors in these natural regenerators following SCI.

P1-B-19 - Pharmacological inhibition of O-GlcNAc hydrolase reduces pS129-α-Synuclein sggregation in the Substantia Nigra of mThy1-hSNCA mice

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The inclusion of phosphorylated α-synuclein – notably phospho-serine 129 (pS129) – within neurotoxic Lewy Bodies (LB) is generally viewed as a biomarker for the progression of Parkinson's disease (PD). Given the resistance of LB against proteolytic clearance, strategies that prevent the accumulation of pS129-α-synuclein from maturing into LB in the brain represent attractive targets in the development of PD treatment. In this study, we investigated the therapeutic benefit of chronic elevation of a unique form of glycosylation, termed β-D *N*-acetylglucosamination (O-GlcNAcylation), in blocking the deposition of pS129-α-synuclein aggregates in the brain of a mThy1-hSNCA mouse model of PD (Line 15). Administration of the pharmacological inhibitor Thiamet-G, which blocks the enzyme O-GlcNAc hydrolase (OGA), for 10 months to both Line 15 and wild-type mice (n=91) increased levels of β-D *N*-acetylglucosamine (O-GlcNAc) by up to 6 folds relative to vehicle treated cohorts (n=60). Thiamet-G treatment also led to significant reductions in pS129-α-synuclein immunoreactive aggregates within the substantia nigra of the Line 15 mice, without impacting the overall level of α-synuclein expression. Line 15 mice also demonstrated better locomotion in open field and pole descending tests within the course of Thiamet-G treatment. Our findings are consistent with other published studies, and strongly encourage the advancement of OGA inhibitors, in which some are already in the clinic, as a disease modifying treatment against PD.

P1-B-21 - Investigating the function of polymorphisms in APOE in chronic pain

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Single cell RNA sequencing of microglia revealed that Apolipoprotein E (Apoe) is the top upregulated gene in spinal cord microglia at chronic time points after peripheral nerve injury in mice1. APOE is a lipoprotein that is essential for the regulation of neuroimmune functions, synaptic activity, and aging. In humans, there are 3 different isoforms of APOE: APOE-Îµ2, APOE-Îµ3 and APOE-Îµ42-5. We have shown that carriers of APOE-Îµ2 have higher risk to develop chronic pain, whereas carriers of APOE-Îµ4 have lower risk to develop distinct chronic pain conditions1. We hypothesize that APOE-Îµ2 increases the risk of developing chronic pain, while APOE-Îµ4 has a protective effect.
To test the functional role of ApoE polymorphisms in chronic pain, we subjected humanized mice expressing APOE-Îµ2, APOE-Îµ3 and APOE-Îµ4 (KI) to spared nerve injury (SNI), a model of neuropathic pain. To test for cell-type-specific effects, these KI mice were crossed to a microglia-specific Cre-inducible mouse line (TMEM119CreERT2) to create a conditional knock out (cKO) in microglia. Following SNI, APOE-Îµ4 mice showed less nerve injury-induced cold hypersensitivity as compared to APOE-Îµ2 and APOE-Îµ3. Furthermore, APOE-Îµ2: TMEM119CreERT2 cKO mice showed less mechanical hypersensitivity as compared to APOE-Îµ2 KI mice, suggesting that APOE-Îµ2 plays a detrimental role in microglia in neuropathic pain. These results further support our hypothesis that APOE-Îµ2 increases the risk of neuropathic pain, while APOE-Îµ4 decreases the risk.

P1-B-22 - The beneficial impacts of SorCS1, a new competitor to Amyloid beta, on addressing synaptic and cognitive impairments in a mouse model of Alzheimer's disease

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Soluble AÎ² oligomers (AÎ²Os) disrupt synapses, causing cognitive decline and memory issues in Alzheimer's disease (AD). Neurexins play vital roles in synaptic functions and bind to AÎ²Os, resulting in neurexin dysregulation. SorCS1 regulates NRX1Î², countering AÎ²Os' impact and rescuing synaptic toxicity. This suggests SorCS1's potential in alleviating AÎ²O-induced synaptic dysfunction. To explore SorCS1's in vivo effects, we developed forebrain-specific SorCS1 transgenic mice crossed with 5xFAD, an AD model. To achieve temporal and forebrain-specific expression of SorCS1, SorCS1KI mice were crossed with CaMKIIa-CreERT2 mice, allowing Cre recombinase expression upon tamoxifen treatment specifically in forebrain excitatory neurons. Through these crossings, we established four genetic groups: 1) non-AD normal control, 2) non-AD with SorCS1 overexpression (OE), 3) AD model, 4) AD with SorCS1 OE rescue model. Structural, biochemical analyses such as immunoblot and immunohistochemistry, and behavioral

tests were conducted to determine SorCS1's impact on synaptic impairment and memory deficits in AD mice. In mice overexpressing SorCS1 in the AD model, no significant differences were observed in long-term spatial learning memory, locomotor activity, or anxiety levels compared to the AD control group. However, SorCS1 overexpression notably improved weakened short-term working memory in the AD mice. Furthermore, SorCS1 overexpression partially restored the expression levels of synaptic proteins in the cortex and hippocampus. In conclusion, in vivo, the overexpression of SorCS1 rescues impaired levels of synaptic proteins and improves deficits in short-term working memory.

P1-B-23 - Determining the roles of AKAP150-anchored PKA and CaN in crosstalk between LTP and LTD and consequently LTP/LTD balance

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Unlike the detailed molecular mechanisms for LTP and LTD, the mechanisms underlying crosstalk and balance between them are much less clear. It is not clear if the AKAP150-anchored PKA and CaN plays any significant role in LTP/LTD balance and crosstalk between them. Thus, we hypothesize that AKAP150 through anchoring PKA and CaN may play a critical role in mediating crosstalk between LTP and LTD, and hence LTP/LTD balance. To experimentally validate this, we developed two interference peptides based on the binding sequences of AKAP150 for PKA or CaN. We first demonstrated their ability to disrupt AKAP150-PKA and AKAP150-CaN in biochem experiments. To directly test the roles of AKAP-PKA and AKAP-CaN in LTP, LTD and their crosstalk, we used the peptides in vitro and in vivo recordings with different synaptic plasticity protocols. We found that unbinding CaN, while having no effect on non-decaying LTP, converted decaying LTP into non-decaying LTP and prevented LTD. In contrary, PKA discussion from AKAP prevented both non-decaying and decaying LTP. Moreover, we found that disrupting the AKAP-PKA interaction also reduced LTD. In conclusion, these results suggest that AKAP complex plays critical roles in the expression of LTP and LTD and their crosstalk. Therefore, we propose that AKAP enables LTP-inducing protocol to activate both LTP and LTD mechanisms. During the weak LTP protocol, the coactivated LTD mechanism counteracts the LTP expression mechanism, causing the LTP to decay. However, during the strong-induced LTP, the non-decaying LTP is ensured by a mechanism that actively inhibits this co-activated LTD mechanism.

P1-B-24 - A female-specific role for the EphA2 receptor within the prefrontal cortex in depression-like behaviours and the modulation of low frequency oscillations in rats

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Eph receptors and their associated ephrin ligands regulate synaptic transmission, dendritic spine morphology and synaptic plasticity, and are largely expressed in brain regions implicated in depression, including the prefrontal cortex (PFC) and hippocampus (HIP). Eph-ephrin signaling in the PFC has been implicated in rodent models of depression, however, the role of EphA2 receptor signaling in depression-like behaviours and the associated sex differences is unknown. In this study, male and female rats received bilateral intra-PFC infusions of a EphA2 receptor peptide ligand agonist and the effect on depression-like behaviours and neural oscillations recorded from the cingulate cortex, nucleus accumbens (NAc), and HIP were assessed. At the end of the study, brains were removed for subsequent protein expression analysis by Western Blotting. Changes in PFC, NAc, and HIP c-fos expression, an additional readout of neuronal activity, following intra-PFC EphA2 receptor activation are also currently being analyzed. EphA2 receptor activation in the PFC induced despair- and anxiety-like behaviours in female animals only, as well as alterations in low frequency band oscillations in the NAc and HIP of male and female rats, respectively. The level of phosphorylated ephexin-1, a downstream substrate of the EphA2 receptor, was significantly elevated in the PFC of females only upon EphA2 receptor activation. Collectively, these results indicate a potential female-specific role for EphA2-ephexin-1 signaling in depression pathology.

P1-B-25 - Sexual dimorphisms in cortical transcriptomics at two time points in a model system of autism spectrum disorders

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In autism spectrum disorders sex differences exist in onset, prevalence, etiology, and presentation of symptoms, yet the mechanisms are unknown. Using the valproic acid (VPA) model, sex differences in prefrontal cortical gene transcription at postnatal day (pnd) 0 and 35 were evaluated. At pnd 0, in female VPA tissue, genes involved in growth factor signaling (*egfr*, *fgf15* and *hgf*) were elevated whereas neuropeptide gene expression (*tac1, cartpt, pdyn,* and *penk*) was downregulated as were many receptor genes (*drd1a*, *drd2*, *adora2a*, *mc4r*, and *htr3a*). In contrast, male VPA tissue showed elevations in the neuropeptide (*tac1, cartpt, pdyn,* and *penk*) and receptor (*drd1a*, *adora2a, and mc4r*) gene expression. At pnd 35, most genes were downregulated. In female VPA tissue, the expression of genes involved in vascular permeability (*gpr116, cldn5, flt1, rgs5, angptl4, esam,* and *decorin*) showed the greatest fold reduction in expression. In male VPA rats, genes that showed the greatest changes were those involved in neurotransmitter/ neuropeptide signalling and included an upregulation of *drd2*, *cartpt*, *adora2a*, and *pdyn*, and a downregulation of *nxph4* and several solute carrier genes. In both sexes, genes associated

with myelination, *rxrg*, *opalin* and *mog*, were also downregulated. Findings indicate that in this model cortical gene expression changes between male and female animals were for the most part distinct. Whereas gene expression associated with myelination appears to be altered in both sexes, processes involving vascular permeability may warrant attention in the female animals.

P1-B-26 - A comparison of neuroplasticity-related markers in the nucleus accumbens of male and female mice models of chronic inflammatory and neuropathic pain

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Chronic pain is a major risk factor for depression and anxiety, and rodent models of chronic pain correspond with altered activity in the Nucleus Accumbens (NAc). Accordingly, activity in the NAc is involved in the maintenance of pain hypersensitivity and emotion-related changes associated with chronic pain. However, the underlying molecular mechanisms driving this process have yet to be fully elucidated. Neuroplasticity is a mechanism that is highly involved in chronic pain development. In this work, differences between neuroplasticity-related markers in the NAc in a chronic pain state were compared using two injury models in C57 male and female mice. A complete Freundâ€™s adjuvant (CFA)-induced chronic inflammatory pain model as well as a spared-nerve injury (SNI)-induced neuropathic pain model was used to assess changes in relative levels of NR2B, pNR2B, CREB, pCREB, and PSD-95. Specific attention was also paid to the temporal aspects of pain development, with assessment of these molecular markers post-injury occurring at time points of 3 hours, 24 hours, 3 days, and 7 days in the CFA model, and time points of 5, 14, 30, and 60 days in the SNI model. Significant differences concerning both time post-injury and sex were found, with elevated levels of specific markers observed in the injury condition. This work not only further reveals the underlying molecular mechanisms occurring in the NAc, but also suggests that chronic-pain models that allow sufficient time for depression-like and anxiety-like changes to develop are more appropriate for assessing changes in the NAc.

P1-B-27 - Identification of microglial receptors responsive to gut Microbiota-Derived metabolites

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Microbes residing in the gastrointestinal tract have been shown to exert multiple immunomodulatory impacts on the brain through the highly interconnected gut-brain axis. Products of bacterial metabolism, such as short chain fatty acids, can cross the blood-brain barrier and interact directly with microglia, resident immune cells in the brain. Artificial removal of the microbiome results in impaired microglial development. Thus, microglia critically require the gut microbiome for normal cellular functions and maintenance of the brain homeostasis. However, the mechanisms and signaling cascades of how microbiota-derived metabolites affect microglia remain unknown. Therefore, using a novel chemical affinity labeling approach, we aim to identify microglial receptors that respond to gut microbiota-derived metabolites. We first investigated how metabolites impact microglial gene expression and phagocytic activity using Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR) and flow cytometry. We observed a mix of stimulatory and inhibitory effects of gut metabolites on microglial immune response. We then synthesized photoaffinity probes that mimic microbe-derived metabolites and tagged microglial receptor-probe complexes with a fluorophore via click chemistry. Followed by mass spectrometry, we identified functional microglial receptors responsive to specific microbe-derived metabolites. Hence, our findings provide an in-depth understanding of gut microbiome-microglia crosstalk, opening the door for new microbiome and microglia receptor targeted therapies.

P1-B-28 - Astrocyte-neuronal lactate shuttling in the anterior cingulate cortex in a murine model of chronic neuropathic pain

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Chronic pain impacts 1 in 4 Canadians over the age of 15, with less than half reporting any pain relief with current treatment options. Our current lack of understanding regarding the molecular mechanisms involved in chronic pain development has contributed to this lack in effective treatment options available. Current research points towards neuroplasticity in the anterior cingulate cortex (ACC) as a critical mechanism for long-term brain changes associated with chronic pain. We previously found that astrocyte-neuronal lactate shuttling (ANLS) is fundamental for hippocampal neuroplastic changes associated with pain-induced fear learning. Here, we used a neuropathic model of chronic pain, the spared nerve injury (SNI) model, to investigate nerve injury induced changes in lactate transport in the ACC of female and male mice to better understand the neural origins of chronic neuropathic pain. Various timepoints were chosen to assess lactate shuttling over time post-injury, and mechanical thresholds of injury were assessed using the Von Frey Test. After completing behavioural assessments, samples were collected from specific brain regions including the ACC and assessed using a lactate colorimetric assay. We found that both sexes showed a robust increase in SNI-induced nociceptive hypersensitivity, but observed a sex-specific increase of lactate in the ACC. These findings suggest that nerve injury engages ANLS in the ACC in a sex dependent manner, indicating sexual dimorphism of the ANLS in chronic pain development.

P1-B-29 - A role for the Lion's Mane mushroom-derived compound Erinacine A in neuronal systems function in vitro

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Lion's mane (*Hericium erinaceus*) is an edible medicinal mushroom whose use is evolving in North America. Often employed as a nootropic, evidence suggests Lion's mane can provide neuroprotection against ischemic injury and inflammatory factor-induced cell death in neurodegenerative disease models. Erinacine A (EA), an abundant and biologically active compound found in Lion's mane, has been shown to produce pharmacologic effects within the CNS of rats. EA acts as a potent stimulator of growth factor synthesis, which may have subsequent effects on neuronal systems activity and learning and memory performance. Using a multielectrode array, the aim of this initial study was to investigate the concentration- and time-dependent effects of EA on neuronal systems activity in primary cortical cultures derived selectively from male or female rat pups (pnd 0). We showed concentration (0, 100nM, 1Î¼M, 10Î¼M) and sex-dependent effects of EA, with the most robust effects occurring at 100nM. In the female-derived neurons, significant increases in the mean firing rate, number of bursts and number of spikes were observed, while conversely inter-spike interval increased in male populations. These effects were long-lasting, persisting to the 48 hr time point. These findings show EA can induce sex-dependent alterations in neuronal activity. Further in vivo studies characterizing in vivo systems function and behavioural alterations remain to be characterized.

P1-B-30 - Elucidating the Ketamine-activated neurocircuitry of the rostral linear nucleus

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Major Depressive Disorder (MDD) is currently one of the most common, disabling mental health disorders with an annual 7-8% of Canadians and a total of 300 million people diagnosed worldwide. Current first-line MDD treatments, such as SSRIs, often take weeks before a noticeable effect is observed, and commonly do not induce remission. Ketamine (KET), however, has shown promising results in providing rapid, robust antidepressant effects after a subanesthetic injection. This research

adds to the mechanistic knowledge of KET bioactivity by examining the rostral linear nucleus (RLi), a dopamine neuron-containing region that lies dorsal to the ventral tegmental area, and which we showed is activated by KET, but not psilocybin. The objective of this project was to build on these findings to determine the neurotransmitter phenotype(s) of RLi neurons that are activated by KET in male and female Wistar rats. Rats were administered KET (10 mg/kg) and brains were taken for processing by RNAScope to determine mRNA co-expression of c-fos, the dopamine neuron marker tyrosine hydroxylase, and the glutamate neuron marker vesicular glutamate transporter 2. Studies are presently ongoing. These studies will highlight the importance of the RLi in KET action although whether this region mediates the antidepressant versus dissociative effects of the drug remains to be determined.

P1-B-31 - Exploring the effects of ischemic stroke and diaschisis on presynaptic dendritic spine networks

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Ischemic stroke is a life-threatening medical condition that can lead to dysfunction in both proximally and distally connected areas in the brain, known as â€˜diaschisisâ€™. Diaschisis can provide important information about recovery after stroke; however, the structural changes that occur at the level of neurons connected to a stroke site are not fully understood. Here, we performed confocal microscopy to visualize dendritic spines after a photothrombotic stroke in the primary somatosensory forelimb cortex (S1FL) of adult mice labeled with a retrograde adeno-associated virus (retro pAAV.CAG.GFP). This allowed for the visualization of presynaptic neurons directly connected to the infarct core. We observed a decrease in presynaptic spine density one week after stroke in basilar dendrites within the peri-infarct region; however, no effects were found in regions distant from the ischemic core. These preliminary results suggest that a retrograde degenerative signal may be localized to the peri-infarct region, whereas other factors such as downstream signaling may be playing a role in the widespread functional changes seen after stroke. These findings add novel information about retrograde signaling in neurons proximal and distal to the infarct core, thereby contributing to research looking for potential therapeutic targets after ischemic stroke.

P1-B-32 - If you don’t use it, you lose it: Temporal dynamics of exercise-induced changes in synaptic plasticity molecules

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Acute exercise has a broad positive effect on human cognition by inducing a physiological response that enhances learning and memory potential. Despite this, a precise exercise protocol remains elusive due to limited data on mechanisms linking single-session exercise to learning and memory correlates. Our goal is to identify an optimal exercise dosage, considering factors like intensity and time post-exercise, to invoke key factors (pNMDAR, pAMPAR, pPKA, pPKB, etc.) involved in hippocampus-dependent learning. C57BL/6J mice (n>9/group, 50:50 male/female) exposed to a treadmill (20 min) were assigned to control or exercise groups of various speed intensities (EX## = m/min: EX15, EX20, EX25, EXHI25+15). Hippocampal synaptic fractions revealed an inverted-U relationship with intensity of exercise, maximal after EX20. Phosphorylation status of PKA, PKB/Akt, and AMPARs were significantly enhanced. We tracked recovery (0, 1, 2h) after EX20 to map the recovery of biochemical responses for proteins of interest. Kinase phosphorylation was short-lived, returning to baseline within the hour, contrasting AMPAR phosphorylation, which remained elevated. These findings suggest heightened hippocampus sensitivity to induction of synaptic plasticity molecules, a subset of which are limited to a short period after exercise. We hypothesize an enhancement of this nature increases the likelihood of synaptic plasticity-related structural changes and may complement cognitive-based therapies during this time window. Work was supported by CIHR and the Reiss Innovation Fund for Healthy Aging Research.

P1-B-33 - Behavioral and genetic evidence that habituation involves dissociable, interstimulus interval-dependent processes

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Habituation is a form of learning that occurs when an organism decreases its response to repeated stimuli that do not predict the arrival of appetitive or aversive stimuli. The time between stimulus presentations greatly impacts both the rate, depth, and memory of habituation. Animals habituate more slowly and to a lesser extent at long interstimulus intervals (ISIs), but the response decrement persists for longer than when the decrement is induced by short ISIs. Thus, at longer ISIs habituation learning is slower, but memory of this learning lasts longer than at shorter ISIs. To investigate whether this is caused by different habituation processes acting at short and long ISIs, we used our Multi-Worm Tracker to simultaneously monitor the behavior of dozens of Caenorhabditis elegans, on Petri plates that receive mechanical taps at different ISIs. The wormâ€™s naive response to a tap is to reverse, moving backwards briefly. While we found that this reversal response habituated more shallowly with long ISIs (60s) than shorter ISIs (10-30s), for long ISIs from 60s-500s there were no further changes in the depth

of habituation. We also found that animals with mutations in cmk-1, homolog of Ca2+/calmodulin-dependent kinases 1/4, ogt-1, homolog of O-GlcNAc transferase, avr-14, a glutamate gated chloride channel alpha subunit, and acy-1, homolog of adenylyl cyclase 9, have a wildtype habituation phenotype at one ISI, but a mutant phenotype at a different ISI. These genes may act in ISI-dependent habituation processes, as their habituation phenotype depends on the ISI.

P1-B-34 - Tau as a sex-specific gatekeeper in long-term depression (LTD): increased LTD in male Mapt KO rats is mediated by group I mGluRs

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Understanding the molecular mechanisms underlying synaptic plasticity aids in developing more efficacious therapeutics for neurodegenerative diseases (PMID:38012296). A synaptic protein of particular interest is tau, whose functions are crucial in comprehending synaptopathology, a hallmark of tauopathies. In field potential recordings from hippocampal slices, we previously found that low-frequency stimulation (LFS)-induced long-term depression (LTD), a form of synaptic weakening, is enhanced in the CA1 region of male but not female *Mapt* knock-out (KO) infant (P14-18) rats compared to WT littermate controls (Ralph et al., 2022, *FENS*). Normally LFS induces *N*-methyl-d-aspartate receptor (NMDAR)-dependent LTD in infant rats. Surprisingly, however, we found that the enhanced component of LTD in male *Mapt* KO rats was resistant to NMDAR blockade by 50 ÂµM D-AP5, suggesting that *Mapt* KO in males unlocks the participation of another receptor. Since group I metabotropic glutamate receptors (mGluRs) give rise to another major form of LTD, we hypothesized that *the NMDAR-independent component of enhanced LTD in infant male Mapt KO rats is mGluR-dependent*. Accordingly, we found that combined antagonism of both group I mGluRs subtypes (mGlu1 and mGlu5) with 1 ÂµM MTEP and YM298198, respectively, or antagonism of either mGlu1 or mGlu5 alone fully blocks this residual NMDAR-independent LTD in male *Mapt* KO rats. These results suggest tau acts as a gatekeeper for preventing the aberrant activation of mGluRs during LTD-inducing patterns of synaptic stimulation in infant male but not female rats.

P1-B-35 - Inhibition of hdac3 during immune stimulus ameliorates sex-specific depressive-like behaviour and aberrant microglial morphology

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Women worldwide are diagnosed with major depressive disorder (MDD) twice as frequently as men. Rodent models highlight sex differences in depression neurobiology. In the brain microglia are primary regulators of inflammation. Acutely, microglial responses are beneficial but chronic microglial-driven inflammation can lead to brain and behavioural impairment. Microglial activity can be controlled through epigenetic regulation of gene expression. Histone deacetylases (Hdacs) are a class of enzymes that control gene expression by removing acetyl groups from lysine residues on histone tails, facilitating DNA-histone contacts. Hdac3 is the most widely expressed Hdac in the brain, and pharmacological inhibition of Hdac3â€™s deacetylase activity has neuroprotective and functional recovery benefits in models of brain injury. Inhibition of Hdac3 in microglia promotes resolution of inflammation, but the role of Hdac3 regulation in microglia in MDD has not been examined. This study hypothesizes that inhibiting Hdac3 could yield neuroprotective effects in a novel repeated lipopolysaccharide (LPS) driven inflammation model of MDD. Hdac3 inhibition during active inflammation ameliorated LPS-induced depressive-like behaviour on the sucrose preference test and increased social behaviour in females. Microglial morphology analysis demonstrated sex-specific changes in microglia morphology with LPS treatment in the frontal cortex and hippocampus. This phenotype was reversed with Hdac3 inhibition. Overall, we demonstrate the neuroprotective effects of Hdac3 inhibition in an inflammation driven model of MDD that supports the development of Hdac3 inhibitors as potential therapeutics for MDD.

P1-B-36 - The cell-type-specific spatial organization of the anterior thalamic nuclei in the mouse brain

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To interpret brain computation and function, it is critical to understand the cell-type composition and spatial organization of brain regions. In the thalamus, the anterior thalamic nuclei (ATN) are involved in a wide variety of functions, yet the cell-type composition of the ATN remains undefined at single-cell and spatial resolution. By combining single-cell RNA sequencing, single-cell spatial transcriptomics, and multiplexed fluorescent *in situ* hybridization, we identify three discrete excitatory cell-type clusters that correspond to each of the known nuclei of the ATN and reveal novel marker genes, molecular pathways, and putative functions of these cell-types. We further illustrate graded spatial gene expression along the dorsomedial-ventrolateral axis for all individual nuclei of the ATN, and additionally demonstrate the

anteroventral nucleus exhibits spatially covarying protein products and long-range inputs. Collectively, our study reveals both novel discrete and continuous cell-type organizational principles of the ATN which will help to guide and interpret experiments on ATN computation and function.

P1-B-37 - Molecular mechanisms underlying regulation of neuronal pannexin1 expression with implications for dendritic spine stability

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BACKGROUND AND AIM: Pannexin 1 (PANX1) is a channel-forming protein and signalling hub enriched in immature neurons. We discovered that PANX1 inhibits dendritic spine stabilization and limits neuronal network size and complexity. Accordingly, mouse cortical synaptic PANX1 levels decrease markedly prior to peak spine formation. The molecular mechanism(s) underlying PANX1 downregulation are unclear. Peri-synaptic astrocyte processes surround synapses and release factors, like ATP, that modulate synapse stabilization and maturation. We recently found that ATP triggers internalization of PANX1 in Neuro2a cells. Together this suggests astrocyte-released ATP could act as molecular trigger for neuronal PANX1 downregulation. METHODS: We interrogated transcriptomics databases to identify ATP-release channels enriched in peri-synaptic astrocyte processes. Using primary cortical mouse neuron-astrocyte cultures and complementary imaging and biochemical approaches, we are now investigating the impact of modulating astrocytic ATP-release channels on post-synaptic PANX1 trafficking and expression levels. RESULTS: Our analysis of open-access transcriptomics datasets identified a likely candidate for the primary ATP-release channel in peri-synaptic astrocyte processes. Our studies of the impact of ATP on PANX1 trafficking and levels are ongoing. CONCLUSIONS: This work will advance our cellular and molecular understanding of neuron-astrocyte interactions in the context of spine stability, with implications for neurodevelopment and disease.

P1-B-38 - Determining the role of sex hormones in microglial migration after cerebral microbleed in the adult mouse cortex

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Sex differences have been observed in the prevalence, clinical presentation, disease trajectory, and prognosis of cerebrovascular conditions such as stroke and vascular dementias. Microglia are the resident immune cells of the brain, which possess the unique capacity to migrate towards sites of injury in the mature brain. Recent work from our lab has shown that this migratory ability is sex-dependent whereby microglia in male mice migrated in greater numbers and distances towards microbleeds than microglia in female mice. Using time-lapse in vivo imaging of sparsely labeled microglia, we tracked migratory movements following cerebral microbleeds in male and female mice subjected to gonadectomy and/or hormone replacement. Our results show that gonadectomy in males had relatively little effect on migration. However, we found that the percentage of mobile microglia was elevated in female ovariectomized mice, compared to sham mice. Currently, we are examining which specific sex hormones, such as estrogen, modulate microglia migration. These findings highlight the sex-dependent variations in microglia migration in response to injury and provide an interesting research avenue to explore signaling mechanisms that contribute to these differences.

P1-B-39 - Astrocytes originated from neural precursor cells promote regenerative remodeling of chondroitin sulfate proteoglycans after spinal cord injury

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Progressive neurodegeneration after spinal cord injury (SCI) causes permanent neurological dysfunctions. Detrimental changes after SCI including upregulation of inhibitory chondroitin sulfate proteoglycans (CSPGs) and impaired neuroglia network restrict the endogenous repair process. Neural precursor cells (NPCs) have the innate capacity to replace damaged neurons after SCI. However, the hostile milieu of damaged spinal cord hinders neurogenesis of endogenous or transplanted NPCs and instead drives their astrogenesis. Astrocytes are critical glial cells for CNS injury and repair as they can exhibit pro-inflammatory or pro-regenerative phenotype. Following SCI, the majority of resident astrocytes become pro-inflammatory; however, our understanding of the role of NPC-derived astrocytes after SCI is limited. Here, we show that engrafted and endogenous NPC-derived astrocytes exhibit pro-regenerative and anti-inflammatory phenotypes in rats with SCI compared to pro-inflammatory resident spinal astrocytes, which

was verified by transcriptomics analysis *in vitro*. Our co-culture system indicates that reactive NPC-derived astrocytes enhance NPC neurogenesis and increases maturity and synapse formation of NPC-derived neurons in a paracrine fashion. Mechanistically, we demonstrate that NPC-derived astrocytes perform regenerative CSPGs remodeling by receptor mediated endocytosis, and degradation of CSPGs through production and release of ADAMTS1/9 enzymes, a capacity that spinal astrocytes lack. Taken together, our novel findings show that newly generated astrocytes from NPCs can promote the repair processes after SCI, at least in part, by regenerative remodeling of CSPGs.

P1-B-40 - Understanding in vivo Ca2+ activities of astrocytes in freely-behaving mice

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The role of astrocytic calcium (Ca2+) activities in neurocircuit modulation and information coding is increasingly appreciated. Recent studies have shown that chemo- or optogenetic manipulation of astrocytic Ca2+ activities can alter animal behaviours, impact memory recall and influence vigilance states. Population Ca2+ activities of astrocytes are also shown to be capable of encoding spatial representation in the hippocampus. While most studies generate fascinating insights into gliobiology and neurocomputational science, they are conducted in head-fixed paradigms, so the repertoire of behaviours investigated was limited.

In this study, we address the capability of astrocytic Ca2+ activities in encoding various animal behaviours by directly recording real-time astrocytic Ca2+ activities in the cortex and dorsal striatum in freely-behaving mice with UCLA miniscopes version (v) 4. We parse behaviour modules from long recordings of animal spontaneous behaviours in an open field arena and a spatially restrictive cylinder. The behaviour modules annotated include running, rearing, wall-leaning, self-grooming, immobility, left-turning, right-turning and tail rattling. Preliminary analysis shows the presence of globally synchronous astrocytic and less synchronous local activities during behaviour modules, with the former strongly correlating with rearing and wall-leaning behaviors. Further computational analysis will be conducted to interrogate the behavioral information encoded with different astrocytic Ca2+ activities.

P1-B-41 - Investigating a role for netrin-1/DCC signaling in excitatory homeostatic synaptic upscaling

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Neuronal circuits require homeostatic mechanisms to counterbalance destabilizing forces and maintain physiologically stable firing rates. Homeostatic synaptic scaling allows neurons to maintain the relative weight of synaptic inputs, while adjusting overall firing levels in response to long-term perturbations in activity. The secreted protein netrin-1 and the netrin receptor deleted in colorectal cancer (DCC) are made by neurons and enriched at synapses in the mature mammalian brain. Recent studies have identified essential roles for neuronal netrin-1 and DCC in long-term potentiation (LTP), a classic form of activity-dependent Hebbian plasticity. Netrin-1 is sufficient to potentiate synapses and induce synaptic insertion of AMPA receptors. As such, netrin-1 is a candidate to regulate homeostatic upscaling, a process that requires similar increases in post-synaptic strength. The present study investigates the involvement of netrin-1 in homeostatic upscaling associated with long-term decreases in neuronal activity. We are investigating the impact of long-term netrin exposure on mini excitatory post-synaptic currents (mEPSCs) and AMPA receptor surface expression. Furthermore we will examine the necessity of DCC signaling for upscaling induced by 48-hour silencing of activity with tetrodotoxin (TTX). These findings aim to identify a novel molecular mechanism involved in homeostatic plasticity and a convergence point between Hebbian and homeostatic plasticity.

P1-B-42 - Investigating the impact of acute vaporized cannabis on oligodendrocyte lineage cells in the forceps minor of adult mice

Colin Murray [1], **Haley Vecchiarelli** [1], **Hayley Thorpe** [2], **Sophia Loewen** [1], **Hakan Kayir** [2], **Jibran Khokhar** [3], **Marie-Eve Tremblay** [1]

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The consumption of cannabis is increasing in Canada, but the cellular impact of this substance on the brain of healthy adults is relatively unknown. The most abundant compounds found in cannabis are the cannabinoids delta-9-tetrahydrocannabinol (THC), and cannabidiol (CBD). Recent findings indicate that consumption of cannabis may impact the process of myelination, which is essential for cognition. However, the impact of cannabis consumption on myelination in the healthy adult brain has not yet been extensively researched. In this study, adult (P70) male and female C57BL/6J mice were exposed to vaporized cannabis high in THC (25-31% THC; <1% CBD), balanced in THC and CBD (~10%THC; ~10% CBD) or a vehicle air for 15 minutes, and were euthanized 30 minutes post-onset. The density of oligodendrocytes and OPCs in the forceps minor was then quantified using fluorescent antibodies. We found no significant difference in oligodendrocyte or OPC density in the forceps minor of male or female mice from acute cannabis exposure. However, female mice did show a significantly higher density of OPCs overall compared to males. Female mice exposed to cannabis high in THC also presented a significant association between density and Bregma, which was not found in any other OPC group. These findings

are supported by nearest neighbour distance results following identical trends. This research provides important information on how cannabis may impact oligodendrocyte lineage cells, which play a significant role in adaptive myelination, in the brain of male and female mice.

P1-B-43 - Astrocyte signaling in long-term depression

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Astrocytes have been implicated in regulating synaptic plasticity via Ca^{2+} transients, but it is unclear how. Here, we aim to decipher how astrocytes control long-term depression in mouse visual cortex.

We used sodium fluoroacetate (NaFAC) to abolish astrocyte function in acute slices. We probed for monosynaptic connections between layer-5 pyramidal cells (L5 PCs) using quadruple patch clamp. We used a spike-timing-dependent long-term depression (tLTD) induction protocol and found that we were unable to induce tLTD when slices were treated with NaFAC, as measured by changes in EPSP amplitude before and after induction (103% Â± 7%, n = 7, p = 0.58), compared to control.

Next, we expressed opto-Î±1AR opsin, a light-sensitive Gq-coupled receptor, in visual cortex astrocytes. We again patched L5 PCs and stimulated presynaptic inputs extracellularly to evoke EPSPs. During tLTD induction, we optogenetically activated astrocytes at 445 nm. We found that mean EPSPs were again not depressed in slices expressing opto-Î±1AR (123% Â± 23%, n=4, $p < 0.05$), compared to control.

Surprisingly, we also found that astrocyte Ca^{2+} signaling seems unaffected by tLTD induction. Preliminary results show no difference in the number or duration of Ca^{2+} events during induction between tLTD and control conditions (145 Â± 12% for control, n = 32 ROIs vs 145 Â± 13%, n = 41 for tLTD, p = 0.32 and 88 Â± 12% for control vs 110 Â± 12% for tLTD, p = 0.083, respectively).

While our results imply that astrocytes are involved in tLTD, whether their Ca^{2+} signals play a critical role in this process remains to be explored.

P1-B-44 - Ionotropic and non-ionotropic NMDA receptor signaling in the regulation of synaptic depotentiation

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During memory formation, hippocampal synapses undergo persistent strengthening (long-term potentiation, LTP) which is thought to be subsequently reversed during forgetting. However, the mechanisms that govern LTP reversal, or synaptic depotentiation, remain largely unknown. The N-methyl-D-aspartate receptor (NMDAR) has been implicated in both synaptic strengthening and weakening by recruiting distinct ionotropic and non-ionotropic signaling cascades, respectively. Therefore, the present study aimed to pharmacologically interrogate the role of non-ionotropic NMDAR signaling in synaptic depotentiation in the hippocampus. Field excitatory postsynaptic potentials (fEPSPs) were recorded at CA3-CA1 synapses in acute hippocampal slices from 7-12-week-old male and female C57Bl/6N mice. LTP was induced using either a spaced or compressed theta burst stimulation (spaced LTP, compressed LTP) and subsequently reversed with a low frequency stimulation. Antagonists targeting either the glutamate or glycine binding site of the receptor were used to probe for ionotropic and non-ionotropic NMDAR contributions during synaptic depotentiation. Our preliminary results suggest that non-ionotropic NMDAR signaling may be involved in the depotentiation of compressed LTP, but not spaced LTP, in both male and female mice. These findings suggest that different NMDAR signaling pathways may be recruited during depotentiation depending on the type of LTP induced. This study will ultimately provide novel insights on the metaplastic regulation of forgetting.

P1-B-45 - Baf53b controls actin dynamics in neuronal development

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The actin cytoskeleton underlies the complex morphology of neurons which is necessary for information processing in the brain. Mutation of Baf53b, a subunit of the mammalian Brg1/Brm-associated factor complex (BAF), has been shown to both impair neuronal morphology and be associated with neurodevelopmental disorders including autism spectrum disorder. Structural and synaptic plasticity are dependent on actin remodelling, of which cofilin is a modulator of filamentous actin (F-actin) length. While the link between Baf53b and neuronal morphology is unclear, failure to inhibit cofilin upon neuronal stimulation has been identified in Baf53b knockout neurons, presumably causing blunted dendrites. Our goal is to directly investigate the F-actin cytoskeleton to determine if loss of Baf53b impairs neuronal branching due to misregulated actin cytoskeleton development. Using primary embryonic mouse cortical neuron cultures, we deleted mouse Baf53b via a Cre-Lox system and virally delivered Cre recombinase. These cells were compared to wild-type by confocal microscopy to quantify F-actin and excitatory synapses and by scanning electron microscopy (SEM) to examine whole-cell actin networks. These SEM analyses enabled ultrastructural visualisation of the actin cytoskeleton, advancing our understanding of how BAF and Baf53b control actin dynamics in neuronal development and in neurodevelopmental disorders.

P1-B-46 - Incomplete remyelination via endogenous or therapeutically enhanced oligodendrogenesis is sufficient to recover visual cortical function

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Myelin loss leads to deficits in action potential propagation and behaviour that contribute to the pathophysiology of neurodegenerative diseases, injury conditions, and aging. Because endogenous myelin repair is often incomplete, better understanding endogenous remyelination and developing remyelination therapies are clinical imperatives. Here, we used in vivo two-photon microscopy and large-scale neural recordings to study the dynamics of endogenous and therapeutic-induced cortical remyelination and functional recovery in the cuprizone model of demyelination in mice. We focused our analyses on the visual pathway, which is uniquely positioned to provide insights into structure-function relationships during de/remyelination. We show that endogenous remyelination is highly efficacious at mild demyelination levels, but fails to regenerate the oligodendrocyte population when oligodendrocyte loss is severe, and that its timing and rate depend on recent oligodendrocyte loss. We demonstrate that remyelination therapies substantially increase oligodendrocyte gain during remyelination, eliminating endogenous recovery deficits and hastening recovery of neuronal function. Lastly, we find that incomplete oligodendrocyte restoration is sufficient to recover visual neuronal function. Together, our findings advance our understanding of the remyelination process and its impact on functional recovery following a demyelinating injury.

P1-B-47 - Transsynaptic IgSF21-Nrxn2α interaction governs dendritic synaptic inhibition to control neuronal network excitability and brain function

Nicolas Chofflet [1], **Benjamin Feller** [2], **Cristina Vasuta** [1], **Samuel Boris Tene Tadoum** [3], **Yusuke Naito** [1], **Ryan Wheeler** [4], **Anthony Pastore** [5], **Nirmala Padmanabhan** [6], **Trang Nguyen** [7], **Steven Clapcote** [8], **Steve Bourgault** [7], **Tabrez Siddiqui** [6], **Gabrielle Rudenko** [5], **Tamara Franklin** [4], **Elsa Rossignol** [3], **Hideto Takahashi** [2]

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Synaptic inhibition is crucial for regulating brain activity. Hence, its dysregulation is linked with several neurodevelopmental disorders. Dendritic and somatic synaptic inhibitions are achieved by different populations of interneurons to control neuronal excitability of principal neurons. While somatic inhibition has been extensively studied, it remains largely unknow what molecular mechanisms underlie the development of dendritic GABAergic synapses. We have previously found that postsynaptic IgSF21 is crucial for the organization of GABAergic synapses in the hippocampus. However, several key questions remain to be addressed, 1) how IgSF21 regulates GABAergic synapse organization, 2) which type of synaptic inhibition IgSF21 is involved in, and 3) what the consequences of its loss on neuronal excitability and mouse behaviors are. Through in silico predictions and in vitro assays, we first show that IgSF21 orchestrates presynaptic GABAergic terminal development through its presynaptic receptor Nrxn2Î±. Second, electrophysiological recordings revealed that IgSF21 deletion impairs dendritic, but not somatic, inhibition in the cortex. Consequently, neuronal circuits of IgSF21 knockout (KO) mice are hyperexcitable, increasing vulnerability to pharmacologically induced seizures. Lastly, IgSF21 KO mice display defects in ultrasound vocalizations and increased anxiety. Thus, IgSF21 governs dendritic inhibition to control neuronal excitability and normal brain functions, deepening our fundamental knowledge of the development of GABAergic synaptic connectivity in the brain.

P1-B-48 - Sex-specific behaviour and microglia dysfunction in inflammatory bowel disease

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In the healthy brain, the gut microbiome releases metabolites that signal to microglia, the brainâ€™s innate immune cells. Inflammatory bowel disease (IBD) produces chronic inflammation, gastrointestinal tract damage, and disruption of the gut microbiota. The gut-brain axis may be disrupted by altered gut microbe composition in IBD, leading to dysfunction in the brain and behaviour. IBD patients experience higher rates of depression, anxiety, and social impairments, with childhood onset often resulting in delayed puberty and altered sex hormone regulation. These impacts may be long-lasting, as adults with IBD report higher incidence of sexual dysfunction. We aimed to characterise how disruptions to gut microbiota converge to influence brain function and behaviour. Using a dextran sulfate sodium (DSS) rodent model of pediatric IBD, we analysed sex-specific behaviours, metabolite and hormone signalling, and microglial morphology. We developed a novel urine preference behaviour task to quantify mate-seeking, revealing a significant male-specific deficit in female urine preference for DSS treated males. This was paralleled by a significant reduction in seminal vesicle mass and lower levels of circulating testosterone. Using our newly developed microglial morphology analysis tool, we identified a decrease in ramified hippocampal microglia in DSS treated females, and a trend towards hypertrophic microglia in

DSS treated males. Our findings indicate early life gut inflammation drives long-term reprogramming of microglia function, hormone regulation, and shifts in male mate-seeking.

P1-B-49 - Investigating the role of PANX1 and TNF-α on the dendritic spine cytoskeleton

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BACKGROUND AND AIM: Impaired cognition in AlzheimerÂ´s disease (AD) involves synaptic dysfunction and loss. Marked reduction in dendritic spine density is a hallmark of post-mortem human AD brain, AD mouse model brain, as well as neuronal cultures exposed to AD triggers. Although the loss of spines is considered a primary cause of AD cognitive decline, the molecular mechanisms are poorly understood. Increased levels of pannexin 1 (PANX1), a channel and signalling hub protein, are observed in AD mouse model brain and with treatment of TNF-Î±, a cytokine upregulated in AD. We discovered that PANX1 inhibits spine stability.â€¯Work from others shows that TNF-Î± can contribute to spine loss, suggesting it might inhibit spine stability. Together, these findings suggest that TNF-Î±, possibly through PANX1, could mediate spine loss in AD. To this end, we investigate the impact of TNF-Î± on spine stability and the impact of TNF-Î± and PANX1 on dendritic spine cytoskeleton.â€¯METHODS: Using cortical neuron-astrocyte cultures, we visualize the spine cytoskeleton following modulation of TNF-Î± and PANX1, in living and fixed cells, using confocal microscopy and a repertoire of optical tools. In parallel experiments we investigate the impact ofâ€¯TNF-Î± on synaptic levels of PANX1, cytoskeletal proteins, and markers of synapse maturation in cortical neuron-astrocyte cultures.â€¯CONCLUSIONS: The outcomes of this work will provide molecular insight into the impact of PANX1 and TNF-Î± on dendritic spines as well as inform on the putative molecular mechanisms regulating spine stability in AD. â€¯

P1-B-50 - Age-related changes in astrocyte and neuron plasma membrane proteomes

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Aging is a natural process involving multiple changes at the cellular and molecular levels, affecting both neurons and astrocytes. This leads to a decline in synaptic function, neuronal signaling, and an overall reduction in cognitive abilities. Studies have explored these processes focusing primarily on gene expression of astrocytes and neurons. However, little is known about how the proteomes of these cells change with normal aging. Such understanding is crucial for unraveling the intricate molecular mechanisms of the aging brain.
We employed in vivo proximity-dependent biotinylation to identify astrocyte and neuron plasma membrane proteomes. Using PHP.eB vectors, we delivered BioID2 probes to the plasma membrane using specific promoters. This method accurately labels proteins near plasma membranes. We systematically evaluated the cortical proteomic changes in aging mice.
In both astrocytes and neurons, we identified cortical plasma membrane proteomes at 6, 12, 18, and 24 months, revealing approximately 1000 proteins in each cell type across age. Unique proteins were identified in the proteomes of astrocytes and neurons, reflecting distinct molecular profiles for each cell type. We analyzed how the proteomes change across ages, and we have identified changes in membrane proteins associated with key cellular functions, including ion balance, metabolism, cellular signaling in astrocytes, and ion transport, synaptic signaling, and structural integrity in neurons.
Our study shows that aging alters important proteins and mechanisms in astrocytes and neurons that are seen at the proteomic level in a manner not accurately reflected in gene expression evaluations. We will report rigorous analyses of proteomic data to show how these cells shift their functions with age.

P1-B-51 - Astrocytic glucocorticoid signaling in the Lateral Hypothalamus mediates the effects of early-life stress on orexin firing to perturb diurnal activity rhythms

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Astrocytes regulate several processes in the brain to integrate local and distal synaptic signals underlying behaviour. The metabolic support role of astrocytes has been shown to coordinate many complex behaviours including sleep-wake cycles. Specifically, astrocytes in the lateral hypothalamus modulate the excitability of orexin neurons by dynamically controlling the availability of energy substrates across night and day to drive sleep-wake cycles. Considering that local energetic substrate shuttling to neurons is significantly impeded in conditions of stress, **we hypothesise that stress, specifically elevations in blood glucocorticoids, impacts lateral hypothalamic astrocytes to influence orexin neuron output.**

We employed an early life stress (ELS) paradigm (maternal separation 4hr/day), which significantly increases blood glucocorticoids in adulthood. We examined astrocyte morphology and associated sleep-wake behaviours and observed a sex-specific effect of ELS on diurnal running wheel behaviour.

Furthermore, we note striking sex differences in neuronal excitability mirroring our behavioural phenotypes. Observed changes in orexin neuron excitability were rescued via bath application of L-lactate suggesting a significant impairment of astrocyte-neuron lactate shuttling by ELS.

Our data suggests that ELS perturbs astrocyte network integrity which in turn can influence the supply of energy substrates to neurons and underlies stress-induced behavioural dysfunction.

P1-B-52 - Mechanisms of neuronal suppression by norepinephrine in the developing visual system

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Norepinephrine (NE), a principal neuromodulator found in the brains of all vertebrates, is also known to intensely activate astrocytes. The optic tectum of the developing *Xenopus laevis* tadpole is populated by radial astrocytes which function both as neural progenitors and active partners in sculpting neuronal responses to visual stimuli. Recent work in our lab shows that NE causes the synchronous activation of radial astrocytes and the suppression of tectal neurons. Targeted chemogenetic activation of radial astrocytes is also sufficient to suppress tectal neurons and both methods of inducing neuronal suppression enhance the tadpolesâ€™ ability to escape threatening visual stimuli, suggesting that NE acts through radial astrocytes to shift the tectum to a state biased for threat detection. Using whole-cell electrophysiology, we investigated the cellular mechanisms underlying this process. We show that NE causes a robust decrease in the frequency of miniature excitatory postsynaptic currents that is only partially replicated by the chemogenetic activation of radial astrocytes, suggesting that NE may also act independently of astroglial activation to reduce probability of neurotransmitter release at synapses. Surprisingly, NE did not cause a change in paired-pulse ratio, suggesting that this effect may be limited to spontaneously released vesicles. Additionally, we show that NE causes no change in the resting membrane potential or intrinsic excitability of tectal neurons.

P1-B-53 - Electrical stimulation of endogenous neural precursor cells for neural repair

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Endogenous neural stem cells and their progeny (termed neural precursor cells (NPCs)) are promising candidates for neural repair. It has been shown that activating the NPCs using drugs (e.g. metformin) is correlated with improved functional outcomes. Interestingly, NPCs are electrosensitive and can be activated with applied electric fields leading to expansion of the NPC pool, directed migration and neuronal differentiation.

We propose electrical stimulation (ES) as a novel approach for enhancing neural repair. With the goal of clinical application, we have demonstrated that charge-balanced biphasic monopolar (BPMP) ES, which results in zero net charge delivery to brain tissue, is a safe and efficient means of activating NPCs, both *in vitro* and *in vivo*. Based on the success of our paradigms for cortical ES, we sought to determine the optimized parameters to activate NPCs with striatal stimulation to broaden the application of ES for neural repair.

Herein, we established a computer simulation model of brain tissue being stimulated with BPMP waveform. We used COMSOL Multiphysics to determine the optimal amplitude of the BPMP necessary for activating resident NPCs in the periventricular region of the forebrain. We discerned that striatal ES with a cathodal amplitude of ~100 ÂµA, a 50% reduction compared to cortical ES, results in the greatest NPC pool expansion. The results from the model were confirmed in ex vivo tissue slices. This model allows for rapid and non-invasive way of optimizing stimulation parameters for brain tissue.

P1-B-54 - Voltage and repetitive gating determine use-dependent inactivation of Ca2+ channels in Aplysia neuroendocrine cells

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Intracellular Ca^{2+} is vital in regulating neuronal excitability, gene expression, and secretion; channels and pumps within the plasma membrane and organelles, such as mitochondria, work to strictly control cytosolic free Ca^{2+}. The neuroendocrine bag cell neurons of the sea snail, *Aplysia californica*, contain voltage-gated Ca^{2+} channels which undergo use-dependent inactivation (aka rundown) during a prolonged afterdischarge. The afterdischarge follows a synaptic input and is a synchronous bursts of action potentials, starting with a ~5-Hz, ~1-min fast-phase and then a ~1-Hz, ~30-min slow-phase, ultimately triggering the Ca^{2+}-dependent secretion of egg-laying hormone. We used whole-cell voltage clamp of individual cultured bag cell neurons within solutions that isolate Ca^{2+} currents to investigate the voltage-dependence of rundown. Cells held at -40 mV and given a 1-min, 1-Hz slow-phase-like train-

stimulus of 75-ms steps to 0 mV presented more rundown (~60% current remaining) than cells held at -60 mV or -80 mV (both ~70% remaining) before stimulation. However, preceding the slow-phase-like stimulus with 1-min, 5-Hz fast-phase-like stimulus of steps from -60 mV, reduced the slow-phase rundown, such that there was ~80% current remaining, suggesting facilitation. Rundown is presumably due to Ca^{2+}-dependent inactivation and may be influenced by membrane pumps and organelles handling Ca^{2+} entry. By controlling voltage-dependent changes to intracellular Ca^{2+}, the extent of Ca^{2+} current rundown is ostensibly a key determinant for hormone release and animal reproduction.

P1-B-55 - How do secreted factors circulating in the bloodstream regulate synapse formation and activity in human neurons?

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Synapses, the connections between neurons that enable information processing and memory storage, decline in number and function as we age. This loss of synaptic connectivity leads to cognitive impairment and predisposes healthy individuals to neurodegenerative diseases. Pioneering experiments showed that exposure of aged mice to young blood reversed impairments in learning and memory. Building upon those findings, I differentiated human neurons from stem cells and treated them with serum extracted from young and aged mice (â€œyoung bloodâ€ and â€œold bloodâ€, respectively). Using electrophysiology and immunocytochemistry, I discovered that young but not old blood increased excitatory synapse numbers and enhanced synaptic responses mediated by AMPA and NMDA glutamate receptors. Using mass spectrometry and biochemistry, I identified secreted proteins enriched in young blood, including the extracellular matrix proteins thrombospondin-4 (THBS4) and SPARC-like protein 1 (SPARCL1). Treatment of human neurons with recombinant THBS4 and SPARCL1 recapitulated the benefits of young blood and even enhanced synapse formation in neurons cultured previously with old blood. Experiments are in progress to identify downstream cell-surface receptors and effectors that mediate human synapse formation and to determine if they rejuvenate synaptic connectivity. Collectively, this research will be important for defining molecular mechanisms that drive changes in synaptic connectivity during healthy aging, for understanding how their perturbation drives neurodegeneration, and for developing effective disease therapeutics.

P1-B-56 - Effects of MDGA2 reduction on Synaptic long-term depression during the synaptic pruning critical period

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Autism Spectrum Disorder (ASD) is a neurodevelopmental condition marked by social interaction impairments, communication challenges, and repetitive behaviours. ASD has been linked to mutations in genes encoding synapse organizer proteins, including MAM domain-containing glycosylphosphatidylinositol anchor 2 (MDGA2) protein. MDGA2 negatively regulates synapse development by binding to neuroligins, thus inhibiting the formation of trans-synaptic neuroligin-neurexin complexes. *Mdga2*$^{+/-}$ mice display phenotypes akin to core ASD symptoms, including reduced sociability, increased repetitive behaviours, and impaired spatial and contextual memory. *Mdga2*$^{+/-}$ hippocampal slices from adult mice display altered LTP; however, the effects of MDGA2 reduction on plasticity during critical periods of brain development have not been explored. Moreover, few studies have investigated mechanisms related to synaptic pruning in autism models, which may reveal novel â€œsignaturesâ€ of glutamatergic synapse perturbation during key stages of brain maturation beyond early development. This study explores the impact of MDGA2 reduction on long-term depression (LTD) during the synaptic pruning critical period using *Mdga2*$^{+/-}$ mice (*Mus musculus*). We hypothesize that MDGA2 reduction will impair NMDAR and/or mGluR-mediated LTD due to premature synapse maturation, reducing susceptibility to activity-dependent weakening. We aim to provide insights into the role of MDGA2 in synaptic plasticity during critical periods, shedding light on its potential contribution to ASD pathology during neural development.

P1-C-57 - Investigating the role of IRE1 in brain regeneration using a larval zebrafish model

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Stimulating endogenous mammalian neural stem cells (NSCs) to regenerate lost neurons may be a potential therapy for traumatic brain injury (TBI). However, the cell responses governing NSCs *in vivo* post-TBI are unclear. The zebrafish is an excellent model to study *in vivo* brain regeneration because its NSCs produce new neurons post-TBI. The Unfolded Protein Response (UPR) is a vertebrate conserved stress response that may regulate NSC-driven regeneration post-TBI. Inositol requiring enzyme 1 (IRE1) is a vital UPR protein that regulates mammalian neurons and astrocytes post-TBI. However, the role of IRE1 during the process of successful regeneration post-TBI remains unknown. The objective of this study is to characterize IRE1 signaling during brain regeneration using a larval zebrafish model. Larval transgenic fish that express GFP upon IRE1 activation (*Tg(xbp1s:eGFP)*) were studied at 5 days post-fertilization. A novel larval TBI model was made by creating a lesion in a single hemisphere of the larval forebrain. Larvae were bathed in EdU (proliferative marker) before sacrifice for immunostaining.

Forebrain tissue was analyzed for GFP expression, NSC proliferation ($EdU^+/Sox2^+$), and neuronal differentiation ($EdU^+/HuC/D^+$). Analysis of GFP signal showed that IRE1 activity rapidly increased by 1-hour post-TBI but decreased one day later. Proliferation increased by 1-day post-TBI, inversely correlating with the decrease in IRE1 activity. Analysis of neuronal differentiation is ongoing. This study will yield valuable insight on the role of the UPR for successful brain regeneration.

P1-C-58 - Investigating the mechanistic link between adolescent repeated mild traumatic brain injuries and later life MS-like pathology

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1 in 400 Canadians have multiple sclerosis (MS), a disease with various genetic, environmental, and infectious contributors. Recent retrospective clinical studies demonstrate a link between repeated mild traumatic brain injuries (RmTBI) and an increased risk for MS, but the mechanisms underlying this link are unknown. We use a lateral impact model (LIM) of RmTBI to mimic dynamics of sport related mTBI and have demonstrated behavioral deficits, neuroinflammatory changes, and alterations to myelin integrity. Here we investigated how RmTBI impacts pathology in a mouse model of MS. We hypothesized that RmTBI causes microglia priming, resulting in a robust inflammatory response that aggravates MS-like pathology. We induced RmTBI or sham injuries in male mice and quantified motor deficits with rotarod, microglia density and cytokine chemokine profiles following 4-week cuprizone (CPZ) feeding. CPZ causes oligodendrocyte death and demyelination. Our results show that RmTBI decreased rotarod performance that sustained up to 4 weeks in CPZ fed animals only. We further show increased microglia density in the corpus callosum in RmTBI/CPZ mice compared to all other treatment groups. RmTBI/CPZ mice had reduced brain-wide concentration of MIP-2, IFN-gamma, IL-10, and M-CSF compared to RmTBI/Vehicle mice. Our study demonstrates that RmTBI may uncover/exacerbate motor dysfunction, increase microgliosis, and alter inflammatory cytokine/chemokine profiles in a rodent model of MS-like pathology.

P1-C-59 - Probing mechanisms underlying enhanced spread of sensory-evoked cortical activity in a Huntington disease mouse model

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Patients with Huntington Disease experience deficits in sensory perception, yet these symptoms are understudied in comparison to well-known motor and cognitive symptoms. In Huntington disease (HD) mouse models, cortical activity provoked by sensory stimulation spreads across a much larger area when compared to responses in wild-type (WT) mice. To assess potential mechanisms underlying the spread of activity, acute sagittal slices from WT and the zQ175 HD model crossed with Thy1-GCaMP6s mice were prepared. This preparation allowed us to simultaneously record epifluorescent calcium signal from cortical pyramidal neurons, and electrical field responses in forelimb sensory cortex following electrical stimulation of primary visual cortex. (2R)-amino-5-phosphonovaleric acid (APV) was applied to the slices, and its effects on activity were observed. Electrical stimulation evoked larger and longer-lasting responses in zQ175 slices compared to WT, indicating enhanced network excitability in the HD model. Both genotypeâ€™s responses were significantly reduced by blocking N-methyl-D-aspartate receptors (NMDARs) with APV, suggesting they are partially dependent on NMDAR activation. Notably, these all-or-none responses were recorded approximately 2 mm away from the stimulation site with a low threshold for activation, and did not increase in amplitude with greater stimulation intensity, reinforcing their classification as network-driven phenomena. These initial results reveal NMDAR-mediated network activity as a mechanism underlying the spread of sensory activity in HD mouse models.

P3-C-429, P1-C-60 - Modulating neuroinflammation in Alzheimer's disease: targeting the LINE-1 retrotransposon with a nucleotide reverse transcriptase inhibitor

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Alzheimerâ€™s disease affects millions worldwide, yet successful pharmacological treatments remain elusive. Neuroinflammation, a hallmark of Alzheimerâ€™s disease, has unclear causality in its relation to the disease. This study explores the molecular basis of neuroinflammation in Alzheimer's, focusing on the involvement of LINE-1 retrotransposable elements. Recent evidence suggests their role in neurodegenerative disorders, including Alzheimer's, in association with neuroinflammation. Using Alzheimerâ€™s patient-derived induced neurons (iNs), we treated cultures with a nucleotide reverse transcriptase inhibitor (NRTI) and assessed LINE-1 expression. The results demonstrated decreased expression of LINE-1 RNA elements in treated neurons compared to controls. Subsequent experiments exposed human astrocytes to conditioned media from NRTI-treated iNs, revealing reduced expression of LINE-1 RNA and senescence-associated secretory phenotype (SASP) factors. These findings highlight NRTIs as potential modulators of LINE-1 and SASP expression, offering a promising avenue for neuroinflammation treatment in Alzheimerâ€™s disease.

P1-C-61 - Exploring the therapeutic potential of sesame indicum oil in bilateral common carotid artery occlusion-induced ischemic/reperfusion injury in Wistar rats

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Ischemic stroke (IS) poses a worldwide challenge with limited treatment options due to a narrow therapeutic window and accessibility issues. Phytotherapy, leveraging natural compounds for drug discovery, holds promise with established efficacy and enhanced safety. Sesame Indicum (S.I) oil, acknowledged for its neuroprotective properties, emerges as an accessible and cost-effective candidate. This study investigates S.I. oil's impact on inflammation and oxidative stress in rats with ischemic/reperfusion (I/R) injury from bilateral common carotid artery occlusion. Gas Chromatography Mass Spectrometry and Fourier Transform Infrared Spectroscopy analyzed bioactive compounds and fatty acid composition. Rats received 5000mg/kg S.I. oil orally for seven days post-I/R, and neurological function was assessed. Results reveal significant antioxidant and anti-inflammatory properties of S.I. oil. Post-treatment, coordination and balance improved compared to untreated ischemic stroke-induced rats. S.I. oil mitigated decreased SOD, CAT, GPX activities and increased TBARS concentrations following stroke induction. IL-6 and CCR2 were down-regulated, while IL-10 and IL-13 were up-regulated post-treatment. The treatment notably reduced infarct volume size, as assessed by TTC staining. Conclusively, S.I. oil demonstrates therapeutic potential in alleviating neurological deficits associated with cerebral ischemia. Its modulation of oxidative stress biomarkers, inflammatory cytokines, and infarct volume size supports further exploration of S.I. oil as a novel intervention for IS.

P1-C-62 - Using the CHIMERA model and 3D brain analysis to investigate Traumatic Brain Injury pathophysiology

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Traumatic Brain Injury (TBI), a major cause of mortality and morbidity, is extraordinarily heterogeneous in terms of injury subtype, severity, and clinical presentation, and results in diffuse white matter injury,

inflammation, vascular damage, and neuronal dysfunction. The lack of reliable temporal molecular signatures and translational biomarkers can limit the relevance of animal models to human TBI. The Closed Head Impact Model of Engineered Rotational Acceleration (CHIMERA) is a non-surgical model of impact-acceleration injury that mimics the biomechanics and pathophysiology of human TBI. We aim to use tissue clearing and light sheet microscopy (LSM) to create 3D brain maps of TBI-induced alterations in neuronal activation using cFosTRAP mice (that express cFos upon tamoxifen injection), axonal damage using Thy1-YFP-16 mice, and vascular damage using lectin-based labelling. Upon CHIMERA, neurological deficits were assessed, and brains were harvested at 6h post-TBI for SHIELD fixation and optical clearing. A LSM imaging and analysis pipeline (stitching, 3D rendering, segmentation and registration with the Allen Mouse Brain Atlas) was established to acquire whole-brain and region-specific cell counts, axonal varicosities and abnormal vascular densities. We have thus established whole brain labelling and imaging methods to improve analysis of diffuse brain injury mechanisms and their association with translational biomarkers to improve the relevance of model systems and increase our understanding of human TBI pathophysiology.

P1-C-63 - Consequences of elevated anandamide via FAAH inhibition on the neural correlates of emotional processing in post-traumatic stress disorder

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It has been posited that post-traumatic stress disorder (PTSD) develops from dysregulated fear memory learning and consolidation, which are facilitated by the amygdala and the ventromedial prefrontal cortex (vmPFC). The endocannabinoid (eCB) system is an important, though often overlooked, physiological mechanism supporting stress and fear processing. eCB receptors are involved in the modulation of neuronal activity in the amygdala and medial PFC, and eCB receptor ligands, such as anandamide (AEA), support fear extinction in both animals and humans. AEA is broken down in the synapse by an enzyme called fatty acid amide hydrolase (FAAH), and AEA can be increased pharmacologically through the use of FAAH inhibitors. We tested the hypothesis that FAAH inhibition affects the function of the amygdala and vmPFC in the context of a randomized controlled clinical trial (n = 101). Participants received 25 mg of drug or placebo twice a day for 3 weeks and then completed an emotional conflict task during an fMRI scan. Stimuli were either congruent (a happy or fearful face with the corresponding word) or incongruent (word opposite to the facial expression). In the whole sample, we found 4 clusters with a significant interaction between treatment group and facial emotion, in the fusiform gyrus, ACC, insula, and lingual gyrus. Within the treatment group, there was a significant interaction between blood drug level, facial emotion, and trial congruence in the vmPFC and the parahippocampal gyrus. These findings shed light on the eCB mechanisms supporting emotional processing in PTSD.

P1-C-64 - Post fall neuropsychiatric issues and cognitive dysfunction in patients with neurodegenerative diseases

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Objective: Falls are the most common mechanism of injury faced by millions of patients with neurodegenerative diseases (ND) each year. However, there are large gaps in literature regarding fall related cognitive and neuropsychiatric symptoms (NPS). We hypothesized that patients with NDs who have experienced a fall will have greater cognitive deficits and worse NPS symptoms than patients without falls.

Methods: We used data from the Ontario Neurodegenerative Disease Research Initiative dataset (ONDRI) on 482 individuals in five ND types. We compared frequency and severity of different NPS, and performance in 24 tests in five major cognitive domains, between patients with and without falls in the past 12 months.

Results: Comparing those who experienced falls in the last year (n=169; mean-age=68.3Â±9; 36%Female), to those who had no falls (n=314; mean-age=68.7Â±7; 32%Female), there was significantly higher total NPS severity (p=0.0061), frequency of anxiety (p=0.00026); and anxiety severity (p=0.002) in addition to other symptoms. Patients who had a fall also had significantly lower (p<0.001) scores in attention, working memory, and executive function domains, this was not the case in non-fallers.

Conclusion: ND patients with falls had significantly worse NPS and cognitive function. Such issues post fall event must be assessed, as they can be important prognostic factors in disease stage, impact baseline treatment for NDs, and possibly worsen symptoms from previously undiagnosed neuropsychiatric disorders or post concussion symptoms.

P1-C-65 - Dietary methionine restriction limits neuroinflammatory and neurodegenerative processes in preclinical models of multiple sclerosis

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Multiple Sclerosis (MS) is an inflammatory disease of the central nervous system (CNS). Biological sex, obesity and age-related cardiovascular comorbidities affect MS course. Dietary methionine restriction (MR) displays anti-aging and anti-inflammatory properties and improves metabolic health through sexually dimorphic mechanisms. We previously showed that MR ameliorates EAE and limits the proliferation of pathogenic TH17 cells in MS and its animal model, experimental autoimmune encephalomyelitis (EAE). We now aimed to investigate the mechanisms mediating the benefits of MR on CNS-targeted inflammation in both sexes using two different EAE models. In male and female C57BL/6 mice exposed to MR compared to control diet, we observed a significant delay in symptom onset following immunization with MOG to induce active EAE. In the sex-biased spontaneous EAE model (transgenic TCR1640), MR compared to control or methionine supplemented (M+) diet reduced by more than half the proportion of females developing neurological deficits and nearly abrogated disease onset in males. Clinical amelioration was paralleled by lower numbers of peripheral and CNS-infiltrating pro-inflammatory immune cells during EAE and induced major shifts in the transcriptional profile of immune cells. In addition, MR was associated with lower levels of biomarker of neuroaxonal injury (serum neurofilament light chain) in active and spontaneous EAE. Finally, MR significantly influenced the composition of the gut microbiota compared to control and M+ diets in a sex-biased manner. In conclusion, MR impacts gut bacteria, ameliorates EAE clinical course, limits neuroinflammatory processes and reduces neuroaxonal injury in two distinct MS preclinical models, representing a promising therapeutic avenue in MS.

P1-C-66 - Neuropathology of cognitive impairment in elderly patients with schizophrenia

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Cognitive impairment is a core tenet of chronic schizophrenia, which is only partially explained by neurodegenerative processes such as Alzheimerâ€™s Disease. Further, currently available therapies are not effective against such cognitive symptoms. This study seeks to understand this disease-specific cognitive impairment. Clinical, demographic, and autopsy data were obtained for 55 elderly patients with chronic schizophrenia, with additional neuropsychological testing conducted in a subset. Standard evaluation of neurodegenerative conditions and initial exploratory data analyses were performed. On average, patients experienced multiple decades of schizophrenia symptoms. Their average functional level showcased marked disruption of reality by hallucinations and delusions, and impaired communication and judgment. Scoring of negative symptoms at this advanced disease stage outweighed that of positive symptoms, although both were present. Significant cognitive impairment was noted across several different testing methods. On autopsy, only half of patients had sufficiently severe neuropathology to explain their cognitive impairment, and clinical test scores did not correlate with the severity of such changes. Marked impairment in functioning was not entirely explained by neuropathological changes typically associated with dementia, suggesting the presence of a unique mechanism. Better understanding of the existence and pathophysiology of such a process may provide the basis for novel treatment methods to target the cognitive impairment observed widely in schizophrenia.

P1-C-67 - Opening of KATP channels augments neurite outgrowth and AMPK activity in adult DRG sensory neurons: Implications in peripheral neuropathy

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Dorsal root ganglion (DRG) sensory neurons play a critical role in processing and modulation of sensory information, including that experienced in peripheral neuropathy. In response to injury of peripheral nerves the DRG undergo changes in potassium channel (K^+) function including K_{ATP} channels. K_{ATP} channels are integral in the regulation of membrane excitability and inhibited by physiological ATP and activated during events of energy depletion, such as metabolic stress. K_{ATP} activation has been found to play a neuroprotective role in peripheral neuropathy via membrane hyperpolarization, decreased cell excitability and prevention of cell death. Similarly, the AMP-activated protein kinase (AMPK) signaling pathway has been found to enhance mitochondrial functioning and play a neuroprotective role in peripheral neuropathy. *Hypothesis:* agonists of K_{ATP} channels enhance mitochondrial function and neurite outgrowth via the AMPK pathway and modulate neuronal excitability in DRG neurons. DRG neurons from adult SD male rats were isolated and cultured under defined conditions. K_{ATP} channel opener pinacidil increased levels of phosphorylated AMPK. Additionally, pinacidil augmented neurite outgrowth in the DRG neurons. Interestingly, pinacidil treatment resulted in decreased ATP production as well as coupling efficiency. This study reveals that opening of K_{ATP} channels augments neurite

outgrowth and increases AMPK activity through means not effecting mitochondrial respiration and disclosing its potential therapeutic application in peripheral neuropathy. Funded by CIHR # PJT-162172.

P1-C-68 - Can lifestyle change the progression of a genetic disease? Results from an enriched environment study in an animal model of neurodegenerative disorders

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Introduction: Huntingtonâ€™s disease (HD) is a genetic neurodegenerative disease that shares a lot of similarities with Alzheimer's and Parkinsonâ€™s disease, including cognitive, psychiatric, motor, and peripheral impairments, in addition to common mechanisms such as protein aggregation, excitotoxicity, and monoamine imbalance. Despite lacking effective treatments, emerging evidence suggests lifestyle factors may influence symptom onset age. **Aims:** This study aims to assess the impact of Environmental Enrichment (EE) on disease progression and neuroplasticity in HD YAC128 mice (Ethics Committee on the Use of Animals: 4502210318). **Materials and Methods:** WT and YAC128 mice were exposed to EE and evaluated at 2 and 4 months at the behavioral, cellular, and molecular levels. **Results:** EE exerts an antidepressant-like effect in the tail suspension test and enhances motor performance in the rotarod test in the YAC128 mice at 4 and 2 months old, respectively. EE reduces norepinephrine levels in the striatum, revealing a correlation between improved motor performance and lower norepinephrine levels in 2-month-old YAC128 mice. Furthermore, EE enhances neuronal differentiation, increasing DCX-positive cells in the hippocampus in the YAC128 mice at 4 months old. **Conclusions:** These findings support the potential therapeutic role of lifestyle enrichment in mitigating neuroplasticity deficits and slowing disease progression in HD.

P1-C-69 - Insights from a super seniors population: Plasma biomarkers increase in magnitude and variability with age

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Objective: Plasma neurofilament light (NfL), glial fibrillary acidic protein (GFAP), and phosphorylated tau-181 (p-tau-181) have been thoroughly investigated in Alzheimerâ€™s disease. Further research into how biological variables, such as age, can influence biomarker concentrations in both normative and cognitively resilient populations will be important for more robust interpretations.

Methods: Biomarkers were analysed on the Quanterix Simoa HD-X analyzer using Neurology 4-plex E and p-tau-181 assays. N=900 Canadian Health Measures Survey plasma specimens were analyzed as a normative population. Using smoothed quantile regression, the 5th, 50th and 95th percentiles were determined. N=480 plasma specimens were analyzed from cognitively healthy Super Seniors Study participants with a median age of 88 years old, who had never been diagnosed with dementia, diabetes, cardiovascular or major pulmonary disease.

Results: In the normative population, median concentrations increased between 50 to 70 years old by 88% for NfL, 79% for GFAP, and 38% for p-tau-181. Compared to 50-year-olds, median biomarkers were also higher in Super Seniors; 3.4 times for NfL, 3.3 for GFAP, 2.2 for p-tau-181. The variability of plasma biomarkers increased with age, with a larger concentration range between the 5th and 95th percentiles in the Super Seniors than 50-year-olds. The rages were 5.3 times greater for NfL, 3.4 for GFAP, and 2.8 for p-tau-181.

Conclusions: Cognitively healthy seniors have higher and more variable plasma biomarker concentrations than normative 50-year-olds.

P1-C-70 - A scoping review of comorbidity etiology in multiple sclerosis

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Multiple sclerosis (MS) is an immune-mediated inflammatory and degenerative disease affecting the central nervous system. Co-existing conditions, or comorbidities, are common in MS and include depression, anxiety, and hypertension. Compared to people without MS, those with MS have an increased risk of many comorbidities. The precise reasons for this are unknown. We undertook a scoping review to identify the extent of the literature on the specific etiological mechanisms of comorbidities in MS. We conducted a search for comorbidities in MS published up to September, 2023 using PubMed

and uploaded 6,613 articles to COVIDENCE for title and abstract screening. After screening in duplicate and removing inapplicable articles and those not published in English, 200 articles went to full-text review and 84 to data extraction. Of the 84 articles included, the mechanisms for comorbidity etiology could be grouped into any of: shared molecular (cellular, genetic), environmental, and behavioral factors or due to one condition and/or its treatment causing another. MS and comorbidities may share genetic architecture, as highlighted by positive genetic correlations between MS with amyotrophic lateral sclerosis and inflammatory bowel disease. Mendelian randomization studies identified MS as playing a causal role towards lung cancer and cardiovascular diseases. This review aims to enhance the understanding of comorbidity mechanisms in MS.

P1-C-71 - Induction and monitoring of ischemic stroke in freely behaving mice reveals behavioural sex differences of spreading depolarization

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Stroke is a leading cause of death and disability in Canada. During ischemic stroke, an obstructed cerebral blood vessel causes focal metabolic failure and eventual necrosis in brain tissue. Large depolarizing and slowly propagating waves, spreading depolarizations (SD), emanate from the ischemic core to adjacent hypoperfused (penumbra) and into remote healthy tissue. In penumbra, SD triggers breakdown of ionic gradients, increasing metabolic demand, challenging already vulnerable tissue, and expanding the core in the hours following stroke onset. **While deleterious in penumbra, there is emerging evidence that SD is benign or even beneficial in healthy tissue**. Here, understanding SD following stroke could vastly improve outcomes.

We created an all-optical method for inducing and monitoring stroke and SD in freely behaving mice, using photothrombosis to evoke unilateral stroke and fibre photometry in Thy1-GCaMP6f mice to record neuronal Ca^{2+} dynamics via bilateral fibreoptic implants. During unilateral hippocampal stroke, ipsilesional Ca^{2+} influx was similar across sexes, yet females had larger, more frequent contralesional SD, coincident with increased environmental exploration. Hippocampal stroke generated retrograde amnesia, but **when contralesional SD occurred during stroke, mice had improved functional recovery**. In paired local field potential recordings, epileptiform activity always preceded SD. Here, if SD was disrupted, seizures persisted and generalized, worsening outcomes. It may be the case that contralesional SD acts as an antiseizure mechanism post-stroke.

P1-C-72 - Nitrative stress decreases axonal transport of BDNF in young basal forebrain cholinergic neurons but increases BDNF transport in aged neurons

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Axonal transport of BDNF is critical for basal forebrain cholinergic neuron (BFCN) function. Aging impairs BDNF transport, but the mechanism is unknown. Nitrative stress is a type of oxidative stress that increases with age, but whether this affects BDNF transport is unclear. Addressing these unknowns is critical, as loss of BFCN function contributes to cognitive decline. We investigated if oxidative and nitrative stress affect BDNF transport in aged and young BFCNs. Rat BFCNs were cultured in microfluidic chambers and aged for 9-21 days. Quantum dot-labelled BDNF was added to BFCN axons prior to analysis of its transport via fluorescence microscopy. Nitrative stress was altered using L-NAME, a nitric oxide synthase inhibitor, DEA NONOate, a nitric oxide generator, and SIN-1, a peroxynitrite generator. Oxidative stress was induced by antioxidant deprivation (AOD). SIN-1, DEA NONOate, and AOD reduced transport of BDNF in DIV9 BFCNs, indicating that oxidative and nitrative stress decrease BDNF transport in young BFCNs. BDNF transport was reduced in DIV21 BFCNs and was further decreased by L-NAME, indicating that nitrative stress enhances BDNF transport in aged BFCNs. The BDNF receptor, TrkB, is decreased in aged BFCNs while the p75NTR receptor is maintained. Nitrative stress increases factors required for transport of BDNF via p75NTR. Nitrative stress may increase BDNF transport via p75NTR when TrkB is decreased, as in aging. Other processes that increase with age, such as oxidative stress, reduce BDNF transport, which may explain the overall loss in BDNF transport with age.

P1-C-73 - The CNS cell-specific proteome in health and neurodegeneration

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Several progressive neurodegenerative disorders (NDDs) such as Huntingtonâ€™s disease and Parkinsonâ€™s disease share common pathological mechanisms, including brain region-specific neuronal death, proteinopathy, neuroinflammation, and cognitive decline. Identifying triggers of neuronal decline as well as protective and pathological contributions of surrounding glial cells is key for elucidating early disease mechanisms that can facilitate disease-modifying drug design and enable more precise monitoring of disease progression and therapeutic efficacy. Using magnetic activated cell sorting and mass spectrometry-based proteomics to study both acutely isolated and cultured primary single cell CNS populations from NDD mouse models, we aim to resolve CNS cell-specific proteomes within

vulnerable brain regions and over time to identify early disease changes, novel therapeutic targets, and more precise biomarker candidates. Here, we present preliminary findings in simultaneously isolated cortical and striatal neurons, astrocytes, oligodendrocytes, and microglia isolated from adult NDD mice and age-matched wild-type littermates. Up to 6500 proteins were identified detailing a clear separation of the four different cell types and the two brain regions with our optimized methodology, confirming known and identifying several novel cell type protein markers. This data establishes a solid basis for investigating temporal changes and provides invaluable molecular insight into the spatial aspects of neuron-glial interactions in both healthy conditions and upon neurodegeneration.

P1-C-74 - Increased fear-related behaviors following alpha-synuclein preformed fibrils injected into the basolateral amygdala or striatum in mice

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Alpha-synuclein (Î±Syn)-related pathology crucially contributes to the pathogenesis of Parkinsonâ€™s disease (PD), leading to progressive neuronal populations loss in specific brain regions. This results in the presence of both motor and non-motor symptoms in PD patients. Anxiety occurs at an early stage of the disease and is one of the most frequent neuropsychiatric symptoms in patients with PD, indicating pathology in the cortico-limbic system. This study aimed to decipher that the spread of Î±Syn pathology to a given brain region is sufficient to induce non-motor symptoms observed in PD patients, while psychiatric symptoms of fear and anxiety may originate from pathology in the basolateral amygdala (BLA).

Bilateral stereotaxic injection of human Î±Syn-preformed amyloid fibrils (PFFs) in BLA or striatum (STR) was conducted in female mice overexpressing human Î±Syn (Thy1-Î±Syn) and wild-type (WT) littermates. We characterized the spread of Î±Syn pathology across brain regions and examined the behavioral and fear responses in mice at one-month post-injection (mpi) and 2 mpi of PFFs.

Injection of PFFs resulted in increased pathological Î±Syn accumulation in Thy1-Î±Syn and WT mice, compared to control (phosphate-buffered saline). Thy1-Î±Syn mice exhibited increased cue-related fear behavior 1 mpi of PFFs into the BLA, while contextual fear was neither affected by treatment nor genotype. Notably, WT displayed enhanced contextual fear 2 mpi of PFFs into the STR compared to PBS control. These findings imply that Î±Syn-related pathology may disrupt the amygdala-striatal pathway, leading to enhanced fear responses in animal models of PD

P1-C-75 - Network function and electrophysiological properties are impaired in corticomotor neurons of C9orf72 loss-of-function and gain-of-function ALS mouse models

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ALS is the most common motor neuron disease in humans, whereby upper and lower motor neurons degenerate. A major hypothesis underlying the mechanistic origin of neurodegeneration in ALS postulates that cortical hyperexcitability facilitates cell death. Previous research has identified the G_4C_2 hexanucleotide repeat expansion in the *C9orf72* gene as the most common genetic cause of ALS; however, little is known about the contribution of the *C9orf72* gene to neuronal excitability and synaptic dysfunction in the primary motor cortex. Thus, using *C9orf72* knockout loss-of-function (C9-KO LOF) and gain-of-function (C9-GOF) mouse models, we assessed the intrinsic excitability of corticomotor neurons using whole-cell patch-clamp recordings made from acute brain slices. We have found that after disease onset, the action potential firing frequency is significantly lower in the C9-GOF mice compared to WT mice. Moreover, we have found a significant reduction in excitatory and inhibitory basal neurotransmission in the C9-GOF mice compared to WT mice. Finally, we have found significant alterations in pre-synaptic short-term plasticity and in the post-synaptic AMPA:NMDA ratio, suggesting both pre- and postsynaptic impairments in neurotransmission. In contrast, the C9-KO mice only exhibit a reduction in excitatory basal synaptic transmission, while intrinsic excitability and inhibitory transmission remain intact. Further investigation into the local circuitry will reveal essential information about the neurophysiological mechanisms underlying neurodegeneration in *C9orf72* ALS patients.

P1-C-76 - In vitro modeling of Parkinson's disease using human pluripotent stem cell-derived midbrain neuron and microglia co-culture with alpha-synuclein fibrils

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In this study, we aimed to reproduce early phenotypes of Parkinsonâ€™s Disease (PD) by treating a human pluripotent stem cell (hPSC)-derived midbrain neuron and microglia co-culture system with exogenous alpha-synuclein preformed fibrils (PFFs). hPSC-derived midbrain neurons were cultured for one week in STEMdiffâ„¢ Midbrain Maturation Medium before treatment with 600 nM of alpha-synuclein PFFs from the wild type (WT) form or the familial linked-PD A53T mutant. We also co-cultured the midbrain neurons with microglia generated using the STEMdiffâ„¢ Microglia system for 2 days. The co-culture was incubated with 600 nM of WT or A53T alpha-synuclein PFFs. After 14 days of treatment, the cells were fixed and stained for phosphorylated alpha-synuclein at serine residue 129 (pS129) as well as Î²III-TUB, TH, and Iba1. After treatment with WT and A53T PFFs, the number of pS129^{+} midbrain neurons increased by 1.59 Â± 0.60-fold and 5.15 Â± 2.39-fold, respectively, while the number of TH^{+} cells decreased by 0.61 Â± 0.16-fold for WT and 0.66 Â± 0.09-fold for A53T PFFs (mean Â± SD; data normalized to untreated control; n = 6 from 3 cell lines). The addition of A53T PFFs also resulted in fewer Î²III-TUB^{+} cells (467 cells) compared to the untreated control (1057 cells), but not when the neurons were co-cultured with microglia (971 cells; n = 2). These results suggest that Î±-synuclein fibrils promote phosphorylated Î±-synuclein accumulation and reduce the number of neurons. Co-culture with microglia may mitigate this effect, suggesting a potential protective role of microglia during early stages of PD.

P1-C-77 - Retinal changes detected by ERG and pupillometry in parkinsonian monkeys

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Diagnosis of Parkinsonâ€™s disease (PD) is currently made following clinical observation of motor symptoms. By this time, around 50% of dopamine neurons are lost in the substantia nigra pars compacta (SNc). Non-motor symptoms, such as vision problems, occur much earlier along the progression of the disease. If altered functioning of the retina causes these vision problems, various techniques could be implemented to detect retinal changes, therefore providing early biomarkers for PD. The aim of this project is to determine potential early biomarkers for PD via the retina by using electroretinography (ERG) and pupillometry. In-vivo measurements were performed on 4 non-human primates (NHP), before and after they were rendered parkinsonian by administration of 1-mÃ©thyl-4-phÃ©nyl- 1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin that induces degeneration of dopamine neurons. Post-mortem retinal analyses were compared against the retina of 4 control NHPs. ERG results showed reduced a-wave amplitudes with slightly increased b-wave amplitudes in both photopic and scotopic conditions. Pupillometry analysis showed a consistently greater increase in pupil diameter during the post flash period. Post-mortem measurement of retinal layers found a significant thinning of the outer nuclear layer. This indicates that MPTP caused retinal changes detectable by ERG and pupillometry and that these changes could be attributed to the observed retinal thinning. These results provide evidence for potential retinal biomarkers that could be used as an earlier or more accurate means of diagnosing PD.

P1-C-78 - Propagation of α-Synuclein strains in a mouse model with amyloid-β co-pathology

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Synucleinopathies constitute a group of neurodegenerative diseases characterized by the presence of insoluble, intracellular Î±-Synuclein (Î±Syn) aggregates within the brain. Different structural conformations (strains) of Î±Syn aggregates are implicated in distinct synucleinopathies. Notably, Amyloid-Î² (AÎ²) aggregates co-occur in a significant proportion of synucleinopathy patients, correlating with cognitive decline and a rapidly deteriorating prognosis. Previous research in our lab demonstrated unique disease phenotypes in $M83^{+/-}$ mice, which overexpress human A53T Î±Syn, when two Î±Syn strains, the S and NS strain, are propagated in the mice. The presence of AÎ² aggregates in synucleinopathy patients raises the question whether Î±Syn strain-specific modulation of AÎ² may contribute to selective AÎ² co-pathology in certain individuals. To explore this hypothesis, we propagated the S and NS strains in $APP^{NL-F}/M83^{+/-}$ mice that produce both human AÎ² and mutant human Î±Syn. Upon presentation of neurological symptoms, the brains of mice were biochemically and histologically analyzed. Propagation of the S strain was unaltered in $APP^{NL-F}/M83^{+/-}$ mice. However, the NS strain exhibited unexpected results, reducing Î±Syn aggregates in male $APP^{NL-F}/M83^{+/-}$ mice while significantly extending the disease incubation period in females. Notably, S strain-inoculated mice displayed diminished AÎ² pathology compared to controls, while NS strain-inoculated mice exhibited comparable AÎ² levels. These findings underscore the potential impacts of the strain-specific modulation of AÎ² co-pathology by Î±Syn, with potential sex-specific effects mirroring human populations.

P1-C-79 - Elucidating the cytoprotective effects of cannabinoids in the SH-SY5Y cell line

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Alzheimerâ€™s disease (AD) is a progressive neurodegenerative disorder characterized by amyloid-Î² (AÎ²) plaques and neurofibrillary tangles. Unfortunately, most currently available treatments display low efficacy and do not target these pathological hallmarks. The amyloidogenic pathway contributes to the extracellular accumulation of AÎ² through the initial cleavage of amyloid precursor protein (APP) by beta-

site APP cleaving enzyme 1 (BACE1). Oxidative stress and epigenetic regulators, such as DNA methylation and histone-acetylation, are implicated in the onset of AD via abnormal regulation of amyloidogenic genes, such as APP and BACE1. Interestingly, the endocannabinoid system, which is affected in AD, is implicated in these phenomena, imploring investigation into the potential therapeutic use of cannabinoids in AD. This interplay led to the hypothesis that cannabinoids will be cytoprotective and reduce AÎ² pathology through the epigenetic modulation of genes that regulate the amyloidogenic pathway. Pre-treatment of SH-SY5Y cells with the phytocannabinoids cannabidiol (CBD) or Î”^{9}-tetrahydrocannabinol (THC) attenuated AÎ² and hydrogen peroxide (H_2O_2)-induced cytotoxicity. Neither CBD nor THC alone altered the mRNA expression levels of DNA methyltransferases or BACE1. The effects of these cannabinoids on AÎ² and H_2O_2-modulated gene and protein expression are currently under investigation. Further investigation is required to better elucidate the potential for cannabinoids to attenuate the effects of toxins on epigenetic regulators and targets in the amyloidogenic pathway.

P1-C-80 - Synaptic modulation of glutamate in striatum of the YAC128 mouse model of Huntington disease

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Altered balance between striatal direct and indirect pathways contributes to early motor symptoms in Huntington disease (HD). While degeneration of striatal D2-expressing indirect pathway medium spiny neurons (iMSNs) precedes that of D1-expressing direct pathway ones, altered corticostriatal synaptic function precedes degeneration. D2-mediated signaling on iMSNs reduces their excitability and promotes endocannabinoid (eCB) synthesis, suppressing glutamate release from cortical afferents. D2 receptors also reduce striatal neurotransmitter release from dopaminergic substantia nigra terminals, glutamatergic cortical terminals, and cholinergic interneurons, and these cell types may contribute to early striatal dysfunction in HD. We used corticostriatal brain slice and optogenetic probes to explore neuromodulatory signaling in the transgenic YAC128 HD mouse model. 0.5uM and 5uM of the D2 agonist quinpirole reduced cortically-evoked glutamate release in striatum of YAC128 slices, whereas only 5uM quinpirole reduced glutamate release in WT. Blocking type 1 cannabinoid receptors mitigated this effect in YAC128 slices. We also found that a lower stimulation intensity was sufficient to evoke either dopamine or acetylcholine release in YAC128 compared to WT. This suggests that YAC128 corticostriatal slices show increased D2 signaling to enhance eCB release, as well as a heightened dopaminergic and cholinergic response to stimulation. We provide a proposed mechanism of key neuromodulators that may be impaired early in HD, resulting in the manifestation of motor symptoms later on.

P1-C-81 - Comparing motor and cellular changes after spinal cord injury in male and female mice

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Traumatic spinal cord injury (SCI) is a devastating condition for which there is no treatment. Clinical evidence shows that females recover better than males following SCI. However, little is known about the mechanisms for this disparity. This study explored how biological sex affects motor recovery and tissue pathology in 12-week-old mice following 70 kdyne T9 contusion SCI. Open-field hindlimb motor testing and advanced dynamic weight bearing tasks were used to monitor hindlimb recovery at 1, 3, 7, 14, 21, and 25 days post-injury (dpi). At 28 dpi, spinal cords were immunohistochemically stained and imaged via confocal microscopy to assess lesion pathology. At 1 dpi in both sexes, SCI caused hindlimb paralysis and weightbearing was predominantly supported by the forelimbs. Stepping and hindlimb weightbearing gradually improved until 14 dpi, plateauing thereafter. At 28 dpi, both sexes showed ~60% white matter reduction around the lesion epicentre, an ~80% increase in CD68+ and Iba1+ macrophages, and ~75% increase in laminin, indicating chronic inflammation and vessel remodeling. While both sexes showed increased GFAP+ astrogliosis around the lesion core, females displayed a more robust response over males. Together, these findings suggest that while male and female mice recover similarly from SCI, there may be differences in astroglial responses to neurotrauma that underlie clinically observed sex differences. Future studies will replicate these data and use RNA sequencing to better understand mechanisms driving sex differences in SCI.

P1-C-82 - Deconstruction of complex zebrafish seizure behaviors using machine learning and a massively parallelized multi-camera microscope

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Convulsive seizure behaviors, characteristic of epilepsy, play a pivotal role in clinical diagnosis and the development of new therapies in preclinical animal model studies. Accurate identification of these behaviors is critical, however, detection of seizure behaviors in freely moving animals is largely subjective and prone to bias. Here we evaluated seizure behaviors in 2500 larval zebrafish exposed to

pentylenetetrazole (PTZ), a common acute seizure model. To identify complex behaviors, we acquired high-speed videos with a high-resolution multi-camera array microscope (MCAM) and utilized skeletal multi-point tracking and custom machine-learning (ML) algorithms. Video acquisition rates at 160 frames per second, enabled movement analysis of larvae at 3-7 days post-fertilization to reveal clear increases in activity, distance traveled and speed, with seizure activities exceeding 100 mm/s, after PTZ exposure. Eight-point skeletal tracking of individual larvae revealed reliable, quantitative, and age-dependent changes in head and tail angle measurements and decreased inter-eye distances correlating with seizure severity. Custom supervised ML algorithms enabled automatic identification of unique behavioral categories from normal swimming to convulsive seizure-like events and removed experimenter bias. These results demonstrate that high-resolution imaging coupled to ML effectively parsed complex larval zebrafish behavioral data, setting a new standard for automated detection of larval seizure behaviors crucial for epilepsy research and therapeutic development.

P1-C-83 - Mechanism of Rac1 in contributing to LTP and social memory impairments in Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative disease that entails memory loss, including deficits in social memory. The memory loss is believed to be attributed to synaptic deficits caused by the accumulation of amyloid-beta (AÎ²) peptides. Long-term potentiation (LTP), a long-lasting synaptic mechanism believed to underlie memory, has been widely reported to be impaired in AD. However, how AÎ² peptides lead to synaptic impairment and memory loss is unclear. Rac1 is a Rho family small GTPase, serving as a signalling center in regulating actin dynamics and kinase activity, and plays an important role in LTP and memory. Our investigation showed that the elevated Rac1 activity in the hippocampus of APP/PS1 mice correlates with deficits in ventral CA3-CA1 LTP and social memory, while the suppression of Rac1 activity via its dominant-negative mutant rescues these deficits. Therefore, our hypothesis posits Rac1 interactome undergoes substantial alterations in response to heightened protein activity within the APP/PS1 mouse model, influencing pathways governing activity-dependent surface expression and phosphorylation of AMPA receptors (AMPAR), critical for synaptic plasticity, given that our recording experiments indicate unchanged basal transmission and presynaptic function in 3â€“4-month-old APP/PS1 mice. To explore this, we aim to create and overexpress a novel protein (Rac1 fused with a biotin ligase) for mass spectrometry screening of dysregulated Rac1 factors in APP/PS1 mice. Unravelling Rac1 dysfunction mechanisms in AD may unveil therapeutic strategies for memory impairments in AD, offering insights into novel drug development.

P1-C-84 - 17-beta estradiol increases neurite outgrowth in adult DRG sensory neurons through the AMPK/ATF3 signaling pathway

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Adult rat dorsal root ganglion (DRG) sensory neurons express Î± and Î² estrogen receptors (ERs). 17-beta estradiol (E2) regulates development, survival, and axonal outgrowth of DRG neurons. Cellular energy sensor AMP-activated protein kinase (AMPK) can regulate the expression of both activating transcription factor 3 (ATF3), which is involved in neuronal regeneration as well as peroxisome proliferator-activated receptor Î³ coactivator-1Î± (PGC-1Î±), which is involved in mitochondrial biogenesis. *Hypothesis:* Activation of ERs in DRG neurons will enhance their energy metabolism and axonal sprouting. DRG neurons from adult SD rats were isolated and cultured under defined conditions. E2 treatment increased the levels of phosphorylated AMPK, ATF3, PGC-1Î± and ETC complex I. Additionally, E2 also elevated total neurite outgrowth and basal respiration levels. Inhibiting AMPK using compound C also inhibited E2-mediated increases in ATF3 expression and neurite outgrowth, suggestive of AMPK acting upstream of ATF3. STO-609 was used to block CAMKKÎ²; an upstream activator of AMPK. Blockade of CAMKK caused inhibition of E2-mediated AMPK activation confirming that E2 activated AMPK via the CAMKK pathway. Our work with specific ERÎ± and ERÎ² agonists revealed that these effects were mediated via ERÎ±. This study unveils that E2 acts through ERÎ± to promote neurite outgrowth via a pathway involving activation of CAMKKÎ²/AMPK and ATF3 in adult DRG neurons and highlights its potential therapeutic applications in alleviating neurodegenerative diseases. Funded by CIHR-grant#PJT-162144.

P1-C-85 - Preliminary results of high frequency transcranial alternating current stimulation and transcranial direct current stimulation with cognitive exercise as a potential treatment for dementia

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A double-blind study aimed to compare the effects of three electrical stimulations as potential dementia treatments: transcranial direct current stimulation (tDCS), transcranial alternating current stimulation (tACS) at 70 Hz, and tACS at 90 Hz. During electrical stimulation, all participants performed cognitive exercises using the MindTriggers application. Participants received two 30-min treatments daily, 5 days/week for 4 weeks. Electrical stimulation was applied with the anode electrode over the dorsolateral prefrontal cortex and the cathode electrode over the contralateral supraorbital area. The

primary outcome measure was the Alzheimer Disease Assessment Scale-Cognitive Subscale (ADAS-Cog) measured at baseline, post-treatment, and 1-month follow-up. Preliminary results include 10 (7 males) participants (mean age = 73.3; SD = 8.6) who completed one randomly selected treatment. On average, participants who received tDCS (n = 4) demonstrated improved cognition by 6.7 points post-treatment based on the ADAS-Cog score and 5.2 points at 1-month follow-up when compared to baseline. Participants who received tACS at 70 Hz (n = 4) demonstrated an average of 1.2 points cognitive improvement post-treatment and 1.7 points cognitive decline at 1-month follow-up. Lastly, for tACS at 90 Hz (n = 2), participantsâ€™ cognition declined by 0.3 points post-treatment and 2 points at 1-month follow-up. From the limited data, it appears that tACS at high frequencies (above 50 Hz) may not be the optimal treatment option for people living with dementia over the prefrontal cortex.

P1-C-86 - The fate of brainstem cholinergic neurons in Parkinson's disease

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Parkinsonâ€™s disease (PD) is a neurodegenerative disorder that primarily targets the basal ganglia (BG), a set of sub-cortical structures involved in motor behavior. The subthalamic nucleus (STN) is the main driving force of the BG and the firing pattern of STN neurons is known to be altered in PD. The main objective of this study is to characterize the neuroadaptative changes of brainstem cholinergic (ACh) neurons that send their axons to the STN, potentially contributing to the abnormal firing pattern of STN neurons. Using post-mortem human brains from PD patients and healthy individuals, we estimated the density of ACh neurons (ChAT+) that contain the phosphorylated Î±-synuclein protein (Î±-syn pSer129+) in the pedunculopontine nucleus pars compacta (PPNc) and pars dissipata (PPNd), as well as in the laterodorsal tegmental nucleus (LDTg) of the brainstem. Our results indicate no significant differences in ChAT+ neuronal densities measured in the PPNc, PPNd and LDTg between PD and controls. In PD brains, the proportion of ChAT+ neurons containing Î±-syn is similar between the three brainstem regions examined but Î±-syn aggregates are more numerous in the PPNc and LDTg, compared to the PPNd. Also, within the PPNd and LDTg, Lewy bodies are found in greater proportion in non-ACh neurons. Our previous work showed a reduced number of ACh axonal projections in the STN of PD patients. The present study indicate that ACh parent cell bodies located in the brainstem are preserved and that nearby non-ACh neurons are more susceptible to neuropathological processes at play in PD.

P1-C-87 - Lipid peroxidation within the hippocampus of neonatal rats with febrile-seizure-induced hyperoxia

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Rationale

Febrile seizures in neonatal rat models result in a hyperoxic (excessive oxygen) response in the hippocampus. Hyperoxia can cause oxidative stress and the formation of reactive oxygen species (ROS) which can lead to long term changes in neuronal functioning including lipid peroxidation. Neonatal febrile seizures have been shown to cause long-term recognition memory impairments in female adult rats and lipid peroxidation could be the mechanism behind this impairment. Therefore, this study examined the effects febrile seizures on ROS production in the hippocampus of neonatal rats.

Methods

Sprague-Dawley female and male rat pups were used for this experiment. Pups received four days of a daily injection of lipopolysaccharide starting on postnatal day 9. Three hours after the injection on day 12, they were exposed to exogenous heat until they had a clonic-tonic seizure. Three hours after the initial heat exposure, the pups were euthanized. The brains were harvested, sliced, and stained for 4-hydroxynonenal lipid peroxidation which is a by-product of ROS.

Results

Preliminary results show cells expressing lipid peroxidation in the hippocampus of the febrile seizure group.

Conclusions

Hippocampal dysfunction due to lipid peroxidation is implicated in the link between febrile-seizure-induced hyperoxia and recognition memory dysfunction in female rats.

P1-C-88 - Serotonergic dysfunction in early Alzheimer's disease progression: unraveling neurobehavioral pathways for therapeutic interventions

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Alzheimerâ€™s disease (AD) is a neurodegenerative disorder that affects millions globally, with over half a million individuals affected in Canada alone. In approximately 90% of patients diagnosed with AD, neuropsychiatric symptoms (NPS) manifest themselves years before the onset of cognitive decline. Serotonin is a neurotransmitter crucial for emotional regulation and is hypothesized to play a critical role in the early stages of AD. Treatment with selective serotonin reuptake inhibitors (SSRIs) has shown promising results in delaying cognitive decline, highlighting the significance of serotonergic modulation in disease progression. Here, we hypothesize that serotonergic dysfunction at the early stages of AD progression is a significant contributor to the neurobehavioral symptoms of AD. To test this, we expressed a hyperphosphorylation-prone human tau protein (hTauP301L) exclusively in serotonin neurons of adult female and male mice (ePet1^{hTauP301L}). We demonstrate that ePet1^{hTauP301L} mice show heightened anxiety-like behaviour, altered stress coping strategies, sex-dependent social disinhibition, and spatial working memory impairment. Built upon these observations, we aim to investigate the mechanisms underlying neural circuits modulated by serotonin in the early onset of AD. This knowledge will have important implications for the development of new treatment strategies that target serotonergic pathways, which may be a promising approach for early intervention in the disease.

P1-C-89 - Investigating the neural basis of sensory learning with mesoscale microscopy in a mouse model of Huntington's disease

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Huntington's disease (HD) is a neurodegenerative disorder that affects numerous brain functions, but the impact of altered sensory processing on sensory-based learning in HD remains poorly understood. Previously, we imaged cortical activity through a wide cranial window in anesthetized Q175/B6-GCaMP6s mice under a widefield mesoscale microscope and found that sensory stimulation-induced activity spread across more brain regions for a longer duration in HD compared to wildtype (WT) mice. Further experiments on 6 to 9-month-old awake mice produced similar results, and the amplitude and spread of cortical activation was reduced in WT over the course of repeated trials (habituation) compared to HD mice. This suggests that sensory processing networks of visual stimuli may be altered in HD. Additionally, previous studies have reported a visual learning deficit in a go/no-go discrimination task. As such, we hypothesize that the lack of precise cortical sensory processing in HD mice may interfere with the accurate encoding of learned information. To test this, we designed a visual discrimination learning task where the mice must distinguish between 1 or 2 LED pulses to receive a reward. During this task, we are monitoring the change in network connectivity and circuit functions, to determine the behavioural relevance of sensory spread. We analyzed data using conventional methodologies and attentional networks in machine learning. These alterations to HD cortical activity

provide insights into neuronal network mechanism of HD and other neurodegenerative diseases at an early stage.

P1-C-90 - On-Tract for recovery? Using diffusion tensor imaging, salivary biomarkers and neuropsychological assessment to better understand history of concussion in aging adults

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Background: Each year, around 42 million individuals experience a concussion, a type of mild traumatic brain injury. While symptoms typically subside in two weeks, the long-term impact on brain health is uncertain. Research shows concussion history nearly doubles the risk of later-life dementia, highlighting the need to discover biomarkers linking concussions to neurodegeneration.

Methods: 20 individuals aged 50+ with at least one prior concussion but no neurodegenerative disease were included in this preliminary analysis. Participants underwent a concussion history interview, diffusion tensor imaging, neuropsychological tests, and saliva sampling.

Results: A significant correlation was found between the number of concussions and variables from demographic, neurostructural, cognitive, and biological categories. A regression model including symptom severity and hippocampal fractional anisotropy (FA) predicted concussion history ($F(2,17) = 14.15$, $p < 0.001$), with an R-squared of 0.6584, suggesting that around 65.84% of the variability can be explained by the model. Further, using general linear modelling and whole brain FA, we identified that experiencing more concussions and having the first injury at an older age are together associated with higher FA values in the fornix and anterior thalamic radiation.

Conclusion: Certain biological, structural, and cognitive factors are associated with concussion history in older adults. This association strengthens when these variables are combined, offering insights into potential biomarkers for concussion-related neurodegeneration.

P1-C-91 - The impact of the integrated stress response on dark microglia in 5xFAD mice

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Microglia, the resident immune cells of the central nervous system, are now recognized as critical contributors to maintaining homeostasis and as influential players in various pathological conditions, most notably Alzheimer's disease (AD). Microglia exhibit distinct states, and recent ultrastructural studies have identified a state named dark microglia (DM) in cellular stress and neurodegenerative disease models. Pervious ultrastructural findings illustrated that DM contain more electron-dense components accompanied by other cellular stress features. The integrated stress response (ISR) pathway is activated in the brain of patients with AD, and ISR activation correlates with an increased AD pathology. Nevertheless, the association between cellular stress and alterations in microglial states in AD remains elusive. By performing scanning electron microscopy, we aimed to 1) Characterize the induction of the ISR pathway in DM of 5xFAD mice, a model of AD pathology, and 2) Investigate the impact of ISR activation on the abundance of DM in these mice. Through immunohistochemistry staining, we demonstrated the expression of phospho-eIF2Î±, an ISR marker, in the endoplasmic reticulum and Golgi apparatus of DM. We confirmed the increased presence of DM in 5xFAD mice compared with age-matched wild-type controls, and using chemogenetic mice to induce phospho-eIF2Î±, we showed ISR increased the abundance of DM. In summary, our study provides novel insights into ISR as a molecular basis for stressed DM in AD pathology.

P1-C-92 - Glutamate co-transmission by serotonin neurons of the dorsal raphe nucleus contributes to L-Dopa-induced dyskinesia

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Parkinsonâ€™s disease is characterized by the progressive loss of dopamine neurons. The dopamine precursor L-3,4-dihydroxyphenylalanine (L-Dopa) is the most effective pharmacotherapy but its chronic use is hampered by side effects such as abnormal involuntary movements (AIMs), also termed L-Dopa-induced dyskinesia (LID). Previous studies have shown the crucial role of serotonin (5-HT) neurons in the conversion of exogenous L-Dopa into dopamine and in LID expression. Here, we specifically addressed the

functional role of glutamate co-transmission by 5-HT neurons of the dorsal raphe nucleus (DRN) in LID expression. In 6-hydroxydopamine-intoxicated mice, a chemogenetic approach was first used to alter the neuronal activity of DRN 5-HT neurons while administering L-Dopa to address the role of these neurons on LID expression. Using the same mouse model of Parkinsonâ€™s disease, we used CRIPSR-Cas9 technology and virus injections to knock-out or overexpress the atypical vesicular glutamate transporter 3 (VGluT3) in 5-HT neurons of the DRN. To examine the effect of VGluT3 on AIMs expression, these mice were treated with L-Dopa for one month. Compared to control conditions, AIMs severity was increased after chemogenetic stimulation of DRN 5-HT neurons, and reduced by chemogenetic inhibition of DRN 5-HT neurons. Our CRIPSR-Cas9 manipulations led to exacerbated AIMs in dopamine-lesioned VGluT3-conditional knock-out mice that were treated with a non-dyskinetic dose of L-Dopa (1mg/kg), compared to controls and to transgenic mice overexpressing VGluT3. At higher doses of L-Dopa (3, 6, 12 mg/kg), mice overexpressing VGluT3 showed more severe orofacial AIMs. Overall, these results indicate that glutamate co-released by 5-HT neurons of the DRN contributes to the expression of LID.

P1-C-93 - Behavioural effects of novel microglia-stimulating agents in a mouse model of depression induced by chronic corticosterone exposure

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Ketamine (KET) was approved as the first fast-acting antidepressant. Evidence points to its ability to stimulate microglia to recover brain plasticity. Low doses of immunogens, such as lipopolysaccharide (LPS), have shown similar effect in stimulating microglia upon chronic stress to reverse depression-like behaviour. We aimed to test the effects of novel microglia-stimulating agents (ketamine and LPS) to quickly reverse depression-like behaviour induced by chronic corticosterone (CORT) exposure in mice. Male BALB/C mice were exposed to CORT (20 mg/kg) or vehicle (VEH, 0.1% dimethyl sulfoxide and 0.3% tween-80) for 21 days. On day 21, mice received a single injection of ketamine (10 mg/kg), LPS (100 Âµg/kg) or saline (SAL), and 2 hours later, were evaluated for the open field test (OFT) and forced swim test (FST). CORT+LPS showed a reduced time mobile in the periphery and in the center compared to CORT+SAL and CORT+KET. CORT+LPS presented a reduction in the distance travelled in the center/full arena compared to CORT+VEH. CORT+LPS displayed an increase in the average swimming speed (cm/s) at the time-bins of 30, 90, 150, 210 s compared to VEH+LPS. VEH+KET significantly increased the time mobile at 210, 270, 300, 330, and 360 s compared to the CORT+SAL and VEH+SAL groups. Also, VEH+KET showed a reduction in the number of immobile episodes compared to VEH+LPS. Our results suggest the potential of novel microglia-stimulatory agents to quickly reverse depression-like behaviour observed after CORT exposure, also serving as promising tools for depression treatment.

P1-C-94 - Repeated mild traumatic brain injuries during adolescence could contribute to the development of MS-like pathology later in life

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Retrospective patient studies have identified single or repeated mild traumatic brain injuries (RmTBIs), especially during childhood and adolescence, as risk factors for MS. In addition, there is accumulating evidence that RmTBIs can drive chronic neuroinflammation, myelin- and axonopathy, and increase the risk of developing neurodegenerative diseases later in life. The overarching hypothesis of this project proposes that RmTBIs during adolescence can drive the development of autoreactive immune responses against CNS-derived antigens, resulting in MS-like pathology. I aim to investigate this using the highly translational lateral impact model of RmTBIs in adolescent mice, given prior to the induction of EAE. I expect that early-life injuries will exacerbate, or alter the onset and progression of, EAE pathology, and drive the development of more subtle EAE-like pathology in the absence of certain induction factors. Furthermore, I will investigate the extent of myelin- and axonopathy following our RmTBI-EAE model, as well as the neuroinflammatory responses following RmTBIs that may drive the development or exacerbation of EAE pathology. Overall, I anticipate this research will extend the understanding of MS etiology, as well as inform research into the underlying neurodegeneration in MS, that will ultimately improve treatment strategies for progressive MS patients.

P1-C-95 - Cell-type specific dysregulations in the epileptic human brain

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Epilepsy is a life-altering disease, affecting up to 1 million people worldwide. Despite the introduction of new anti-epileptic drugs in recent decades, 30% of patients remain pharmacoresistant. Specifically in temporal lobe epilepsy (TLE), the most common form of focal epilepsy, 80% of patients do not respond to available medication. One of the challenges in understanding and treating epilepsy is the differences in fundamental properties between mice and human neurons, as well as the difficulty in translating therapeutic approaches from rodent models to clinical trials. We therefore studied the underlying pathological mechanisms of TLE directly in living human brain tissue from patients undergoing surgery for pharmacoresistent TLE.

In this study, we employed single-cell spatial transcriptomics (10x Genomics Xenium) to analyze the molecular landscape of cell types in the epileptic human hippocampus, a structure known to undergo pathological changes in TLE. Through this analysis, we identified the molecular profile and spatial organization of cell types affected by epilepsy. Additionally, we used calcium activity imaging (GCaMP8m) to record seizure-like events at the single-cell level in human brain slice cultures to investigate the functional involvement of interneuron subtypes in epileptic activity in the human brain.

Our findings provide insight into the molecular and functional changes in cell types in the epileptic human brain and will inform the development of novel therapeutic approaches for the treatment of pharmacoresistant temporal lobe epilepsy. Additionally, our study highlights the importance of investigating disease-relevant mechanisms directly in human brain tissue for a better understanding of neurological disorders.

P1-C-96 - Investigating the mechanisms by which aducanumab facilitates Aβ clearance using iPSC-derived human microglia in 2D and 3D cell cultures

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Antibodies targeting amyloid beta (AÎ²) have recently been developed as promising treatments for Alzheimerâ€™s Disease (AD). Aducanumab (Adu) was the first to receive limited FDA approval in 2021 offering new options for AD patients. Aducanumab clears aggregated AÎ² in the brain, presumably through microglial phagocytosis, although the exact cellular and molecular mechanisms of its effect remain incompletely understood. We employed iPSC-derived microglia in 2D and 3D cultures to study the mechanisms by which Adu facilitates AÎ² clearance in the brain. Microglia plated in 2D were treated with oligomeric AÎ²42 and Adu and imaged live for 24 hours to monitor short term changes. For 3D cell culture, neurospheres were formed from iPSC-derived neuronal progenitor cells, which grew to consist of mature neurons and astrocytes. Microglia were added to the neurospheres and infiltrated into the 3D tissue, followed by longer AÎ² and Adu treatment. In the short term, 2D microglia with Adu have significantly higher levels of phagocytosed AÎ² and at a faster rate. Intriguingly, immunostaining for fibrillar AÎ² revealed that Adu decreased the amount of highly aggregated intracellular AÎ². In the neurospheres, microglia are the primary cell type phagocytosing AÎ². Adu did not cause significant AÎ² reduction in the 3D tissue but prevented the formation of larger AÎ² aggregates, which may in turn facilitate microglia mediated AÎ² clearance. Further analysis of phagocytosed AÎ² using biochemical techniques after anti-AÎ² antibody treatment will help determine the precise molecular mechanisms of its effects.

P1-C-97 - The Impact of a Murine Coronavirus upon Alpha-synuclein pathology in neurons and microglia

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Parkinson's disease (PD) is characterized by a loss of midbrain dopamine neurons and the accumulation of aggregates of oligomeric and fibril forms of the alpha-synuclein protein. The multi-hit hypothesis points to an interaction between genetic and multiple environmental risk factors in the cause of the disease. Much evidence has indicated that mutations in the inflammatory leucine rich repeat 2 (LRRK2) protein is critically linked to PD. Moreover, viral infection may play a role as an environmental trigger and may do so by augmenting the pro-inflammatory consequences of LRRK2. The present study utilized primary midbrain neurons and microglia from wild type and LRRK2-G2019S mutant mice. Murine Hepatitis Virus (MHV) was utilized as a model for coronavirus infection and real-time live cell imaging and immunobiological assessments used to assess changes in microglial morphology, microglia-neurons interactions and alpha-synuclein aggregation in response to MHV. Thus far, we have found that MHV robustly infects midbrain dopamine neurons and microglia, leading to time-dependent neurodegeneration. The virus also caused microglial activation, increased motility, and resulted in cell fusion with the formation of complex syncytial networks. These effects were generally increased in the LRRK2 G2019S derived cells and the mutation appeared to catalyze the spread of alpha-synuclein. Our preliminary data indicate an importance for microglia and LRRK2 in coronaviral neurotoxicity and alpha-synucleinopathy, which has tremendous clinical implications.

P1-C-98 - Complement opsonin C1q protein and mRNA expression in prefrontal cortex in schizophrenia and bipolar disorder

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The complement system is a major player in the immune system. Studies have revealed that the complement system plays a vital role in normal brain development and homeostasis, including contributions to the regulation of neurogenesis, neuronal migration, synaptic pruning, and plasticity. C1q is the initiating protein of complementâ€™s classical pathway and has been shown to tag vulnerable synaptic elements for microglial engulfment. The complement system has been implicated in the pathophysiology of schizophrenia (SCZ), leading to the hypothesis that levels of complement opsonins,

including C1q, are increased in the disorder, contributing to synaptic dysfunction. However, it is not clear whether C1q protein and mRNA expression is altered in the brain in SCZ.

Frozen tissue comprising frontal cortex of control (n=35), SCZ (n=35) and bipolar disorder (BD) (n=34) subjects was obtained from the Stanley Medical Research Institute. Western blotting and qPCR were utilized to quantify protein and mRNA levels, respectively. ANOVA was used to examine differences in expression levels between groups.

C1q protein was significantly higher in the SCZ group compared to controls. A significant relationship between C1q protein and serum C-reactive protein levels was observed. C1q mRNA did not differ between control and SCZ groups but was significantly higher in the SCZ group compared to the BD group. Significant relationships were observed between mRNA and post-mortem interval.

Significant alterations in C1q mRNA expression and protein were found in post-mortem tissue of individuals with SCZ. Further studies are required to fully elucidate the role of neuroinflammation and complement opsonins in SCZ.

P1-C-99 - Central and peripheral targets of ketogenic dietary interventions in preclinical models of Alzheimer's disease

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Alzheimerâ€™s Disease (AD) remains a major global health concern, requiring innovative therapeutic strategies. Increasing evidence support benefits of ketogenic dietary interventions on cognition. In this study, we investigate the central and peripheral targets of such diets in AD mouse models. 3xTg-AD and 5xFAD mice and their control strain/genotype mice were fed a standard carbohydrate-rich diet (Control diet, 70% carbohydrate, 20% fat, 10% protein), an identical diet supplemented with ketogenic medium-chain triglycerides (MCT, a ketogenic substrate), or challenged with an extreme ketogenic diet (CFHF,carbohydrate-free high fat diet). AD mice on the MCT and CFHF ketogenic interventions showed improved learning performances after 1 month. After 6 months on the ketogenic interventions, the hippocampus of AD mice retained increases in the number of dendritic spines and correction of 41% (with MCT) and 56% (with CFHF) of differentially expressed genes. The analysis of peripheral metabolism revealed a distinct vulnerability of AD mice to hyperleptinemia and body weight gain when submitted to CFHF, whereas MCT showed evidence of improving peripheral energy metabolism. AD mice showed hundreds of deregulated genes in the liver and microbiome alterations, and these were also differentially

affected by the MCT and CFHF diets. This study highlights the cognitive benefits of ketogenic diets in AD models and reveals similarities and differences between MCT and CFHF interventions. It underscores the intricate relationship between diet, metabolism, gene expression, and the gut microbiome in AD.

P1-C-100 - The bidirectional effect of 2-AG on hyperdopaminergic states: implications of the CB2 receptor

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Multiple components of endocannabinoid system are dysregulated in schizophrenia (SCZ); Cannabinoid Receptor 2 (CB2) gene is associated with SCZ. Mice lacking CB2 on striatal dopamine neurons exhibit a hyperdopaminergic tone, presenting as hyperactive. Hyperdopaminergia is an accepted etiology of SCZ. Previously, we showed that increasing 2-arachidonoylglycerol (2-AG, major endocannabinoid) led to exacerbation of high dopamine states in two models (amphetamine, AMPH; Dopamine Transporter Knockout, DATKO). This was attenuated by Cannabinoid Receptor 1 (CB1) inverse agonist, rimonabant. Interestingly, decreasing 2-AG presented opposite effects in both models, highlighting a therapeutic avenue for novel SCZ treatments. However, 2-AG is an agonist for both CB1 and CB2. Here we investigate the role of CB2 in the response to 2-AG alterations in states of high dopamine.

DATKO present with subcortical hyperdopaminergia; exploratory hyperactivity, impaired sensorimotor gating; increased 2-AG exacerbated both. This was recapitulated in AMPH model of high dopamine; increased 2-AG exacerbated psychostimulant responses. Acute administration of the CB2 inverse agonist, AM630, saw a partial reversal of the exacerbating effects of 2-AG in both models. However, we observed that this effect appears to be biphasic, dependent on the magnitude of synaptic dopamine and on sex. This preliminary data suggests that, while blockade of 2-AGâ€™s effects on CB1 put a break on hyperdopamine exacerbation, CB2 has a more nuanced role in moderating dopamine levels dependent on synaptic concentration.

P1-C-101 - From concept to practice: assessing the implementation of 3D-multiple object tracking cognitive training for brain injury rehabilitation

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Objectives: The aim of this study was to evaluate the implementation and feasibility of a cognitive training program using Three-Dimensional Multiple Object Tracking (3D-MOT) for rehabilitation in brain injury survivors. Brain injury symptoms and perceived life adaptability were also assessed as outcomes of the program.
Methods: Twelve individuals with a history of brain injury were recruited through the Victoria Brain Injury Society for this patient-oriented study. Participants underwent five weeks of the 3D-MOT program with training sessions once or twice per week. Brain injury-related symptoms and perceived life adaptability were recorded before and after the 3D-MOT program. Implementation outcomes of acceptability, appropriateness, and feasibility were assessed through semi-structured interviews and a 5-point Likert Scale questionnaire.
Results: Participantsâ€™ perceived life-adaptability significantly improved (M=-7.75, 95%CI[-13.59, -1.91], SD=9.19, t(11)=-2.92, p=0.014) and reported symptom severity significantly decreased (M=-4.67, 95%CI[-27.8; 17.2], p=0.023) after the 3D-MOT program. Of the 12 participants, 91.6% rated the 3D-MOT program as highly acceptable and appropriate. More participants (83.3%) found engaging in 3D-MOT sessions once per week to be highly feasible when compared to sessions twice per week (41%).
Conclusions: The 3D-MOT program was found to be acceptable, appropriate, and effective for a population of brain injury survivors. This low-cost, accessible tool has the potential to be integrated into brain injury rehabilitation programs.

P1-D-102 - Superficial Tac1-lineage spinal cord neurons are polymodal nociceptors with low excitability

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The dorsal spinal cord is one of the first structures relaying somatosensory information from the primary afferent to the brain to form various sensations. Despite its importance, the neuronal circuits and mechanisms involved in this process remain to be fully investigated. Recent studies identified a population of neurons expressing Tachykinin 1 gene (Tac 1) which is essential for coping behavior induced by intense noxious stimuli. However, the nature and functional properties of theses neurons are still unclear. Our study aimed to thoroughly characterize Tac 1 neurons using slice electrophysiology and *in vivo* imaging techniques.

First, using tdTomato mice, our immunohistochemical results show that Tac1-lineage neurons are scattered across multiple laminae in the dorsal horn. Whole-cell patch recordings from parasagittal spinal cord slices demonstrated that superficial Tac1-lineage neurons have heterogeneous properties. Indeed, half of them are phasic neurons, while the other are single-spike, reluctant or tonic neurons. The high and activation threshold of action potential indicated that Tac1-lineage neurons have low excitability.

In vivo calcium imaging of superficial Tac1-lineage neurons with two-photon microscopy showed that only a small subset is mechanosensitive, but most of them are thermosensitive. They respond to both heat and cold stimuli applied to the hind paw and they are activated preferably by noxious heat stimulation. Interestingly, their thermal sensory profile is very similar to the global population of thermosensitive primary afferents, suggesting a direct connection between thermoreceptors and Tac1-lineage neurons. Collectively, our data demonstrated that superficial Tac1-lineage spinal cord neurons are predominantly heat-nociceptors, which is consistent with their role in the nocifensive behavior.

P1-D-103 - The causal role of the human posterior thalamus in the control of visual attention

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Traditionally seen as a passive relay, the thalamus transmits information to the cortex. Recent animal studies challenge this, revealing thalamic manipulations impacting executive functions. Historically, studying the human thalamus in executive functions faced limitations of non-invasive methods. Transcranial ultrasound stimulation (TUS) now enables precise targeting of deep brain areas. This study explores the hypothesis that the human pulvinar, a posterior thalamic nucleus, actively controls visual attention, shedding light on its cognitive role beyond a passive relay.

Fifteen participants performed a visual search task, identifying a "T" among "L" distractors with varying counts. Response time and accuracy gauged the impact of distractor count on visual attention. The study included a control task involving the globus pallidus internus (GPi), linked to response inhibition, using a stop signal task. BabelBrain facilitated optimal sonication trajectory planning for TUS, involving 120-second sequences targeting the pulvinar or GPi bilaterally.

Our results unveil a double dissociation of effects: TUS targeting the pulvinar enhances visual attention, as evidenced by improved search efficiency, without affecting response inhibition. Conversely, TUS

directed at the globus pallidus interna selectively impairs response inhibition, as indicated by prolonged SSRTs, while leaving visual attention unaffected. This dissociation highlights the distinct cognitive functions of the pulvinar and the globus pallidus internus, while establishing a causal link between pulvinar activity and attentional modulation. Moreover, our study demonstrates that TUS can causally investigate functions of deep brain areas that cannot be targeted by other noninvasive brain stimulation techniques.

P1-D-104 - Claudin-5 supplementation restores blood-retina barrier integrity and prevents neurodegeneration in Glaucoma

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Vascular dysfunction is a major component of glaucoma pathophysiology. The contribution of blood-retinal barrier (BRB) disruption in glaucoma is poorly understood. We tested the hypotheses that: i) BRB dysfunction occurs in the early stages of glaucoma, and ii) rescue of BRB integrity promotes retinal ganglion cell (RGC) survival.

Ocular hypertension (OHT) was induced in C57BL/6 mice by intracameral injection of magnetic microbeads. Longitudinal fundus angiography showed a progressive increase in fluorescein leakage into the retinal parenchyma starting at one-week post-OHT (OHT-1w) which remained elevated thereafter. Whole-retina and peripapillary confocal imaging at OHT-2w, when OHT is stable but no significant RGC loss is detected, showed a substantial increase of intravenously administered dextran (3K, 10K, 70K MW) leakage into all vascular plexuses of the retina. Immunoblotting of endothelial tight junction proteins revealed reduced Claudin-5 expression in eyes with OHT relative to sham controls. Endothelium-specific gene supplementation of Claudin-5 (Cldn5) by intravenous delivery of recombinant adeno-associated virus reduced fluorescein leakage and improved RGC survival (OHT-3w) compared to eyes treated with a control virus.

Our data demonstrate early disruption of BRB integrity during OHT-induced damage. We show that Cldn5 gene transfer to endothelial cells effectively reduces vascular leakage and improves RGC survival in glaucomatous eyes. These findings suggest that strategies that restore vascular integrity are beneficial to counter neurodegeneration in glaucoma.

P1-D-105 - An inhibitory pathway mediates motional contextual modulation in the midbrain

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Our perception of objects is not only determined by the sensory inputs encoding them, but also influenced by their contexts. One common visual context is background motion, which occurs when our heads or the environment moves. It is well known that background motion profoundly influences the perception of objects of interest, and related neural activity. However, it remains unclear how this contextual motion is represented in the brain and employed to modulate visual processing. To address this question, we use the circuit of the superior colliculus (SC), which is a prominent visual processing and integration center in the midbrain. We developed a novel model of visual contextual modulation and found that background motion largely suppressed SC activity evoked by flashing objects. To search for the source of visual suppression, we performed retrograde tracing and found that the SC receives prominent inhibition from the nucleus of the optic tract (NOT) in the midbrain. Moreover, with optogenetics and electrophysiology we discovered that SC-projecting inhibitory NOT neurons innervate SC neurons and are strongly responsive to background motion. Last, we optogenetically perturbed the activity of this pathway, and found that it substantially contributes to the contextual modulation of SC neurons, and in turn influences visual-guided behaviours. Overall, this study uncovers a novel midbrain projection pathway that conducts the inhibitory motion signal to the SC, suppressing SC visual processing and related visual behaviours. These findings will provide insight into the functions of the visual circuits processing background motion and computational principles underlying their functions.

P1-D-106 - Medial entorhinal cortex and thalamic HD cells are coordinated during sleep

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Successful navigation requires the production of signals that remain consistent, irrespective of changes in the environment. This can be achieved by constraining neuronal activity to low-dimensional subspaces that are mapped onto spatial features of an animal's behavior. During sleep, a period of reduced external input, pairwise coordination is maintained within different areas of the spatial navigation system. This coordination is notably seen between grid cells of the entorhinal cortex (MEC) and head-direction (HD) cells in the anterodorsal nucleus (ADn) of the thalamus. This supports the idea that activity in this network remains organized in all conditions. Since the ADn is necessary for the formation of grid cells, we hypothesized that the organization of neuronal activity within MEC requires a coherent HD input signal. To test this possibility, we performed simultaneous electrophysiological

recordings in the ADn and MEC during periods of wakefulness and sleep. We first showed that coordination between HD cell pairs in ADn and MEC is preserved after environmental changes, as demonstrated in a cue rotation experiment. In addition, the preferred direction angular offset of ADn-MEC HD cell pairs predicted pairwise correlation during sleep. Lastly, we showed that the correlation among cell pairs in MEC depended, at least partially, on the signal coming from ADn. In conclusion, our findings suggest that organized activity in the MEC is, at least in part, controlled by the coherent HD signal arising from the ADn across brain states.

P1-D-107 - Comparative 2-photon optogenetic mapping of layer-5 visual and motor cortex microcircuits

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Microcircuit connectivity dictates information processing. In primary visual cortex (V1) and primary motor cortex (M1), layer(L) 5 mediates cortical output. Therefore, we asked how L5 microcircuits differ in these distinct regions. The current state-of-the-art technique for probing microcircuit connectivity â€“ multiple patch clamp â€“ is challenging and low throughput. We therefore created optomapping, a high throughput connectivity mapping method relying on 2-photon optogenetics and patch-clamp electrophysiology. We performed viral injections in neonatal Emx1-Cre mice to express the soma-targeted opsin, ChroME, in neocortical pyramidal cells (PCs). In P18-P26 acute slices, we elicited action potentials in ChroME-expressing PCs with 1040-nm laser spiral scans. We patched PCs, basket cells (BCs), and Martinotti cells (MCs) in L5 of V1 and M1 while sequentially spiral-scanning hundreds of surrounding PCs to search for presynaptically connected PCs across all cortical layers. In V1, L5 BCs received a denser, stronger, and farther number of PC inputs than L5 PCs (connectivity: $p<0.001$; amplitude: $p<0.05$) and MCs (connectivity: $p<0.01$; amplitude: $p<0.05$). In M1, L5 PCs received denser ($p<0.05$) and stronger ($p<0.001$) PC inputs from descending connections than ascending connections. V1 L5 PCs had more descending and intralaminar inputs ($p<0.05$) and stronger ascending inputs than M1 L5 PCs (<0.001). The lognormal distributions of PC amplitudes were similar across both regions ($p=0.6$). Despite their different functions, V1 and M1 L5 microcircuits were not vastly different.

P1-D-108 - Comparative 2-photon optogenetic mapping of layer-2/3 visual and motor cortex microcircuits

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Connectivity patterns determine information processing in the brain. Layer (L) 2/3 is known as the computational layer, where key information processing occurs. Current state-of-the-art methods such as multiple patch clamp are inefficient for large-scale microcircuit mapping. So, to elucidate cortical microcircuit structure, we created optomapping, a high-throughput, two-photon optogenetic mapping method that tests hundreds of connections in one experiment. Here, we compare L2/3 in primary visual cortex (V1)â€"a key cortical input areaâ€"to primary motor cortex (M1)â€"a key cortical output area. We injected P1-2 Emx1-Cre mice with the opsin ChroME to target pyramidal cell (PC) somas. In P18-26 acute slices, we whole-cell recorded from PCs, basket cells, and Martinotti cells, spiral scanning surrounding ChroME-expressing PCs with a 1040nm laser to elicit EPSPs in the patched cell. We found higher and stronger L5â†'L2/3 PC connectivity in V1 than in M1 (connectivity: 7.9% Â± 2% vs. 0.87% Â± 0.5%, $p < 0.01$; amplitude: 0.34 Â± 0.007 mV vs. 0.21 Â± 0.002 mV, $p < 0.05$). However, there were no synaptic strength differences across layers in V1 ($p = 0.23$). M1 L2/3 had more intralaminar than interlaminar connections (8.9% Â± 2% vs. 0.87% Â± 0.5%, $p < 0.05$), whereas V1 L2/3 had higher interlaminar connectivity from L4 than within L2/3 (24% Â± 5% vs. 7% Â± 1%, $p < 0.001$) consistent with the canonical V1 circuit. M1 as well as V1 PCâ†'PC synaptic weights distributed log-normally. Overall, despite their functional differences, V1 and M1 L2/3 microcircuits are not uniquely distinct.

P1-D-110 - Depletion of the teleost ortholog of connexin-36 causes hyperopia and visual-motor deficiencies during zebrafish development

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Connexins (Cx) form electrical synapses that mediate bi-directional communication between neurons and is thought to be essential in several developmental events, including neural circuit formation. Though Cx36 is a major constituent of electrical synapses, how it contributes to development is unknown. Zebrafish are a powerful model to investigate the neurodevelopmental roles of electrical synapses as many signaling cascades are highly conserved in mammals. Since Cx36 is robustly expressed in the retina, we hypothesized that gjd2b/Cx35.1 electrical synapses, the teleost ortholog of Cx36, contributed to the development of the visual-motor circuitry.

Targeted ablation (knock-out, KO) of Cx35.1 was achieved using Cas9/CRISPR technology and confirmed by the loss of expression in the inner plexiform layer and select photoreceptor cells (PRCs). Behavioral assays capitalized on the larval innate ability to avoid light transitions through the startle-burst locomotor response. Here, KO larvae were hyperactive in response to abrupt light transitions; this was recovered under low light and absent during the onset of darkness. qRT-PCR revealed that two rhodopsin genes were downregulated, thus we speculated that rod PRCs were less sensitive to bright light transitions and instead cone PRC activity was enhanced in KOs. Optical coherence tomography analysis of the adult retina confirmed changes to the refractive properties, leading to hyperopic shifts. Together, this study provides evidence for the role of Cx35.1 in the development of the visual system and visual-motor behaviors.

P1-D-111 - Development of a polygenic score to predict cisplatin-induced sensorineural hearing loss

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Introduction: Cisplatin is used to treat several cancers, including brain tumors. However, cisplatin is limited by off-target toxicities, such as ototoxicity (i.e., sensorineural hearing loss). Genetics play a significant role in the variability observed in patientâ€™s susceptibility to cisplatin-induced ototoxicity.

Objective: To use genomic data to predict cisplatin-induced ototoxicity (CIO).

Methods: Using SBayesR, we developed a polygenic score (PGS) to predict hearing loss based on a hearing loss multi-trait meta-analysis genome-wide association study conducted on the UKBiobank dataset (n=353,983). The relevance of this PGS to CIO was tested in a pediatric CIO cohort (n=390). Enrichment analyses were conducted on murine inner ear single-cell-RNA-sequencing data from gEAR to determine whether variants annotated to genes expressed in specific inner ear cell types are more likely to be associated with hearing loss traits.

Results: The hearing loss PGS, which included 2,753,914 genetic variants, was significantly associated with CIO (P=3.8x10^{-3}, R^2 = 0.02). Enrichment analyses revealed that variants mapping to genes expressed in cell subtypes in the epithelial cochlea and stria vascularis were more likely to be associated with hearing loss (P<2.0x10^{-16}).

Conclusion: This is the first PGS that was developed using a large-scale hearing loss dataset that is associated with CIO. Future analyses will focus on replicating these results and incorporating data from

single-nuclei RNA-sequencing of cisplatin-treated mouse cochlea samples to improve the biological relevance of the PGS.

P1-D-112 - Incorporating novel audiogram classification strategies to identify genes and pathways involved in subtype components of age-related hearing loss

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Background: Age-related hearing loss (ARHL) is heterogeneous 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.

P1-E-113 - Targeting nutrient-induced satiating vagal afferent neurons via TRAP2 mice

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The gut-brain axis is critical for control of food intake, as intestinal nutrients activate vagal afferent neurons to control meal size via release of gut peptides. However, the specific population of vagal neurons responsible for control of food intake, and the specific signaling pathways which mediate this response is still not fully understood. We utilized TRAP2 ($Fos^{2A\text{-}iCreER}$) mice, which express a tamoxifen-inducible Cre recombinase downstream from the Fos promoter, to target specific vagal neuronal populations. TRAP2 mice received a bilateral nodose injection of either an excitatory DREADD (pAAV-hSyn-DIO-hM4D(Gq)-mCherry) or an inhibitory DREADD (pAAV-hSyn-DIO-hM4D(Gi)-mCherry), and one week later were implanted with a small intestinal catheter. One week following recovery, lipid-responding neurons were â€˜trappedâ€™ with an i.p. injection of 4-hydroxytamoxifen immediately prior to a 15min infusion of intralipid (IL) or saline control after a 12hr fast, ultimately resulting in DREADD expression selectively in vagal afferents neurons activated following infusion. Utilizing these mice, we demonstrated that injection of clozapine N-oxide (CNO) following a 12hr fast significantly decreased food intake in Gq-nodose-IL mice compared to Gq-nodose-saline mice. Conversely, CNO partially reduced the satiating effect of an IL infusion in Gi-nodose-IL mice compared to Gi-nodose-saline mice. These data demonstrate the ability to selectively target nutrient-induced satiating vagal neurons in-vivo, and future studies are aimed at better understanding these specific neuronal populations.

P1-E-114 - Epilepsy-linked GATOR1 Mutations Impair PI3 Kinase-Dependent Growth Factor-Signaling Regulation of mTORC1.

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GATOR1 is an evolutionarily-conserved GTPase-activating protein complex that controls the activity of mTORC1 in response to intracellular amino acid availability in cells. Genetic mutations in the GATOR1 subunits, NPRL2, NPRL3, and DEPDC5, have been associated with familial forms of epilepsy, autism spectrum disorders, and sudden unexpected death of epilepsy (SUDEP) in humans, however, the specific effects of these mutations on GATOR1 function and mTORC1 regulation are not well understood. Here, we report that epilepsy-linked mutations in the NPRL2 subunit of GATOR1, NPRL2-L105P, -T110S, and -D214H, increase basal mTORC1 signal transduction in cells. Notably, we show that NPRL2-L105P is a loss-of-function mutation that disrupts protein interactions with NPRL3 and DEPDC5, impairing GATOR1 complex assembly and resulting in high mTORC1 activity even under conditions of amino acid deprivation. Furthermore, our work shows that the GATOR1 complex is necessary for mediating the rapid and robust inhibition of mTORC1 in response to growth factor withdrawal or pharmacological inhibition of PI3 kinase, in the presence or absence of amino acids. We propose that cell autonomous

defects in GATOR1 contribute to seizure sensitivities since deleting the NPRL2 â€“subunit of GATOR1 in glutamatergic neurons cause tonic and tonic-clonic seizures in mice, providing insight into the neurological cell-types involved in GATOR1-dependent seizures.

P1-E-115 - Role of astrocytic glucocorticoid receptor (GR) signaling in the adaptive response to metabolic stress

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Energy homeostasis and stress response involve constant adaptation, recruiting much of the same circuitry. Disruption of this dynamic neuroendocrine circuitry directly contributes to the development of metabolic disorders and anxiety. Recent results from our lab suggest an important role of astrocytic GR activity in regulating synaptic and behavioural responses to stress. Despite these data, little is known about astrocytes implication in the adaptation to metabolic stress. Therefore, we set out to test the hypothesis that astrocytic GR signalling may be involved in response to metabolic stress.

For that, we generated transgenic mice to induce astrocyte-specific deletion of GR in a brain wide manner (AstroGR^{KO} mice) before performing metabolic phenotyping of these mice before and after a metabolic challenge. In basal conditions, we found that AstroGR^{KO} mice have an increased fat mass associated with a higher ZT2 corticosteronemia than controls. After a 16h fasting/refeeding test, we observed a slower recovery from body weight loss in AstroGR^{KO} males and the absence of the characteristic corticosterone response to fasting. Interestingly, this phenotype was absent in females, suggesting distinct roles of astrocyte GR signalling between sexes. To determine the mechanism underlying astrocytic GR dependant corticosterone secretion, we investigated astrocytic morphology and c-fos immunoreactivity changes in critical hypothalamic regions.

Overall, our results suggest that astrocytic GR signalling plays a critical role in the adaptation to metabolic stress in a sex-dependant manner.

P1-E-116 - Pregnancy high-sucrose diet intake does not alter glucocorticoids and aldosterone in the fetal brain and blood of rats

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Sucrose (table sugar) intake is high globally, yet little is known about how it affects the brain and hormones. Glucocorticoids (GCs) and mineralocorticoids (MCs) are steroid hormones that mediate stress responses. The primary GC in rats is corticosterone, which can be converted into the MC, aldosterone (ALDO). In rats, maternal high-sucrose diet (HSD, 26% sucrose) from 10 wk before mating to weaning increases blood and brain corticosterone levels in adult female offspring, compared to isocaloric- nutrient-matched control diet (CON, 1% sucrose). Maternal HSD from 10 wk before mating to embryonic day 19.5 (E19.5) increases maternal serum GC levels and fetal blood and brain ALDO levels. It is unclear whether a maternal HSD during pregnancy alone is sufficient to alter GCs and ALDO in fetal blood and brain. To test this, pregnant rats were fed CON or HSD starting at E0.5 (n=15/diet). On E19.5, we collected fetal blood and brain. Fetal brain was microdissected for 5 regions. We quantified steroids via highly specific and sensitive liquid chromatography-tandem mass spectrometry. Maternal HSD during pregnancy alone did not alter maternal food intake, body mass, or litter size but increased % males per litter. HSD during pregnancy alone did not alter GCs in fetal blood and brain regions. ALDO was not detected. Our results suggest that maternal HSD during pregnancy alone does not alter fetal GCs and ALDO levels and thus, a longer duration of maternal HSD before mating is needed to alter fetal endocrine physiology. Our study will clarify how nutrition affects neurodevelopment.

P1-E-117 - Bridging the gap: ghrelin and the effects of social stress on male and female mice

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Social stress, a common risk factor for depression & anxiety, disproportionately affects females. Interestingly, the hormone ghrelin seems to attenuate the effects of social stressors in mice, but this work has been done predominantly in males. In this project, we examined the role of ghrelin receptor signalling in mediating the protective effects of ghrelin in the face of social stressors using the chronic non-discriminatory social defeat stress paradigm on male/female WT mice & mice with target deletion of the growth hormone secretagogue receptor (GHSR KO mice), the only known ghrelin receptor. Male/female mice pairs were introduced into the cage of an aggressive sexually experienced male CD-1 mouse for 10 min & housed with this mouse overnight, separated by a screen that allowed for olfactory/visual cues to be detected. This was repeated with a new CD-1 each day for 21 days. Experimental mice had 4-hr daytime access to a high-fat diet (HFD) to test their preference for palatable food. Results showed that stressed males ate more chow & less HFD than controls, but female WT mice ate more HFD than KO mice. Moreover, stressed WT males consumed more calories/day than controls while stressed WT females consumed fewer calories/day, effects not observed in GHSR KO mice. Looking at BW, males did not change with stress. Control KO females did not gain weight as WTs did. When

stressed, female KOs lost weight, but not WTs. These data highlight a differential feeding response to social stressors in males & females, potentially related to sex differences in ghrelin & GHSR function.

P1-E-118 - Ghrelin receptor expression in hypothalamic tanycytes

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Hypothalamic tanycytes are glial cells that line the walls of the third ventricle and form the blood-cerebrospinal fluid (CSF) barrier. They are involved in the metabolic effects of hormones such as leptin and ghrelin. Ghrelin, the only known orexigenic hormone, is also involved in regulating metabolism, and it exerts central metabolic control by acting on the hypothalamus. However, the role of tanycytes on ghrelin function has not yet been fully determined. Here we attempted to determine the potential for ghrelin to act directly on tanycytes by assessing tanycytic expression of ghrelin receptor (GHSR) mRNA. Experiments were approved by the Carleton University Animal Care Committee (#118381). In four C57BL/6 mice (n = 2 male/female), we injected an adeno-associated virus encoding green fluorescent protein (GFP) under the control of the deiodinase II (Dio2) promoter (pAAV-Dio2-iCre-2A-GFP; BrainVTA) into the lateral ventricle, targeting GFP expression to hypothalamic tanycytes. We then used in situ hybridization to assess the colocalization of GHSR mRNA with GFP-positive tanycytes. We saw no expression of GHSR on tanycytic cell bodies anywhere along the ventricular wall. We did consistently observe GHSR-expressing cells in close proximity to tanycytic processes, including in the arcuate nucleus, which is directly exposed to peripheral ghrelin. We hypothesize that ghrelin may not be interacting directly with tanycytic GHSR but may interact with tanycytes either directly or indirectly at the sites of GHSR-expressing neurons with tanycyte projections in close proximity.

P1-E-119 - Loss of neuroendocrine feedback in hypothalamic appetite neurons in obesity exacerbates weight gain

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Obesity is the result of chronic excess energy intake through feeding, which is prevented by leptinâ€™s inhibition of hunger neurons in the hypothalamus. However, the brain can become leptin-resistant in overfeeding, exacerbating weight gain. Preventing leptin resistance is a key target for anti-obesity

therapeutics, but previous studies used RNA sequencing, which lacks spatial resolution and thus cannot resolve neural appetite circuits. We therefore sought to uncover the transcriptomic changes driving leptin resistance in a spatial context, to understand how this impairs appetite circuits. We used multiplexed RNA in situ hybridization in brains from obese mice to test the hypothesis that mRNAs encoding receptors of key neuroendocrine hormones would decrease in appetite-stimulating neurons, while mRNAs encoding their negative feedback pathways would increase. Our results showed that obese mice had reduced expression of receptors for leptin, insulin, and glucagon-like peptide in the arcuate nucleus. Conversely, negative feedback mechanisms for leptin and insulin signaling were upregulated. This resulted in satiety neurons shifting transcriptionally to resemble hunger neurons. These data suggest that appetite-regulating neurons of the hypothalamus have reduced endocrine receptivity in obesity, which may cause increased appetite and drive the development of type 2 diabetes. Our findings shed light on the progression of obesity and its comorbidities, which we hope will help inform new therapeutic strategies for the management of obesity and diabetes to ease patient suffering.

P1-E-120 - Exploring central BDNF profiling in an anorexia nervosa-like mouse model: Insights into diagnostic and prognostic implications

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Anorexia Nervosa (AN) is a multifactorial mental disorder characterized by voluntary food restriction and excessive physical activity leading to dramatic weight loss. The Brain-Derived Neurotropic Factor gene has been associated with AN. In rodents, both central and peripheral BDNF have been found to induce weight loss and anorexic effects. Thus, besides its function in neuronal development and mood, BDNF also acts as a metabolic regulator, making it a candidate biomarker for AN diagnosis. We used C57BL/6J female mice under 2-week 50% food restriction with access to running wheels to mimic the metabolic environment of AN patients. One-week of progressive refeeding and 1-day binge eating were also done. We measured mRNA levels of BDNF and related genes in brain regions (prefrontal cortex, hypothalamus, dorsal striatum, etc.) using qPCR and RNA sequencing. To date, we have found a significant decrease in BDNF expression in PFC and DS of food-restricted mice, which persisted in the PFC but disappeared in the DS after progressive refeeding. Although the results suggest the potential of BDNF as a biomarker for the diagnosis and prognosis of AN, further investigation is needed to determine whether circulating BDNF levels reflect a detailed BDNF profile.

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P1-E-121 - Local encoding of stress memory by PVN CRH neurons

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An aversive experience leaves a trail of memories. In rodents, fear memory is encoded in cortical and limbic structures and apparent through freezing behavior elicited upon recall. Changes in physiology accompany the fear memory, but other aspects of the aversive memory related to the internal state of the animal are less understood. We have recently shown that CRH^{PVN} neurons express associative, physiological stress memory during recall. In addition, CRH^{PVN} neuronal activity controls stereotyped, repetitive behaviors that appear immediately after stress. This critical period aligns closely with the process of memory consolidation. Whether this means that hypothalamic CRH neurons contribute to locally and independently encoded physiological aspects of aversive memory is not known. Here, we show that the time period immediately after stress is accompanied by increased blood oxygenation in the PVN, elevated CRH release in the median eminence and a persistent activity state in CRH^{PVN} neurons. Specific inhibition of the CRH^{PVN} neurons either using optogenetics or through exposure to an appetitive stimulus immediately after stress blocks the contextual stress memory but leaves the fear memory intact. Our observations reveal the existence of a secondary memory storage system independently encoding memory of an aversive experience.

P1-E-122 - Effects of the stimulant drugs methamphetamine and MDMA on circulating endocannabinoid levels in healthy humans

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Background

The endocannabinoid (eCB) system plays a critical role in stress and reward, both of which are impacted by repeated drug use. Recently, humans with chronic stimulant use were found to have higher levels of the eCB ligand 2-Arachidonoylglycerol (2-AG), as compared to both recreational users and stimulant-naÃ¯ve controls. Recently abstinent stimulant users had decreased 2-AG levels, while levels of the other primary eCB ligand anandamide (AEA) were increased. However, little is known about the acute effects of stimulants on eCB levels in humans.

Methods

The present study investigated the acute effects of the stimulants methamphetamine and MDMA on plasma eCB levels in healthy adult humans. Healthy participants (N=22) received MDMA (100mg), methamphetamine (20mg), and placebo on three separate sessions in a counter-balanced, double-blind, within-participant design. Blood samples assessing levels of the eCBs AEA and 2-AG were collected at approximately 165 minutes post-drug administration.

Results

Repeated-measures general lineal models revealed significantly lower 2-AG plasma levels in the methamphetamine condition, compared to placebo ($F = 4.508$, $p = 0.046$). No significant differences in 2-AG levels were found between the MDMA and placebo conditions, nor between AEA levels in either condition.

Acute administration of the stimulant methamphetamine was associated with significantly lower levels of the eCB ligand 2-Arachidonoylglycerol, in contrast to studies with chronic stimulant users, suggesting that the eCB response to stimulants may change with repeated use.

P1-F-123 - Investigation of prefrontal dopamine dynamics during a novel model of context fear learning

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Identifying an environment as threatening or safe is essential for survival and depends on integrating past experiences with the present situation to guide context-appropriate behaviour. Individuals experiencing post-traumatic stress disorder (PTSD) often generalize their fear responses to safe environments. The medial prefrontal cortex (mPFC) may be a key structure to investigate during context-guided fear behaviour because a) itâ€™s engaged during fear expression b) its neural representation changes when the meaning of a context is altered and c) mesocortical dopamine is associated with guiding adaptive actions. To further characterize the role of the mPFC during context

threat uncertainty, we developed an apparatus that â€œteleportsâ€ the mouse between contexts to measure discrimination. We expressed the biosensor GRABDA in the mPFC to monitor dopamine signalling with fiber photometry during context fear learning. Our results indicate that mice discriminate between threatening and neutral contexts following â€œteleportationâ€. Further, we observed transient and prolonged increases in dopamine signalling during context fear conditioning. In addition, â€œteleportationâ€ between threatening and neutral contexts elicited increased dopamine signaling. These results suggest the â€œteleporterâ€ apparatus can be used to model contextual processing and that prefrontal dopamine dynamics are altered during context fear memory encoding and retrieval. Together our findings indicate prefrontal dopamine signalling may be a useful target for developing therapeutic interventions for those with PTSD.

P1-F-124 - The effects of acute moderate-intensity exercise on emotional conflict processing in healthy young adults

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This study aims to investigate the physiological mechanisms underlying emotional conflict processing in healthy young adults following acute moderate-intensity exercise. Event-related potential was used to explore the effects of acute exercise on conflict processing in 15 healthy young adults with an age range between 18 and 23 years, based on 32 electrodes. Measurements were taken before and after a 20-minute acute moderate-intensity exercise session using the Emotional Stroop Task. Statistical analysis employed a 2-way repeated ANOVA to understand the impact of acute exercise intervention on behavioral performance (reaction time) and event-related potential (N450 amplitude) in conflict situations.

The experiment indeed induced situations of conflict interference, as reaction time were longer in incongruent conditions compared to congruent conditions (F = 51.039, p = .000, Î·^{2} =.785) in 2Ã—2 ANOVA, confirming the stability and validity of this experimental paradigm. From a single bout of exercise intervention, we observed significant interaction for intervention by condition (F = 9.737, p = .008, Î·^{2} =.410). The condition in incongruent of N450 amplitude was selectively recruited more neural resources (larger negative amplitude) after a single 20-minute exercise session compared with control condition (t = 2.217, p = .044).

Healthy young adults, after a single exercise session, recruited more neural resources to improve behavioral performance selectively in conflict situations. Previous studies showed that the N450 component indicates conflict detection and resolution, and it is commonly observed during tasks like the

Stroop test. However, the current behavioral results did not reach significance, possibly due to the single bout of exercise not involving enough neural resources.

P1-F-125 - Significant impact of supramammillary nucleus on alertness and memory deficits in ADHD

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ADHD affects a significant number of individuals with considerable impact on memory and alertness. Optimal stimulation theory suggests that the symptoms of ADHD arise from a deficiency in external stimulation. While interventions like multimedia learning have demonstrated some success in mitigating these symptoms, the underlying neural circuitry remains poorly understood.

In this study, we utilized looming stimulation and novel environment exposure tests to transiently enhance alertness in spontaneously hypertensive rats (SHR), an established model of ADHD to search for new neurosubstrates involved in ADHD. We observed that looming resulted in a temporary reduction in response time and improved recognition memory during a subsequent novel object recognition test in SHR rats. Considering the involvement of the hypothalamic-pituitary-adrenal axis in ADHD, we investigated the role of the hypothalamus in alertness modulation and discovered a direct association between the activity of neurons in the SuM and the animals' alerting level. By manipulating SuM neurons with optogenetics and chemogenetics, we achieved bidirectional controls over alertness, emphasizing the significance of these SuM neurons in altering responses and possibly in ADHD. Furthermore, through viral neural tracing, we identified the critical role of SuM neurons projecting to the dentate gyrus (DG) in the manifestation of recognition memory deficits observed in ADHD.

These findings offer valuable insights into the previously uncharacterized neural circuitry underlying alertness and cognitive impairments in an ADHD rat model. Additionally, the identified neurons in the SuM as potential therapeutic targets for enhancing cognitive impairments associated with ADHD.

P1-F-126 - Projection specific plasticity in central amygdala for mouse maternal care

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Because mammalian infants depend on care from adults for all aspects of survival and wellbeing, parental behaviors are highly consequential to species survival. Despite their critical importance, however, infant directed behaviors in many species are highly flexible and can range from caregiving to infanticide depending on a range of external and internal factors. In mice, pup-naÃ¯ve females initially avoid or attack pups whereas experienced females will approach and care for pups. While this transition from avoidance to approach suggests a reversal in the affective valence of pups, how valence-encoding circuits in the brain respond to and are altered by experience with pups has not yet been examined. Given its role in processing valence, we investigated neurons in the central amygdala (CeA) and discovered a population that project to the locus coeruleus (LC) that respond strongly to pups in naÃ¯ve but not experienced mice. We found that these responses contributed to initial pup aversion as inhibiting CeA-LC neurons abolished pup avoidance. We then found that oxytocin released into CeA depresses excitatory inputs and that this was required for increased approach following social experience with pups. Downstream, we found that stimulation of CeA increased spontaneous firing in LC, which was blocked by corticotropin releasing factor (CRF) antagonism. Taken together, our results suggest that oxytocin released during cohousing reduces pup-responses in CeA-LC neurons through synaptic depression of excitatory inputs, resulting in lowered CRH release in LC and reduced pup avoidance.

P1-F-127 - Acute D-serine rescues spatial pattern separation deficits in aged adult mice and is sufficient to improve pattern separation in young adult mice

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Memory impairments are hallmark symptoms of dementia and mild cognitive impairment, as well as healthy aging. Many of these impairments likely occur through deficits in memory discrimination as opposed to deficits in memory retention. Pattern separation, the process of orthogonalizing similar inputs into distinct memories, is impaired in healthy age-related and disease-based cognitive decline. Finding treatments to alleviate declines in pattern separation provides hope for therapeutics. D-serine, an amino acid and co-agonist of NMDARs, has shown promise as a pro-cognitive treatment in aging, brain injury, and neurodegenerative disease. We demonstrate that an acute dose (100mg/kg, 2h prior to testing) is sufficient to improve pattern separation in young adult (3-4 months old) male and female mice. Aged male and female mice (19-20 months old) could not discriminate between novel and previous-encountered object locations in an easier version of the task in which the younger cohort could, indicating impaired pattern separation. Acute D-serine was sufficient to rescue pattern separation

in these aged mice. Immediate early gene analysis is underway to determine whether changes in neuronal dentate gyrus activity underlie impairments with aging and pro-cognitive effects of D-serine and whether mature or immature neuron activity is differentially affected, as the dentate gyrus and immature adult-born neurons therein are essential for pattern separation. These findings suggest that D-serine may be useful as a novel therapeutic to combat age-related declines in memory discrimination.

P1-F-128 - Functional characterization of the medial mammillary nuclei in spatial memory

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The mammillary bodies (MB) have been implicated in processing spatial information; however, the role of distinct MB nuclei remains elusive. The organization of inputs and outputs of the medial (MM) and lateral (LM) MB nuclei are parallel to one another and are topographically organized, which may represent a functional differentiation within the structure. To selectively investigate the role of the MM in spatial memory, we coupled behavioral testing across differently cued spatial memory tasks with functional inhibition of the MM soma using synaptic silencing and optogenetics. The spatial memory tasks employed in our investigation evaluate egocentric and allocentric reference frames, as well as the resolution of conflict between both reference frames during naturalistic escape behaviour. To selectively target the MM, we procured the Nts-Cre mouse line that selectively expresses Cre in the MM. We began by synaptically silencing the MM soma using Cre-responsive AAV expressing tetanus toxin light chain. At 3-4 weeks post-op, mice displayed profound impairments in visuospatial abilities, including egocentric spatial memory and balance, a prominent head tilt, and impaired nest building in the home cage. Attempts to retest the mice on the egocentric spatial task at 5 weeks were unsuccessful due to progressive visuospatial decline. To transiently inhibit the MM soma across varying parts of the behavioral tests, we used optogenetics by expressing Cre-responsive ArchT in the nuclei. Our results suggest the MM may serve a functional role in the integration of spatial cues necessary for spatial memory and navigation in an environment more broadly.

P1-F-129 - Glutamatergic neurons in medial prefrontal and orbitofrontal cortices are involved in odour-based incidental memory in rats

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Incidental memories are encoded through spontaneous interaction and exploration in an environment and can contribute to higher cognitive functions like working memory. The recent development of spontaneous incidental memory tests, the Identical Stimuli Test (IST) and the Different Stimuli Test (DST) with both objects and odors, allows for cognitive testing using variable memory loads in rats. Using the IST and DST with odors and chemogenetic techniques, we tested the involvement of the medial prefrontal cortex (mPFC) and the orbitofrontal cortex (OFC) in odor-based incidental memory. Rats underwent stereotactic surgery to bilaterally inject inhibitory Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) targeting glutamatergic neurons in the above brain regions. After recovery, both male and female rats were tested on the IST and DST following either a saline or a DREADD agonist (Compound 21, 1mg/kg, i.p.) injection. In both sexes, we observed novelty preference deficits under high-memory loads of the DST following inhibitory DREADD activation in both the mPFC and the OFC. Total exploration times of the items in both the IST and DST remained unaffected by treatment. Ultimately, the mPFC and the OFC may play unique roles in supporting incidental memory as test complexity increases. Future experiments will work to delineate the involvement of these regions in the sample, delay, or test phase in the DST, which will further contribute to our understanding of the circuitry involved in incidental memory.

P1-F-131 - A review and meta-analysis of fMRI studies of proactive and reactive cognitive control

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The dual mechanisms of control (DMC) theory posits that cognitive control has two modes, proactive and reactive control. Proactive control is thought of as an â€˜early selectionâ€™ process whereby goal-relevant information is sustained in anticipation of conflict or interference. In contrast, reactive control is a â€˜late correctionâ€™ process whereby control processes are recruited after conflict is detected. The two modes are thought to be subserved by different brain regions. Specifically, proactive control is thought to involve sustained and anticipatory engagement of the lateral prefrontal cortex (lPFC). Reactive control is associated with transient, stimulus-driven activation of the lPFC and other brain regions. We conducted a review and activation likelihood estimation (ALE) meta-analysis of studies investigating the brain networks involved in the two modes of control in healthy adults. The ALE meta-analysis was used to identify the brain regions consistently recruited across the studies during proactive and reactive control. The lPFC was found to be consistently recruited during both proactive and reactive control. Reactive control was also associated with activations in a wider set of brain regions, including the insula and the inferior parietal lobule. Proactive control was also associated with activations in a broader set of brain regions (e.g., cingulate gyrus and inferior parietal lobule), indicating that the

conceptualization of the neural correlates of proactive control might need to be modified to include more brain regions than the lPFC.

P1-F-132 - TRAPing hippocampal-dependent fear memories

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Fear conditioning is a widely used behavioral paradigm used to study learning and memory in rodents. Contextual fear conditioning is a simple form of fear learning where a footshock is presented in a specific environment. Auditory trace fear conditioning is a more complex version of fear learning where an auditory tone and a footshock are separated in time by a trace interval (20 seconds). Although lesion studies have shown that an intact hippocampus is required for contextual and auditory trace fear conditioning, the processes involved in encoding and retrieval fear memories in each of these tasks is not fully understood. Here, we used the Targeted Recombination In Active Population (TRAP2) mouse crossed with a tdTtomato reporter line to permanently label neurons activated during contextual or auditory trace fear learning with 4-hydroxytamoxifen. Mice underwent fear memory retrieval 14d later and euthanized 90 minutes later. To evaluate neurons involved in fear encoding and retrieval, brains were processed for tdTomato and c-Fos, respectively. We found that male and female mice that underwent contextual or auditory trace fear conditioning showed greater freezing during learning and memory retrieval tests compared to no-shock controls. We also found that mice that received footshocks in either paradigm had a significantly greater number of hippocampal tdTomato+ neurons compared to no-shock controls. We are currently analyzing colocalization of tdTomato+ and c-Fos+ neurons to identify potential regional differences in fear encoding vs retrieval across both tasks.

P1-F-133 - The anterior retrosplenial cortex and recognition of objects, locations, and object-in-place associations: role of glutamatergic neurons in male and female Long Evans rats

Dan Mcelroy [1], **Ilne Barnard** [1], **Veronica Kryachko** [1], **Justin Botterill** [1], **John Howland** [1]

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Previous research shows ionotropic glutamate receptors in anterior retrosplenial cortex (aRSC) are critical for short-term (1-hour) object-in-place (OiP) retrieval. Here, we extend these findings using a chemogenetic approach to inhibit aRSC CaMKIIÎ±-expressing glutamatergic neurons in three recognition memory tests. Using a viral vector, we expressed inhibitory designer receptors into the aRSC of male (n=12) and female (n=12) Long Evans rats using stereotaxic surgery techniques, enabling selective inhibition of glutamatergic neurons with Compound-21 (C-21). Following surgery recovery, rats were repeatedly tested in object recognition (OR), object location (OL), and OiP tests following injection of either saline or C-21 (1.0mg/kg; i.p.) ~45-min prior to the test phase (6 tests/rat). Preliminary results suggest glutamate neuron activity in aRSC is critical for short-term recognition retrieval in all tests, but highlight a potentially nuanced influence of sex, treatment, and modality in different visuospatial recognition paradigms. Following analysis of exploration times, results indicate that female rats explore significantly less than males in both sample and test phases, but interestingly only in the OR test and this is not associated with performance. Taken together, results confirm that glutamate signalling in the aRSC plays a crucial role in short-term visuospatial recognition retrieval, however, ongoing experiments are crucial to further characterize the mechanisms and circumstances by which the aRSC supports OR, OL, and OiP recognition memory in male and female rats.

P1-F-134 - Role of early-life stress in fear memory acquisition and extinction

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Stress has been shown to induce lasting memory alterations, with specific enhancement of emotional memory. Indeed, people that have previously experienced extreme adversity during childhood, i.e. early-life stress (ELS; e.g. neglect, abuse) have a higher incidence of psychiatric disorders. Many behavioural dysfunctions associated with ELS are faithfully reflected in rodent models, including enhancement of fear memories that are resistant to extinction. Exposure therapy, a widely used form of therapy for stress disorders, shows a great variability of success, with some studies reporting up to 50% of patients as non-responders or relapsing at follow up. In order to improve this therapeutic strategy, we must first understand the biological underpinnings of stress-induced enhancement of fear memory. To do this we will use an ELS model that we have shown to have dramatic impacts on both neurons and astrocytes. We will compare the efficacy of short and long fear extinction paradigms between naÃ¯ve and ELS mice. In addition, we will characterise the contribution of astrocytes in fear extinction using a genetic approach specifically targeting these cells. We hypothesise that the ratio of extinction-resistant individuals will be higher in ELS mice, requiring a longer protocol to extinguish fear memory. As the success rates of current treatments for affective disorders remain low, we hope to shed light upon the biological underpinnings of stress-induced fear enhancement and identify novel therapeutic targets such as astrocytes.

P1-F-135 - Dopamine at D1 receptors is involved in overcoming boundary conditions for object location memory destabilization

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Consolidated long-term memories are subject to strength or content modification via reconsolidation when a reminder cue initiates reactivation of that memory trace triggering destabilization. The present study investigates the role of dopamine at D1-receptors in object location memory destabilization, with focus on overcoming boundary conditions for destabilization. Using male rats in a modified object location task, D1-receptor antagonist SCH23390 was administered systemically (0.1mg/kg) prior to reactivation of recent and relatively remote object location memories. Subsequent injection of NMDA receptor antagonist MK801 was used to impair reconsolidation. Older (remote) memories do not destabilize unless a salient novel cue is presented at reactivation to signal destabilization. SCH23390 appeared to block destabilization of recent, and relatively remote object memories with a novel floor insert at reactivation. Using the same paradigm, we administered D1-receptor agonist SKF38393 (5mg/kg; systemic) to induce destabilization of remote memories in the absence of salient novelty. The results revealed that SKF38393 promoted destabilization of the relatively remote trace, supporting a potentially crucial role for dopamine in this â€œboundary condition overrideâ€ process. Consistent with findings implicating dopamine in memory destabilization, this study shows evidence for the role of D1-receptors in destabilization of boundary condition protected-object location memories and a potentially pivotal role in adaptive modification of newly encoded and relatively remote spatial memories.

P1-F-136 - Investigating covert awareness in individuals with Alzheimer's disease using functional near infrared spectroscopy

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Alzheimerâ€™s disease (AD) is a form of dementia that impairs memory. With disease progression, AD patients reportedly experience disturbances in conscious awareness. These disturbances are linked to

poor clinical outcomes, which raises concerns about how AD patients experience the world. The present study uses a neuroimaging paradigm to â€˜mapâ€™ conscious experiences via activity changes in the fronto-parietal areas associated with higher-order processing. Studies using this paradigm reported similarity (i.e., synchrony) in internal mental experiences of behaviourally non-responsive patients and healthy controls. In this study, patients with mild-moderate AD (n = 9) and age-matched healthy controls (n = 29) watched intact- and scrambled-plot (i.e., condition that only produces synchrony in sensory regions) versions of two movies while undergoing a functional near-infrared spectroscopy (fNIRS) scan. Inter-subject correlations were used as the metric for synchrony. At the group level, controls displayed robust synchronization in the frontal and parietal areas during intact-plot versions, whereas AD patients lacked consistent synchronization. Single-subject analyses revealed that only one AD patient exhibited some synchronization with the controls. These preliminary results indicate that AD patients may be experiencing the world differently from healthy individuals, with the variability reflecting the heterogeneity in disease-related impairments. Understanding deficits and patient needs from the lens of impaired conscious processing could improve prognosis and personalized care.

P1-F-137 - A role for astrocytes in active avoidance

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Maladaptive avoidance is a hallmark of many psychiatric disorders, ranging from anxiety disorders and major depression to schizophrenia. Over the decades, research has shown that neurons of the lateral amygdala, by encoding threat memory, are crucial to the acquisition of avoidance behaviors. However, a growing body of evidence suggests that non-neuronal cells such as astrocytes might also be implicated in amygdala-dependent memory and behaviour. Here, we assess the role of astrocytes in avoidance behaviours using a combination genetic manipulation to impair astrocytic function in the lateral amygdala and 2-way active avoidance behavioural paradigm. We report that disruption of lateral amygdala astrocyte activity leads to impairments in avoidance learning dynamics, with mice exhibiting higher avoidance performance on first day of 2way AA but decreased progress throughout training. These results further support a role for astrocytes in fear-related memory and behaviours and highlight their potential as a promising target in the treatment of stress disorders.

P1-F-138 - Circuit-specific mechanisms and regulation for emotion recognition

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Understanding and interpreting subtle emotions conveyed through facial expressions is crucial for effective social communication. This process involves the interplay of bottom-up and top-down processes, primarily engaging the amygdala and prefrontal cortex (Sun et al., Translational Psychiatry, 2023). Our previous research has shown that individuals with neuropsychiatric disorders, such as schizophrenia, exhibit reduced emotional sensitivity and decision confidence, with the dmPFC-amygdala circuit being over-connected. With this in mind, we aim to develop evidence-based treatment strategies to enhance social functioning using neuromodulation, a method that selectively alters brain function.

The current HD-tDCS study aimed to explore the potential of stimulating the dmPFC to improve emotion perception and decision-making in individuals with schizophrenia. Participants received stimulation over the dlPFC (n=9), dmPFC (n=9), or sham (n=9) for five consecutive working days. The findings revealed a decrease in decision confidence in schizophrenia inpatients, but stimulating the dmPFC led to a slight increase in decision confidence compared to sham stimulation. Additionally, tDCS over the dlPFC significantly reduced response time for resolving perceptual ambiguity compared to sham stimulation. However, there were no improvements in emotion perception (i.e., sensitivity or specificity in emotional discrimination) before and after tDCS intervention for each group. These results suggest that targeting the dmPFC through neuromodulation may have modest implications for enhancing social functioning in individuals with schizophrenia by improving the ability to resolve emotional ambiguity and increasing decision confidence.

P1-F-139 - Deciphering distinct signaling networks that regulate dopamine-mediated learned and innate behaviours

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A critical function of the brain is to elicit optimal and adaptive behavioural responses needed to survive. Adaptive behaviours are based on past events (memory) or internal state (starvation-altered innate behaviour). Interestingly, in *Drosophila melanogaster*, dopaminergic (DA) signalling through a receptor, Dop1R1(R1), regulates both learned and state driven behavioural plasticity. R1 is critical for encoding memories through adenylyl cyclase (ADCY1 ortholog) driven synaptic depression, whereas R1 signals through a separate adenylyl cyclase (ADCY2 ortholog) to drive state dependent changes to innate odour preference via synaptic potentiation. Itâ€™s unclear how these R1 pathways bi-directionally regulate memory synapses. To understand R1 signaling, we used proximity labelling proteomics and RNAi

screening to identify interactors that regulate these two behaviours. By labelling in active neurons *in vivo* using a R1-Turbo-V5 fly line, we identified candidate proteins significantly enriched around the R1 receptor. Disruption of many candidates in the memory circuits altered memory and state dependent behavioural plasticity, but not both, indicating we labelled two distinct pathways. Future *in vivo* imaging will reveal if candidate interactors alter synaptic depression, potentiation, memory encoding, and downstream cAMP signalling. Our study will characterize novel pathways regulating DA signalling to illicit distinct behaviours through bi-directional control of memory synapses. This will illuminate mechanisms the brain uses to fine-tunes and adapt behaviours through one receptor.

P1-F-140 - Neuronal dynamics in Olfactory memory: In vivo calcium imaging of the Anterior Olfactory Nucleus

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The anterior olfactory nucleus (AON) serves a central role in early olfactory processing, integrating bottom-up inputs from primary olfactory structures and top-down inputs from higher-order limbic structures to modulate odor-guided behaviors. We previously demonstrated that hippocampal projections to the AON form an experimentally tractable neural circuit model of odor-context memory, highlighting the AON as a repository for odor-context engrams. However, the temporal dynamics of AON activity during odor memory processes remain unknown. In this study, we coupled *in vivo* fiber photometry with an olfactory go/no-go paradigm to analyze AON activity in Thy1-GCaMP6 mice during the development and expression of odor-context memory. We found that AON activity dynamics shift significantly when odor-context associations are necessary to receive a reward. Specifically, the AON is pre-emptively activated before odor sampling in a context-dependent go/no-go task, but not in a single-context context-independent task. This supports our hypothesis that the AON functions as an odor-context memory repository. We also discovered a robust suppression of AON activity corresponding to reward anticipation. Finally, we performed brain-wide cFos mapping to compare circuits activated by context-dependent and context-independent odor memory tasks. This study provides novel insights into the functional importance of AON circuits in odor-context memory and how the brain processes sensory elements of episodic memory, which has significant implications for the field of memory research.

P1-F-141 - Exploring the use of probiotics as treatment for major depressive disorder: neuroimaging findings from CAN-BIND-12

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In efforts to explore alternative treatment options for mood-related disturbances and symptoms of psychiatric disorders, manipulation of the gut microbiome has recently gained traction for its unique influence on mood via the gut-brain axis (GBA). Microbial-based therapeutics are now being investigated as potential treatment options for psychiatric disorders, such as major depressive disorder (MDD). To better understand the dynamic influence of the GBA on symptoms of depression, the incorporation of neuroimaging measures may serve as a tool to explore and interpret the complexity of this relationship.

Probioâ€™Stick is a daily, orally-administered, probiotic product that was assessed for its use as treatment for symptoms of depression. Individuals aged 18-65 with MDD received either Probioâ€™Stick (n=13) or placebo (n=9) for 8 weeks. Participants completed numerous clinical assessments evaluating symptoms of depression, along with multimodal neuroimaging measures such as magnetic resonance imaging (MRI), functional MRI, and diffusion tensor imaging. All preliminary analyses and results will be reported.

Given the novelty of targeting the gut microbiome within a psychiatric scope, the efficacy and feasibility of microbial-based treatments for psychiatric disorders remains uncertain. Any findings from this study will aid in distinguishing the neural mechanisms involved with probiotics and their influence on depression, while also giving insight on the future potential of gut therapeutics within psychiatry.

P1-F-143 - Representational drift of contextual fear neuronal representations across the brain

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Traditionally, it has been believed that neural representations of a learning experience must remain stable to maintain memories and support correlated behaviour (Guzowski et al., 1999; Rejimers et al., 2007). However, memories are dynamic and recent investigations have revealed that neural representations are more fluid than formerly thought. Neurons previously recruited during an

experience show variation through time, a process called representational drift (Driscoll et al., 2022). Understanding how representations drift over longer intervals is crucial to comprehending how short-term and long-term memories continue to guide future behaviour as they are reorganized in the brain through mechanisms such as systems consolidation. As perception and memory rely on a myriad of brain regions (Driscoll et al., 2022), it is essential to adopt a broad network-level approach to truly understand the stability of neural representations. Here, to characterize representational drift across the brain, FosTRAP2 mice and activity-dependent tagging are used to indelibly label activated neuronal populations during two identical contextual fear conditioning sessions, at recent and remote timepoints. Identical learning experiences were used to elicit maximum reactivation of neuronal representations. While animals showed context-specific learning behaviour, this was not reflected in neuronal activation results. Among the 11 brain regions analyzed, activation and context-specific reactivation in the retrosplenial cortex increased over time, while ventral CA1 reactivation decreased, aligning with the theory of systems consolidation.

P1-F-144 - The role of the hippocampus in anxiety: A behavioural study of early life stress events in the context of infantile amnesia.

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Background: Early life stress events (ELS), such as forms of child abuse, have enduring effects into adulthood, increasing risks of depression, anxiety disorders, and more. The paradoxical link with Infantile Amnesia, where children cannot recall events from their first three years, prompts inquiry into the hippocampus's dual role in memory formation and anxiety. This raises questions about its contribution to both learned and innate behaviours. We used a mouse model to understand how traumatic experiences heighten anxiety even when the memory of that event is forgotten.

Objectives: Our study aims to investigate the impact of ELS events on memory retention in infant mice and assess the long-term behavioural effects of ELS experiences on anxiety levels in adult mice.

Methods: Seventeen-day-old (P17) C57BL/6 infant mice underwent contextual fear conditioning training and were tested for freezing one and thirty days later to assess short- and long-term memory. Anxiety levels were evaluated one day after long-term retention in the Open Field, Elevated Plus Maze, and Light and Dark box paradigms.

Results: P17 mice exposed to ELS show short-term but not long-term memory of the ELS event. Adult mice exposed to ELS during infancy did not exhibit heightened anxiety compared to controls.

Conclusion: Despite short-term memory recall in infant mice, these memories do not have behavioural repercussions in adulthood. Contrary to our hypothesis, adult mice exposed to ELS during infancy did not show increased anxiety levels compared to controls.

P1-F-145 - Investigating top-down inhibition in chemistry learning: A neuroscience perspective

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Students commonly hold beliefs that contradict the facts of science. These misconceptions are routinely found prior to formal scientific training; however, research postulates that students retain them long after obtaining scientific knowledge and that subject matter experts actively censor misconceptions when relevant information is presented. Managing this conflict in learning is central to conceptual change and learning in the sciences. Well-documented inhibitory control structures as well as N2 and late positive potential (LPP) event-related potential (ERP) waveforms are key neural markers of misconception. This research uses neuroimaging combined with eye-tracking to study the presence and persistence of a common chemistry misconception. EEG and screen-based eye-tracking techniques were implemented. EEG analysis included N2 and LPP signal strength and latency in the pre-frontal cortex. Comparisons of oscillatory EEG alpha/theta ratio shifts among experts in congruent and non-congruent stimuli were also implemented to provide evidence for inhibitory control as more than a mechanism of attentional focus. Areas of interest and fixation/transition metrics were incorporated to compare visual behaviour between novice and expert participants. This study will provide insight into an area of chemistry cognition research that has yet to be explored outside educational settings. The existence of this misconception contributes to the current neuroscientific understanding of conceptual change for abstract concepts, such as those in chemistry.

P1-F-146 - Atypical excitatory neurons the subiculum reveal robust responses to novelty and toggle novelty seeking

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Hippocampal neural representations are typically studied from the perspective of pyramidal cells. Recent work has highlighted discrete subtypes of excitatory hippocampal neurons, suggesting that the diverse functions of the hippocampus may be driven in part by distinct subtypes of neurons.

Here, we reveal an excitatory non-pyramidal subtype that defies conventional cell-type structure and functionality flexibility of the hippocampus. This neuron subtype â€” the "ovoid" neuron â€ is spatially adjacent to subiculum pyramidal cells, yet the morphology of ovoid neurons significantly differs from other excitatory cells in the subiculum, suggestive of a specialized function. Ovoid cells show slow, sustained activity when imaged with 1-photon calcium imaging. Despite the subiculum being heavily implicated in spatial navigation, ovoid neurons show tuning to object novelty, but not spatial novelty. Specifically, novel object encounters drive ovoid neur on responses, while familiar objects fail to elicit activity even months after learning. Remarkably, when optogenetically manipulated, pyramidal and ovoid neurons play complementary roles in novelty recognition, with pyramidal cells displaying a primary role in spatial novelty and ovoid neurons playing a pivotal role in object novelty. Silencing ovoid neurons prevented object learning while activation was sufficient to toggle behaviour from novelty seeking to familiarity seeking.

These results highlight a novel cell type that defies conventional hippocampal timescales, functions, and illustrates subtype-specific feature selectivity.

P1-G-147 - Interpersonal synchrony in psychotherapy: Towards social biomarkers of therapeutic alliance

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Over 5 million Canadians aged 15 and older (18%) are diagnosed with mental health conditions, highlighting the need for effective psychiatric interventions, including psychotherapy. Current research struggles to pinpoint mechanisms driving the effectiveness of therapy, often finding similar efficacy across different types of psychotherapy. This challenge, coupled with the neuroscientific community's hesitation to study psychotherapy due to perceived measurement difficulties, underscores the need for innovative approaches.

This project adopts a novel social neuroscience approach to quantify a key factor in psychotherapy outcomes: the therapeutic alliance. Using biosensors, we aim to capture behavioral, autonomic, and neural synchrony during therapy sessions, surpassing the existing limitations of self-reports. Our proof-of-concept study will analyze cardiovascular synchrony between patient and therapist in ketamine-

assisted therapy for depression, as well as physiological and brain synchrony between family and therapist in systemic family therapy.

Our project will demonstrate the promise and feasibility of recording and analyzing multi-modal, multi-person time-series data in real-life therapy settings. This innovative methodology will pave the road to a more scientific understanding of the therapeutic process by providing a multimodal, moment-to-moment assessment of the alliance between patient and therapist. If validated, these â€˜social biomarkersâ€™ could revolutionize the study and optimization of interpersonal dynamics in psychotherapy, offering a tangible, physiological leverage point for assessing and enhancing the effectiveness of therapy.

P1-G-148 - A labeled clinical-MRI dataset of Nigerian brains

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There is currently a paucity of neuroimaging data from the African continent, limiting the diversity of data from a significant proportion of the global population. This in turn diminishes global health research and innovation. To address this issue, we present and describe the first Magnetic Resonance Imaging (MRI) dataset from individuals in the African nation of Nigeria. This dataset contains pseudonymized structural MRI (T1w, T2w, FLAIR) data of clinical quality, with 33 images from healthy control subjects, 32 images from individuals diagnosed with age-related dementia and 21 from individuals with Parkinson's Disease. Given the potential for Africa to contribute to the global neuroscience community, this unique MRI dataset represents both an opportunity and benchmark for future studies to share data from the African continent.

P1-G-149 - FARESHARE: An open-source device for measuring drinking microstructure in socially housed rats

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Aim: To develop an open-source device for tracking fluid intake in socially-housed rats.

Methods: The device uses RFID to identify rats, a lickometer to activate fluid delivery, a custom low-profile PCB that sits on top of an Arduino-based microcontroller, fluid delivery via custom peristaltic pump for accurate measurement of consumption volume, OLED display, and continuous data logging to an SD card. A validation of the design was conducted with two devices (one water and one 10% ethanol) using four female rats. Data was collected for nine days.

Results: Pump accuracy following 20 measurements of 1.00 mL was high, with an average error of 0.5% (95% CI 0.3-0.7) based on weight and remained stable across tests. Rats consumed a much greater volume of alcohol during the dark cycle ($F(1,64)=494.7$, $P<0.001$). Alcohol resulted in greater average bout size ($t(3)=3.659$, $P=0.0353$), max bout size ($t(3)=7.088$, $P=0.0058$), and volume per lick ($t(3)=5.255$, $P=0.0134$)). Importantly, the device was also able to delineate individual variation in fluid consumption.

Conclusions: Having a robust, affordable method for measuring drinking microstructure in socially housed animals will be of considerable use in preclinical addiction research and a step toward more translationally relevant animal models of fluid consumption. The added dimension of time allows for the analysis of circadian-linked consumption and the discrimination of continuous or binge-like drinking behaviours. Additionally, being open-source enables researchers to customize the device for more advanced applications such as sending signals to additional peripherals (e.g., optogenetic stimulation) or software on drinking initiation for time-locked or closed-loop interventions, manipulations, and measurements.

P1-G-150 - Effects of low intensity focused ultrasound stimulation combined with functional electrical stimulation on corticospinal excitability in Parkinson disease

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Objective: To examine the effects of combining theta burst transcranial focused ultrasound (tbTUS) of bilateral motor cortex (M1) with functional electrical stimulation (FES) of hand muscles on corticospinal excitability in individuals with Parkinson disease (PD). **Patients and Methods:** 15 individuals with PD will attend 3 visits in a random order. The visits consist of 1) Real tbTUS (120s train of 20ms ultrasound bursts, repeated every 200ms) followed by Real FES (bilateral stimulation of the opponens pollicis brevis (OP) and first dorsal interossei (FDI) muscles during execution of functional tasks involving a pinch grip), 2) Real tbTUS+ Sham FES or 3) Sham tbTUS+ Real FES. Corticospinal excitability is measured using transcranial magnetic stimulation with motor-evoked potentials (MEP) recorded from bilateral FDI, OP and abductor digiti minimi

(ADM) muscles at baseline (BL), immediately after tbTUS (T0), after 30 min of FES training (T30) and at 30 mins (T60) post FES and clinical changes are measured using MDS-UPDRS-III scores and finger accelerometry. To-date 4 participants have completed the study. **Results:** The participants showed increased MEP amplitude in the FDI and OP muscles at T30 compared to BL in both arms in all study conditions, except the OP muscle in the less affected arm which showed no change in MEP amplitude in the Real tbTUS +Sham FES condition. Changes in MDS-UPDRS-III scores were not significant. **Conclusion:** Real tbTUS, real FES and their combination resulted in an immediate increase in corticospinal excitability suggesting LTP-like changes in PD.

P1-G-151 - Development of a novel orange calcium biosensor for monitoring brain activity

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Significance Genetically encoded calcium ion (Ca^{2+}) indicators (GECIs) are powerful tools for monitoring intracellular Ca^{2+} concentration changes in living cells and model organisms. In particular, GECIs have found particular utility for monitoring the transient increase of Ca^{2+}concentration that is associated with the neuronal action potential. Despite advances, current imaging is limited by inadequate sensitivity, insufficient tissue penetration, and a lack of capability for multiplex detection.

Approach We fused a circularly permuted mOrange2 fluorescent protein to a calcium sensing (Calmodulin) and binding domain (eNOS or M13) to develop an allosteric protein, termed OCaMP. We characterize the purified protein and assess its performance *in vitro* using one-photon excitation in cultured rat hippocampal neurons, *in vivo* using one-photon excitation fiber photometry in mice, *ex vivo* using two-photon Ca^{2+} imaging in hippocampal slices, and in vivo with co-expression of a glutamate indicator, iGluSnFR3.

Results OCaMP has enhanced sensitivity (âˆ†F/F0 = 74, kd = 87nM) and improved tissue penetration due to longer wavelengths (excitation = 545nm in 1-photon; excitation = 1040nm in 2-photon). It displays an emission peak of 565 nm. Under one-photon excitation conditions, OCaMP consistently enabled detection of action potentials with higher signal-to-noise (SNR) than the existing yellow (jYCaMP1) and red (jRGECO1a, jRCaMP1a, XCaMP-R) variants.

Conclusion OCaMP is a high-performance orange GECI that, under one-photon and two-photon conditions, provides advantages relative to the state-of-the-art yellow and red calcium indicators.

P1-G-152 - Generation of functional 3D spinal cord organoids from human pluripotent stem cells

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There is no cure for motor neuron (MN) diseases and having a reliable model would be useful for understanding disease mechanisms. Spinal cord organoids (SCO) contain a variety of cell types including MNs, glial cells, and interneurons, which improve their physiological relevance. Here, we present STEMdiffâ„¢ Spinal Cord Organoid Differentiation Kit which generates SCOs from human pluripotent stem cells (hPSCs) at a high efficiency. A single-cell suspension of hPSCs was cultured for 6 days in AggreWellâ„¢800 plates containing organoid formation medium. The resulting organoids were cultured in expansion medium from days 6 - 19, followed by differentiation medium from days 19 - 43, and maintenance medium from day 43 onwards. We evaluated the cell identity at day 19 for neural progenitor cells, day 30 for MNs, and day 75 for glial cells by qPCR and immunocytochemistry (ICC). We also measured spontaneous electrophysiological currents using the Axion multielectrode array system. The SCOs expressed HOXB4 and OLIG2 on day 19, MNX1 and ISL1 on day 30 at significantly higher levels than hPSCs (fold changes relative to hPSCs of 29103, 1568, 1105, and 3059, respectively, $p < 0.0001$, $n = 11$), and contained a significant proportion of ISL1+, MNX1+, and/or CHAT+ MNs by ICC. The SCOs displayed spontaneous firing after 4 weeks in culture (0.72 Â± 0.20 Hz, weighted mean firing rate mean Â± SEM), which continued as they matured in BrainPhysâ„¢. Taken together, STEMdiffâ„¢ Spinal Cord Organoid Differentiation Kit provides a powerful tool to generate hPSC-derived SCOs for studies of human MN diseases.

P1-G-153 - The influence of geometric model selection on estimates of axon diameters in the corpus callosum of a mouse

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Previous research has linked numerous neurological disorders *post-mortem* to abnormalities in axon integrity within white matter tracts. Therefore, it is of high interest to investigate methods with the potential to infer axon diameters *in vivo*. Axon diameters can be estimated with magnetic resonance

imaging (MRI) using temporal diffusion spectroscopy (TDS). Oscillating Gradients in a Spin Echo (OGSE) pulse sequence at higher frequencies can replace the Pulsed Gradient Spin Echo (PGSE) pulse sequence to achieve shorter diffusion times and probe smaller axons, which constitute most cortical connections. The most common geometric model used to estimate axonal dimensions assumes axons are parallel cylinders and tends to overestimate intra-axonal diameters. In this project, we compare results in the genu of the corpus callosum in a mouse using cylindrical and spherical geometries. Imaging was performed using a 15.2 T Bruker BioSpec MRI System equipped with a triaxial gradient system with a maximum gradient strength of 1000 mT/m. The signals were fitted to the ActiveAx model, modelled either as cylinders or spheres. The mean effective axon diameter inferences for the signal fitting to the cylindrical model is 4 Â± 43 Î¼m, compared to 2 Â± 4 Î¼m for the spherical model. The results suggest that modelling axons using a spherical geometry as opposed to a cylindrical geometry produces smaller and less variable estimates of the diameters of smaller axons (1-2 Î¼m). Spherical models may thus be less susceptible to overestimation of intra-axonal diameters, as seen in cylindrical modelling.

P1-G-154 - A simple and high-throughput analysis pipeline to automatically quantify neurite outgrowth

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The investigation of cellular and molecular processes controlling neurite outgrowth is pivotal to understanding nervous system structure and function. However, the manual analysis of neurite outgrowth remains an exceptionally labor-intensive task that limits statistical robustness and constrains the scope of analyses. Advancements in microscopy afford the opportunity to generate high-resolution images of tens of thousands of neurons quickly; however, the potential of this data remains untapped due to the lack of efficient analysis methods.
Here, we present a pipeline for the automated and high-throughput analysis of neurite outgrowth. This system can analyze hundreds of thousands of neurons in the time it would take to quantify hundreds manually. Employing custom-made scripts in ImageJ, we process these images with minimal hands-on time investment, setting initial values empirically and applying them uniformly across the dataset. Images are constrained by quality control checks for the pipeline to isolate, skeletonize, trace, and perform complexity analyses. This allows for the identification of overall changes in morphology, such as branching behavior, radius of neuronal domains, and correlations to size and intensity of secondary markers.
Crucially, our approach minimizes manual labour, provides access to files for verification, and limits bias in a tool for efficient, reproducible, and quality-centric neurite outgrowth analysis. We anticipate that this tool will be a valuable approach to discern changes in neurite outgrowth following genetic or pharmacological manipulations.

P1-G-155 - Direct measurement of free glucocorticoids in mouse serum using ultrafiltration and LC-MS/MS

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In mice and rats, corticosterone (CORT) is a steroid hormone secreted during stress that has profound impacts on the brain. Circulating CORT binds to corticosteroid-binding globulin and albumin, and only unbound (free) CORT enters the brain. Current methods to measure free CORT levels are time-consuming and require high sample volumes (250+ Âµl serum). We developed a rapid method to quantify free CORT in small volumes of mouse serum using commercially available ultrafiltration columns and liquid chromatography-tandem mass spectrometry (LC-MS/MS). We also measured free 11-deoxycorticosterone (DOC, CORT precursor) and free 11-dehydrocorticosterone (DHC, CORT metabolite). We used 30, 60, 90, 120, and 150 ÂµL of pooled C57BL/6J mouse serum (n=5/volume) in ultrafiltration columns with a 30 kDa molecular weight cut-off cellulose membrane. We quantified DOC, CORT, and DHC in filtrate using liquid-liquid extraction and LC-MS/MS. We will use this method to measure free CORT in serum from mice administered the endotoxin lipopolysaccharide (LPS) (50 Î¼g/kg, i.p.) or saline (n=10/group/sex). This method allows measurement of free DOC, CORT, and DHC in 30-150 ÂµL of mouse serum with high precision, estimating free CORT to be 1% of total CORT in controls. Preliminary results show an increase of free to total CORT ratio in LPS mice compared to controls, matching previous reports. We show that ultrafiltration columns can be used to measure free steroids in as little as 30 ÂµL of serum. This method can be applied to correlate free steroid levels with changes in brain and behaviour.

P1-G-156 - Transcriptomic insights: Fine-tuning cellular responses in biomaterials for enhanced nerve regeneration

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The peripheral nervous system is capable of regenerating injured axons and rebuilding functional connections. This unique potential relies on Schwann cells (SC) that support axon regeneration. However, axon growth is a slow process limited to a few millimeters in humans. To ensure a functional nerve recovery, nerve guide conduits (NGC) are used as a potential alternative to bridge cut nerves and promote

axonal regeneration. We developed a novel composite NGC featuring interconnected microchannels. Our overarching goal is to assess its potential for enhancing nerve regeneration by evaluating the impact of NGC composition and coating on cellular behavior and identity. To do so, we used human SC and induced pluripotent stem cells (iPSC) and cultured them in a 3D biomaterial microenvironment. We evaluated cell engraftment via immunohistochemistry and immunofluorescence assays on biomaterial cuts. Cell survival and proliferation were evaluated with viability-cytotoxicity differential staining. An RNA-seq analysis was performed to determine the changes in cell identity and behavior within the biomaterial and identify a transcriptomic shift following cell contact with the NGC. Our findings give insights into the biological mechanisms by which cells sense and interact with their surrounding mechanical environment. Our results highlight how physical stimulation plays an important role in regulating cell behavior and will help develop effective solutions for peripheral nerve injuries.

P1-G-157 - Modeling firing rate dynamics of Thalamic-DBS network and model-based closed-loop DBS control

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Thalamic ventral intermediate nucleus (Vim) is the primary surgical target of deep brain stimulation (DBS) for reducing symptoms of essential tremor. Yet, the neural mechanisms underlying Vim-DBS induced firing rate dynamics are not fully uncovered. We developed a model of the firing rate dynamics of Vim-network in response to different frequencies of DBS. Based on the Vim-network model, we further developed a closed-loop control system to automatically update the DBS frequency.
We have access to single-unit recordings of Vim neurons during Vim-DBS in human. DBS pulses are fed into a synapse model of short-term synaptic plasticity to generate inputs to Vim neurons. The Vim-network model consists of recurrent connections among three neural groups: DBS-targeted Vim neurons, external excitatory nuclei and inhibitory nuclei. We then develop a macroscopic model connecting DBS, Vim, motor cortex, motor neurons in the spinal cord, and muscle fibers to generate muscle activities, which are represented by EMG. This macroscopic model is used to predict EMG response to DBS pulses, and the model-predicted EMG is used in a closed-loop controller to provide feedback information for updating the DBS frequency.
Our model can accurately track the instantaneous firing rate of Vim neurons in response to varying Vim-DBS frequencies (5 to 200Hz). Our closed-loop control system can automatically update the appropriate DBS frequency so that EMG power reaches a desired target, and can be potentially implemented in and out of the clinic to automatically update the appropriate DBS frequency.

P1-G-158 - Novel standardized social interaction arena for determination of brain circuits mediating social behavior in mice

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Intro:

Methods for studying brain circuits involved in mice social interaction are either difficult to quantify and standardize, or are forced. We create a standardized, homecage social interaction arena (**SIA**) for long-term fiberphotometry recordings.

Method:

We create 3 distinct sections in a homecage setup with partitions. Mice are placed in either the left-most or right-most section. Both can enter a middle section (SIA) for water (~3Î¼L/entry). Male (n=4) and female (n=4) C57BL/6 mice were water-restricted for 3d before individual training (15mins) to learn where water is dispensed and paired (35mins) training, where mice were placed in opposing sides and rewarded for any SIA entry. Variables recorded include entry time, licks, duration of stay in the SIA and video recordings of the SIA. Fiberphotometry recordings were performed in a subset of mice (n=6) for up to 8h.

Results:

Preliminary data show that mice learn to voluntarily enter the SIA within 1d. Males have more entries for individual tasks. An average of 67.5 social interactions (avg=6.97s; males), or whisker to whisker interactions, were made in paired tasks. No behavioral differences were seen in prolonged fiberphotometry recordings.

Conclusion:

The homecage setup encourages social interaction in freely-moving mice, illustrating its feasibility for studying social interaction. Next steps include extending to a cooperative task that rewards only synchronized entries, and integrating long-term fiberphotometry recordings where rewards can be dependent on brain activity across subjects and within particular brain regions.

P1-G-159 - TBISeq: A spatial transcriptomics atlas of the mouse brain following traumatic brain injury

Aditya Swaro [1], **Brianna Bristow** [1], **Anqi (Angela) Zhang** [1], **Larissa Kraus** [1], **Sarah Erwin** [1], **Mehwish Anwer** [1], **Cheryl Wellington** [1], **Mark Cembrowski** [1]

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Individuals that receive a Traumatic Brain Injury (TBI) can suffer from a wide range of debilitating physical and mental symptoms. However, little is known about how TBI mechanistically changes brain structure and function. Using the Closed Head Impact Model of Engineered Rotational Acceleration (CHIMERA) model of impact-acceleration injury, we aimed to identify spatio-temporal molecular changes in diffusely injured mouse brain. Using Visium, spatial transcriptomic information with strong replicate reproducibility for 55-Âµm circular regions tiling entire brain sections (2 sections/mouse; 4992 sequenced regions/section; ~40,000 RNA-seq datasets) was acquired. Within an initial dataset of >1B reads, we identified cell clusters with dysregulated genes previously identified in TBI as well as novel differentially expressed genes. To facilitate these data being used by the community in a computationally efficient and easy-to-use manner, we built the TBISeq web portal. TBISeq facilitates data use by computing complex machine learning analysis in the backend, and displaying friendly low-dimensional analysis and visualizations at the user interface. Using TBISeq, researchers can quickly explore how their favorite gene, brain region, or cell type is differentially expressed following CHIMERA TBI in mice. We believe that spatially enriched transcriptomics data and our highly efficient webapp, TBISeq, will accelerate research into TBIs' molecular dysregulation mechanisms, and set the groundwork for research into targets for therapeutic treatment.

P1-G-160 - Automated prognostication via machine learning in neonates with hypoxic-ischemic encephalopathy

John Lewis [1], **Mehmet Cizmeci** [1], **Helen Branson** [1], **Michelle Stoopler** [1], **Ashley Danguecan** [1], **Atiyeh Miran** [1], **Krishna Raghu** [1], **Linh Ly** [1], **Brian Kalish** [2]

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Neonatal hypoxic-ischemic encephalopathy (HIE) is a serious neurologic condition that occurs due to inadequate cerebral oxygen delivery around the time of birth. HIE is a common cause of neonatal death and neurodevelopmental morbidity worldwide. Magnetic resonance imaging (MRI) is routinely used for neuroprognostication, but there is substantial subjectivity and uncertainty about neurodevelopmental

outcome prediction. Therefore, we sought to develop an objective and automated approach for analysis of newborn brain MRI to improve the accuracy of prognostication. We first created an anatomical brain MRI template from a large sample of infants with HIE, and labelled the amygdala, hippocampus, caudate, putamen, globus pallidus, and thalamus in both hemispheres. We extracted quantitative information, including information represented by complex patterns (radiomic measures), from each of these structures in all infants. We trained an elastic net model to use these measures, together with clinical and demographic data, to predict which infants will survive, and which will show motor, language, or cognitive impairment, as measured by Bayley-III scores at 18-24 months. We found sets of measures that predict survival with an area under the curve of over 0.89, and Bayley-III scores with correlations ranging from 0.45 to 0.66, explaining between 18% and 43% of the variance in the scores. We make these tools available to the neuroimaging community, but note that external cohort validation is required in order to demonstrate the validity, generalizability, and utility of this approach.

P1-G-162 - SLAP2: Random access Kilohertz two photon imaging of neuronal activity In vivo

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Understanding how a neuronâ€™s synaptic topography and dendritic structure dictate its computational encoding requires simultaneously measuring all activity across the dendritic arbor. In development, this topography is highly dynamic and constantly shifting, changing a neuron's computational efficiency and encoding properties as it wires into a network. How these inputs are refined during development is not fully understood, and current imaging technologies lack the speed or resolution to image the neuronal activity in vivo to answer these questions. To achieve the measurements needed to observe these dynamic input-output relationships between synaptic and somatic activity, I constructed a Scanned Line Angular Projection 2 Microscope (SLAP2). This custom two-photon microscope combines projection microscopy and high-speed scanning paired with a spatial light modulator to achieve 10,000 Hz imaging. I use this microscope to measure visually evoked activity in the neurons in the optic tectum of albino Xenopus tadpoles during early development to understand how neurons encode visual information and how these properties change over time.

P1-H-163 - Brain death criteria for Nepal

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Human Organ donation and transplantation is one of the greatest medical breakthroughs of this century and it is very challenging in Nepal due to cultural beliefs, legal frameworks, and ethical considerations. The Human Body Organ Transplantation (Regulation and Prohibition) Act (HBOTA) has not met with substantial success after its amendment. This review critically appraises the current state of brain death and organ transplantation in Nepal. In the present context of Nepal, kidney is extracted only from live donors. Further, once people start accepting brain death, it will make avail different organs eg. 1. kidneys, 2 lungs, 3 livers, 4 heart, 5 pancreas and 6 small intestines which will be useful to save so many lives after transplantation of these organs. It explores challenges, evaluates progress, and provides recommendations. Literature review of databases was conducted to find articles on brain death, organ donation, and transplantation in Nepal. Analysis of cultural, legal, ethical, and practical factors influencing implementation. Key challenges include limited awareness, religious beliefs, infrastructure gaps, and family consent barriers. HBOTA amendments in 2016 enabled brain death donations, however, donation rates remain low. Strategies are needed to improve public education, resources, personnel training, and collaboration. Cultural sensitivity and stakeholder engagement are crucial. A multifaceted approach addressing cultural, legal, ethical and practical dimensions is essential to improve organ donation rates in Nepal. Despite progress, substantial challenges persist requiring evidence-based strategies focused on awareness, capacity building, policy improvements, and culturally appropriate community engagement.

P1-H-164 - Neuroscience outreach programmes to secondary school girls and community in Karu, Nasarawa state, Nigeria

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Scientific outreaches (SOs) have been a central theme in scientific societies (SS) and organizations. Many SS and governmental organizations are pushing for the public understanding of science, encouraging and promoting citizens' participation in science. IBRO has been at the forefront of these activities, through its programmes like IBRO/Dana Brain Awareness Week (BAW) and the IBRO Global Engagement Seed Grant (GESG). The authors of this abstract have been engaged in SOs and public awareness campaigns since 2010. Since 2017 we have conducted SOs in Karu, Nasarawa State, Nigeria through our non-profit organization STEM4GirlsNigeria with the support of individuals, organizations and IBRO. Support from

IBRO has helped us to conduct BAW in 2020 for girls from junior and senior secondary schools. The major reason is to encourage young girls to pursue careers in neuroscience. The GESG was conducted in three phases, a radio programme to educate the populace on the science of the brain, a brain healthcare outreach for the community, and an introduction of neuroscience to secondary school teachers who were gifted a brain model to their schools. In the next project, we plan to introduce neuroeducation to primary and secondary schools, to educate the teachers particularly so that they will apply this knowledge to improve the teaching and learning experience, especially for students with disability, this is presently missing. The SOs conducted have been impactful on the attendees as reported in the programme evaluation questionnaires.

P1-H-165 - Critical ethical considerations in patenting neurotechnology

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Patents describe the intended products of innovation and, once granted, give entrepreneurs exclusive commercial rights to their inventions. The number of neuromodulation and neuroimaging patent filings have increased year after year, indicating a worldwide surge in neurotechnology commercialization (Roskams-Edris et al., *Nat Biotech.*, 2017, **35**:19). Patents are crucial in areas such as neurotechnology, where devices and techniques may be reverse engineered or duplicated. However, exclusivity may also introduce roadblocks to healthcare access. Although theoretical frameworks have been proposed to guide responsible and beneficial innovation, few have drawn upon empirical ethical analyses of patent publications themselves (Roskams-Edris et al., *Neuromod.,* 2019, **22**:398). In response, we describe a novel method for characterizing neurotechnology content in the patent record and a rigorous approach to analysing ethical considerations raised by the patent specifications. The search of USTPO patents from 2016 â€“ 2021 returned 779 patents for characterization, and ethics analysis yielded 9 case studies for deep examination. Results validate the method and highlight scientific validity, mental privacy, and mental integrity as critical ethics principles for innovators to incorporate as they enter and progress through the neurotechnology commercialization pipeline.

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Poster Session 02

P2-A-166 - Investigating early-life development of the connections between the prefrontal cortex and the claustrum

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The prefrontal cortex (PFC) is a high-order cortical region that regulates cognitive functions, such as novelty detection. The PFC forms strong reciprocal connections with the claustrum (CLA), a small nucleus in the forebrain that shares overlapping novelty functions, suggesting that PFC interaction with the CLA is involved in cognition. PFC-dependent cognitive abilities emerge during infancy when the brain undergoes immense molecular and structural changes. However, early-life establishment of PFC communication with the CLA is poorly understood, and the role of PFC-CLA networks in cognition remains elusive. To investigate this, we performed *in vivo* electrophysiological recording of CLA neurons. We found that stimulation of PFC input to the CLA excites CLA principal neurons up to postnatal day (P) 12 while generating inhibition during adolescence (P20-P60), suggesting a developmental shift in CLA neuronal activity from excitatory to inhibitory patterns during the third postnatal week (P14-P21). Previous work indicates that in the CLA, parvalbumin-expressing (PV) interneurons potently modulate the response of principal neurons to cortical input. We found that PV interneurons are absent in the CLA until P14, but their numbers reach adult levels at P21. Hence, our data suggests that maturation of PV interneurons coordinates PFC-CLA network transition from newborn-like to adolescent-like patterns between P14-P21. Future studies will examine the effect of occluding PV interneurons in the CLA ~P14 on PFC-CLA wiring, and whether this may result in potential cognitive deficits.

P2-A-167 - Developmental axonal swellings depend on action potentials and calcium signalling

Bruna de Souza [1], **Amy Smith-Dijak** [1], **Alanna Watt** [1]

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Axonal development and the establishment of contacts between neurons are crucial for the proper formation of functional brain circuits. We have demonstrated that axonal swellings appear transiently on cerebellar Purkinje cell axons during postnatal development where they propagate action potentials with higher fidelity, thus enhancing cerebellar function. Therefore, an understanding of how axonal swellings form is important. We first investigated the role of action potentials in the formation of axonal swellings. We performed 2-photon time-lapse imaging in acute cerebellar slices from juvenile mice (P10 - P15) and added sub-saturating levels of tetrodotoxin (TTX; 1, 2.5, 5 and 10 nM) to parametrically impair action

potentials in spontaneously active Purkinje cells. We found that low concentrations of TTX that block action potentials to a lower degree resulted in an enhancement in the rate of formation and the number of axonal swellings. This supports a model in which action potential failures trigger axonal swelling formation, where low failure levels are the optimal signal. Next, we aimed to investigate potential mechanisms involved in the formation of the swellings. We observed that the formation of swellings was impaired in the absence of extracellular Ca^{2+} and in the presence of Nifedipine (100uM), a L-type voltage-gated Ca^{2+} channel blocker. Our results indicate that both action potential activity and extracellular Ca^{2+} influx is required for the formation of axonal swellings during postnatal cerebellar development.

P2-A-168 - Neural synchrony as a mechanism for broader attention in childhood?

Justine Vorvis [1], **Donald Mabbott** [2], **Amy Finn** [1], **Katherine Duncan** [1], **Julie Tseng** [2]

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The ability to selectively attend to "targets" and ignore "distracters" develops slowly into early adulthood, and recent work suggests that this may allow children to attend more broadly and learn more about distracters. But what are the brain mechanisms underpinning broader attention in childhood? Could children's broader attention arise from the more equal propagation of target and distracter information through neural synchrony? To answer this question, we are using magnetoencephalography to measure brain activity in 6- to 30-year-old participants while they selectively attend to either objects or faces in an *n*-back task. We expect adults to show greater neural synchrony (measured as the weighted phase lag index) between the object-selective lateral occipital complex (LOC) and other regions in the ventral stream when the target category is objects as compared to faces, and greater synchrony between the face-selective fusiform face area (FFA) and other regions in the ventral stream when the target category is faces as compared to objects. In children, we expect neural synchrony levels to be less influenced by the target category. Preliminary analyses (n = 7 children and youth) on a subset of connections (the LOC and FFA with the hippocampi and V1) found no effect of target category in either the low or high gamma bands after false discovery rate correction. If this pattern is observed in the full child sample, then less selective neural synchrony patterns may be one way in which the developing brain equips children to process the multitude of information available to them.

P2-A-169 - Behavioural and neuronal consequences of prenatal Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) exposure persist into adulthood

Marieka Devuono [1], **Mina Nashed** [2], **Mohammed Sarikahya** [1], **Erynn Lonnee** [1], **Andrea Kocsis** [1], **Kendrick Lee** [1], **Sebastian Vanin** [1], **Daniel Hardy** [1], **Steven Laviolette** [1]

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Cannabis is widely used during pregnancy and is often perceived as safe; however, prenatal cannabinoid exposure (PCE) is associated with long-term neuropsychiatric consequences, such as cognitive impairments and increased vulnerability to mood disorders. Investigation into the underlying mechanisms of these effects and the impact of different cannabis constituents is needed to understand the potential risks better. Thus, we explored the long-term neuropsychiatric outcomes in male and female offspring of pregnant Wistar rats given daily injections (ip) of Î”9-tetrahydrocannabinol (THC), cannabidiol (CBD), or a combination (THC+CBD) during gestation. At adulthood, offspring underwent behavioural testing to assess changes in cognition, anxiety, and depressive-like behaviours. Then, *in vivo* electrophysiology was used to determine neuronal alterations in the prefrontal cortex (PFC) and ventral hippocampus (vHipp), brain regions whose development is necessary for appropriate cognitive and affective functioning. Results were analysed by sex. Males prenatally exposed to THC and THC+CBD displayed anxiety and depressive-like behaviours, respectfully. Additionally, THC and THC+CBD males displayed impaired object recognition and spatial working memory. Preliminary electrophysiology analysis suggests excitatory/inhibitory imbalances in PFC and vHIPP of male PCE offspring which may underlie the long-term behavioural effects. Conversely, no significant behavioural abnormalities were observed in female PCE offspring, suggesting males may be more susceptible to the protracted effects of PCE.

P2-A-170 - Exposure to HIV antiretroviral therapy during pregnancy leads to hippocampal long-term memory deficits and hyperactivity

Shreya Dhume [1], **Kayode Balogun** [2], **Ambalika Sarkar** [1], **Sebastian Acosta** [3], **Howard Mount** [3], **John Sled** [4], **Lena Serghides** [1]

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Treatment of HIV using antiretrovirals (ARVs) has been pivotal in reducing vertical transmission of HIV from mother to child. Changes in perinatal neurodevelopment can have long-term effects on behavior and cognition in adults. Thus, we hypothesize that early developmental impairments in the brain caused by in-utero exposure to ARVs can manifest as cognitive and motor delays in adulthood. We assessed the long-term effects on neurodevelopment after exposure to ARVs during gestation in a mouse model. Dams were treated with a combination of protease inhibitors with either abacavir-lamivudine (ABC/3TC+ATV/r) or tenofovir-emtricitabine (TDF/FTC+ATV/r). 6-week-old mice were assessed with a battery of behavioral tests that investigated motor performance and cognition. We studied changes in

brain structure and function using magnetic resonance imaging, immunohistochemistry and qPCR. In utero exposure to ARVs showed enhanced motor performance, exploratory drive, delayed working memory, and hippocampal-dependent memory deficits. ABC/3TC+ATV/r exposed mice showed significant volumetric changes in the brain and reduced neuronal counts in the hippocampus. Both treatment groups showed altered expression of neurotransmitter receptors and neurotrophic factor, BDNF and its receptors. Our results show that neurological impairments differ based on the type of drug present in the regimen and display sex differences within treatments. Our findings suggest that in-utero exposure to ARVs can have long-term effects on brain development that lead to cognitive and motor impairments in adulthood.

P2-A-171 - Neocortical cell fate transitions in the early post-natal brain at single-cell resolution

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While neocortical embryonic neurogenesis has been well studied, early postnatal cell fate transitions, including gliogenesis, remain under studied. During the late embryonic/early postnatal (LE/EP) period, ventricular zone (VZ) radial glial progenitor (RGP) cells end production of cortical layer excitatory neurons, and switch to producing glia which populate the neocortex. Moreover, during this same period RGPs are tasked with producing several other cells including adult neural stem cells, ependyma and olfactory bulb interneurons. As a result, fate regulation during the LE/EP period is critical for normal brain development and function with disruptions to these processes contributing to neurodevelopmental disorders (NDDs). To better understand LE/EP fate transitions we used several plasmid-based clonal lineage tracing methods to examine progenitor/progeny relationships in combination with high-throughput single-cell RNA-sequencing. These techniques were further paired with high-resolution spatial transcriptomic and imaging strategies to place RGPs and their progeny in their *in situ* spatial context at times up to post-natal day 22. Using these datasets, we can draw several conclusions about the function of LE/EP RGPs including that individual RGPs are multipotent, capable of producing both glia and neurons, and that RGP fate selection occurs post-mitotically in progeny cells. Through these approaches we shed light on the dynamics of the VZ LE/EP environment and provide further insight into the mechanisms governing LE/EP fate specification which may offer insight into NDDs.

P2-A-172 - Maternal sleep disruption alters neurodevelopmental programs in the embryonic hypothalamus

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Neurodevelopmental disorders are on the rise worldwideâ€”yet the etiology behind this increase is not well understood. Recent scientific advancements show a strong association between prenatal maternal challenge and neurodevelopmental disorders, suggesting that the intrauterine environment is critically sensitive to early life disruptions. Interestingly, a common feature reported across different early life adversity animal models is disruption of the immune system, suggesting that microgliaâ€”the resident immune cells within the developing fetal brainâ€”may sense changes in the intrauterine environment and respond by altering neurodevelopment. To examine the underlying mechanism(s) linking prenatal maternal disruptions to changes in neurodevelopment, we focused our attention on hypothalamic microglia, given their responsiveness to environmental cues. Moreover, our prior analyses showed that E11.5 to E15.5 is a sensitive window for neurodevelopmental disruptions in the hypothalamus; therefore, we used three separate maternal sleep disruption paradigms (i.e., 24 hours of light or dark exposure or a shift in the light cycle) that spanned this time frame. Although total microglia numbers were unchanged, the number of microglia that localized to the caudal domain of the tuberal hypothalamus significantly increased in female embryos collected from the maternal sleep disruption paradigms. Similarly, maternal sleep disruption resulted in a significant elevation in the secretion of G-CSF, CXCL1, and CXCL2, but only in females. Together, these data suggest that maternal sleep disruption may alter microglial signalling in the developing hypothalamus.

P2-A-173 - Developmental trajectory of α-Synuclein-positive neurons in the mouse cerebellar nuclei

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Introduction: Understanding the development of cerebellar nuclei (CN) has the potential to answer various questions when we study neurodevelopmental disorders. Conventionally, glutamatergic excitatory and GABAergic inhibitory CN neurons originate, respectively, from the rhombic lip and ventricular zone post-embryonic day 9 (E9). Our study reveals a novel cell subset in the nuclear transitory zone (NTZ) appearing in NTZ before E9. Our objective is to study the fate of these neurons in the mouse cerebellar nuclei.

Methods: We employed a combination of EdU-based birth-dating analysis, fluorescence-activated cell sorting (FACS), cell fractionation assay, immunofluorescence labeling, and confocal imaging to investigate the developmental trajectory of the early subset of neurons in the NTZ of the SncaGFP transgenic mouse.

Results: First, the early subset of cells in NTZ exhibit SNCA expression that emerge as early as E8.75, most probably originates from the mesencephalon. Second, the colocalization of SNCA in these cells with neuronal and glutamatergic markers hints at their nature as excitatory neurons. Last, the cytosolic localization of SNCA in these neurons points to its potential role in signal transduction, cytoskeletal organization, and trafficking.

Conclusion: In conclusion, our study suggests that early neurons in the CN express SNCA and emerge as early as E8.75. Unraveling the origin and developmental timeline of these early neurons could significantly contribute to advancing our understanding of neurodevelopmental disorders such as autism spectrum disorder (ASD).

P2-A-174 - Parental high-fat/high-sugar diets alter offspring brain development

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Maternal metabolic conditions such as obesity have been linked to an increased offspring risk for neurodevelopmental disorders (NDDs). As diet is closely linked to obesity, we examined the effects of parental high-fat/high-sugar diet consumption on offspring neurodevelopment using longitudinal brain magnetic resonance imaging (MRI) to assess morphology. Cohorts of five-week-old C57Bl/6J mice were fed either control diet (CD), high-fat diet (HFD), or â€œWesternâ€ diet (WD; containing high-fat and high-sugar) for an 8-week acclimation period and subsequently during breeding, gestation, and lactation. All offspring were fed CD post weaning. Male and female offspring were imaged at 8 timepoints from birth to adulthood using MRI, and volumes were extracted to compare across diet groups and offspring sex. Male and female offspring of WD and HFD groups showed altered brain growth patterns compared to CD group over the time span. Alterations that persisted into adulthood included relative volume increases in several cortical structures including the secondary motor cortex (+2.8% WD, +4.4% HFD), and relative volume decreases in subcortical structures such as the hippocampal CA3 subregion (-3.4% WD, -3.9% HFD) and striatum (-2.2% WD, -1.8% HFD), with $q<0.1$ for all structures. In conclusion, parental high-fat/high-sugar diet consumption leads to early and long-term brain structure changes in offspring brain. These data establish an animal model that can be used to identify underlying mechanisms to test preventative/amelioration measures that could have implications for NDDs in humans.

P2-A-175 - Establishing primary fibroblast cell lines from Ursus maritimus to model neural development in vitro

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Polar bears (PB) are highly intelligent, having one of the largest brain-to-body ratios of any land mammal. Understanding the fundamental processes that make the PB brain unique at a cellular and molecular level is important to the future preservation of this species and also offers insights that could lead to medical breakthroughs for humans.
Despite being banned for decades, non-dioxin-like polychlorinated biphenyls (PCBs) - persistent organic pollutants - are still accumulating in the Arctic ecosystem. In humans, PCB exposure, especially during pregnancy, is linked to developmental issues in children, such as memory defects, cognitive dysfunctions, and sensory and motor disorders.
This research focuses on developing PB neuronal cell cultures to study the effects of PCBs on neural development. We have successfully established primary fibroblast cell lines from polar bear skin samples collected in Churchill, Manitoba. These fibroblast cells have been maintained for over 20 passages, and their genetic and structural integrity has been confirmed through DNA fingerprinting and karyotypic analysis, respectively. Furthermore, we have managed to convert these fibroblasts into neurons using a chemical induction process, a transformation validated by immunostaining techniques.
This research program represents important impacts on phylogenetics, conservation, and evolutionary brain research, potentially creating a paradigm shift in how we study and preserve endangered species.

P2-A-176 - Investigating the functions of F-actin regulators in synapse formation in C. elegans

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Branched filamentous actin (F-actin) is a scaffold enriched at presynaptic specializations in mammals, Drosophila and *C. elegans*. Branched F-actin assembly is mediated by a series of proteins, including the actin-nucleating Arp2/3 complex and its regulators, the WASP and WAVE family proteins, and Coronins.

Mutations and dysregulation of branched F-actin regulators are implicated in neurological disorders that exhibit abnormal synaptic functions, such as Alzheimer's disease and Down syndrome. Despite its presynaptic enrichment, the roles of branched F-actin and its regulators at synapses remain elusive because loss of function mutants are embryonic lethal. To overcome the issues with lethality, we use the auxin-inducible degron (AID) system in *C. elegans*. The AID system allows for the degradation of AID-tagged proteins specifically in the nervous system. Using the AID system, we found that neuron-specific degradation of ARX-2/Arp2, a subunit of the Arp2/3 complex, and WVE-1/WAVE resulted in a significant decrease in synapse number compared to wild type, suggesting both ARX-2 and WVE-1 are necessary for synapse formation. Currently, we are investigating the role of ARX-2 and WVE-1 in synapse maintenance and investigating the roles of other branched F-actin regulators in synapse formation and maintenance.

P2-A-177 - Identification of photosensitive cells controlling circadian responses over development in the pineal complex

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In vertebrates, melatonin secretion, orchestrated by the pineal gland, is promoted during the night and blocked by the light of the day. While mammals rely on ocular opsins (i.e. melanopsin) to sense light, non-mammalian vertebrates, such as *Xenopus laevis*, utilize the pineal complex (PC) located on the top of their head as a photosensitive "third" eye. To investigate the role of melatonin and photosensors in the development of the brain, we use *Xenopus laevis* as an excellent experimentally amenable model. *Xenopus laevis* tadpoles can dynamically adjust their skin color in response to light in a manner independent of eye photosensitivity. By using skin color as a readout, we were provided a unique avenue to investigate PC and the development of the PC melatonin-secretion pathway in terms of photosensitivite, melatonin-producing, and projection cells. We determined the neural circuits that control melatonin secretion, with a particular focus on identifying opsin proteins, by using *in situ* hybridization and immunohistochemistry techniques. We find Pinopsin, expressed by pineal photoreceptors, regulates the circadian variation of skin pigmentation. Pinopsin CRISPR F0 mutant tadpoles exhibited paler skin during the light phase as compared to wild type, but displayed comparable skin lightening during the dark phase, suggesting the mutants are unable to synchronize with the environmental light. Our focus now is on PC neural circuit differences in the PC of wild type vs. mutants, opening new insight into understanding the regulation of circadian variation.
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P2-A-178 - Effects of oxygen supplementation on the brain development of mouse fetuses with hypoplastic left heart syndrome

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Hypoplastic Left Heart Syndrome (HLHS), one of the most severe forms of Congenital Heart Disease (CHD), is associated with abnormal fetal brain maturation and postnatal neurodevelopmental deficits. Evidence from Magnetic Resonance Imaging (MRI) studies associates reduced fetal brain volume with reduced cerebral oxygen delivery and consumption. Maternal oxygen supplementation has the potential to raise fetal blood oxygen saturation, increase fetal cerebral oxygenation, and ameliorate abnormal brain growth in the CHD human population. We tested the effect of this intervention on brain development in a recently developed mouse of HLHS.

A surgically induced, cardiac specific HLHS mouse model was created by injecting an embolizing agent on embryonic day 14.5 into the fetal left atrium to block blood flow through the left heart and induce its hypoplasia. Immediately following the HLHS surgeries, pregnant dams carrying these HLHS fetuses were exposed to 75% O_2 (hyperoxia) for four days. Term fetuses (day 18.5) were collected and imaged ex vivo by three-dimensional T2-weighted MRI.

This preliminary study showed no whole brain volume difference between HLHS (n = 13) and sham controls (n=11) under normoxia conditions. In contrast, hyperoxia exposed HLHS fetuses (n=24) had on average 6.3% smaller brain volumes ($p < 0.05$) than normoxia-exposed sham fetuses (n = 11). Regional Brain volume comparisons are in progress to elucidate these findings and further evaluate the safety and feasibility of maternal hyperoxygenation to improve CHD fetal neurodevelopmental outcomes.

P2-A-179 - Maternal high-sucrose consumption disrupts steroid levels in the mother, placenta, and fetus in rats

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Consumption of sucrose (table sugar) is high around the world. The effects of maternal sucrose intake on the placenta and fetal brain remain unknown. In rats, maternal consumption of sucrose at a human-relevant level (26% kcal from sucrose) alters the motherâ€™s brain, metabolism and steroids, as well as the adult offspringâ€™s brain and behaviour in a sex-specific manner. Maternal sucrose intake increases corticosterone levels in adult female offspring and increases motivation for sugar rewards in adult male offspring.

Here, we examined how a maternal high-sucrose diet in rats affects steroids in the dam, placenta, and fetus at embryonic day 19.5. Maternal sucrose intake reduced placenta mass but not fetal weight. Maternal sucrose intake increased maternal and fetal glucocorticoid levels. 11-deoxycorticosterone (DOC) and 11-dehydrocorticosterone (DHC) were increased in maternal serum by sucrose consumption. DHC was also increased in amniotic fluid by maternal high-sucrose intake. The mineralocorticoid aldosterone tended to be increased in maternal serum due to sucrose and was significantly increased in fetal blood, amniotic fluid, and specific brain regions (nucleus accumbens). In addition, maternal sucrose intake decreased levels of androgens (androstenedione and testosterone) in the placenta and specific fetal brain regions (nucleus accumbens). In summary, we found dramatic changes in maternal, placental, and fetal steroids that might mediate the long-term effects of maternal sucrose consumption on adult offspring neuroendocrinology and behaviour.

P2-A-180 - Identifying extrinsic factors to enable human hypothalamic organoid culturing

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Abnormal development of the hypothalamus leads to severe neurodevelopmental diseases such as Prader-Willi syndrome (PWS). Discovery into disease etiology and treatments for PWS are lacking due to a paucity of human models. Currently, we are unable to grow PWS patient-derived brain organoids because little is known about the culturing conditions needed to form the various regions of the hypothalamus. First, we plan to use iPSCs from a healthy female individual to develop the induction medias that drive hypothalamic development in vitro. To start, I used dual SMAD inhibitors with different WNT signaling reagents (inhibition, activation, or both) in the first induction medium to pre-pattern iPSCs to neuroectodermal and potentially hypothalamus fate. Next, I added other morphogens, such as WNTs and SHH to the second media to drive hypothalamic lineages broadly. Quantitative PCR (qPCR) analyses on these organoids at day 60 showed expression of markers for anterior and posterior regions of the hypothalamus. I found that hypothalamus markers were better expressed in the organoids first exposed to WNT inhibitors, suggesting WNT plays an important role in determining hypothalamic fate. Next steps are to test other candidate morphogens involved in hypothalamic development such as BMP7 and TGFÃŸ to be able to generate more region-specific hypothalamus organoids. Over the long term, my goal is to generate organoids from iPSCs of patients with PWS to study the disease.

P2-A-181 - A missense mutation in BIRC6 causes dwarfism in mice

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Our lab identified a mouse line with a small size phenotype in a dominant N-ethyl-N-nitrosourea (ENU) mutagenesis screen for suppressors of the phenotype in methyl-CpG binding protein 2 (*Mecp2*) mice, a valid model for Rett syndrome. Sequence analysis found a missense mutation in a highly conserved arginine in Baculoviral inhibitor of apoptosis repeat-containing 6 (*Birc6)*, a member of the inhibitor of apoptosis protein (IAP) family. BIRC6 plays a role in inhibiting apoptosis, but has been implicated in additional molecular pathways including autophagy, cell cycle regulation and the DNA damage response. Homozygotes for a null allele die at birth due to placental failure, yet heterozygotes for a null allele are normal in size. The causative nature of the lesion was confirmed using CRISPR/Cas genome editing to engineer an identical allele, which also has a dominant small size phenotype. *Birc6*$^{Sum20\text{-}Jus}$/+ mice are smaller than littermates from mid-gestation through adulthood. At three months, the mice are approximately 50% of the size of their wild type littermates, exhibiting proportional dwarfism with pathological changes in bone, thyroid and pituitary. IGF1, but not growth hormone (GH) is decreased, and the mice have primary hypothyroidism (high TSH, normal T4). Tandem mass tag (TMT) spectrometry quantification of proteins in the brain links BIRC6 mutation to perturbations in cell cycle and metabolic pathways. Together, our data suggest that mutations in BIRC6 cause a dominant small stature phenotype due to signalling anomalies that perturb the endocrine pathway. An association between *Birc6* and dwarfism has not been previously reported; therefore, this work identifies a new model of proportional dwarfism and a new function for BIRC6. This finding reinforces the link between the neurological condition Rett syndrome and metabolic perturbations.

P2-A-182 - Floor plate derived netrin-1 is an instructive long-range guidance cue for commissural axons in the embryonic spinal cord

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The distribution and functional significance of long-range axon guidance cues remain controversial. Netrin-1 is essential for commissural axon extension to the ventral midline of the neural tube. It remains

unclear, however, to what extent netrin-1 protein functions as a long-range or short-range cue in the embryonic spinal cord, nor has it been determined if netrin-1 protein has an instructive function *in vivo* that directs axon migration, or is merely permissive and required for axon extension. Here we address how netrin-1 protein is distributed in the developing spinal cord and how that distribution influences axon guidance. In early embryonic chick spinal cord only floor plate cells express *NTN1*, yet the distribution of netrin-1 protein extends ~200 Î¼m dorsal of the floor plate, exemplary of a long-range cue. In the embryonic mouse spinal cord *NTN1* is expressed by cells in the floor plate and ventral ventricular zone. We show that selective deletion of *NTN1* from floor plate cells in mouse flattened the gradient of netrin-1 protein within ~200 Î¼m of the midline and altered commissural axon trajectories across the same distance. In gain-of-function studies, we demonstrate that ectopic expression to change the distribution of netrin-1 in the embryonic spinal cord is sufficient to redirect commissural axons. These findings reveal that netrin-1 secreted by floor plate cells is distributed as a long-range cue in embryonic chick and mouse spinal cord, and demonstrate that the distribution of netrin-1 protein instructs the direction of commissural axon extension.

P2-A-183 - Magnetic resonance imaging of brain myelination in the developing common marmoset

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The common marmoset (Callithrix jacchus) is a promising non-human primate preclinical model to study neurodevelopmental disorders and social behaviors. As a relatively recent model, baseline studies on neurotypical subjects still need to be carried out to understand this species. For example, magnetic resonance imaging (MRI) and histology have been used to map brain myelination, showing a high myelin content in primary sensory areas and in the extrastriate visual areas. However, little is known about brain myelination during the developmental stages of this species. In this cross-sectional study, we scanned 13 in-vivo anesthetized marmosets ranging from early infancy to adulthood at 6 time-points (4-month-old (mo), 5mo, 7mo, 11mo, 18mo and adulthood) on a 3 Tesla Siemens Prisma-Fit MRI scanner. We acquired T1- and T2-weighted images, allowing us to compute the T1w/T2w ratio which is correlated with cortical myelin content in the healthy brain. At 4mo, the T1w/T2w ratio is higher for sensory areas than for prefrontal areas, responsible for high order executive functions, suggesting a lower myelin content in the latter areas. This aligns with the spatio-temporal myelination patterns observed in primates during the critical period. Our T1w/T2w imaging identified early development patterns of the marmosetâ€™s brain that likely correspond to a steep myelination trajectory. It is a first step toward the implementation of more precise methods that I will develop to investigate how disease models, such as autism spectrum disorders, impact this trajectory during the critical period.

P2-B-184 - Sex differences in Astrocyte-Neuron dynamics in chronic neuropathic pain in the Anterior Cingulate cortex

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Chronic pain impacts 25% of Canadians aged fifteen and above, especially marginalized groups, with women making up 67% of those affected. Despite this, pre-clinical research has predominantly prioritized male rodent models, leaving a knowledge gap regarding female chronic pain mechanisms. Mounting evidence indicates neuroplastic changes within the anterior cingulate cortex (ACC) as pivotal in chronic pain development. Responding to neuronal activity, the astrocyte-neuronal lactate shuttling (ANLS) can rapidly provide lactate to neurons, meeting metabolic demands required for neuroplasticity. However, its role in chronic pain-induced neuroplasticity remains unknown.
This study investigates ANLS in the ACC of female and male mice, exploring its involvement in chronic neuropathic pain development. Using the spared nerve injury (SNI) model in adult female and male C57BL/6 mice, we assessed gene expression in the ACC of ANLS pathways at 5, 14, 30, and 60 days post-surgery using RT-qPCR. We also confirmed mechanical allodynia for each timepoint using the Von Frey Test.
Despite similar patterns of SNI-induced pain hypersensitivity in both sexes, we found that long-term SNI increased ANLS-related gene expression in the ACC of male but not female mice. We thus conclude that neuropathic pain affects ANLS in the mouse ACC in a sexually dimorphic manner. Furthermore, these sex differences highlight the need to include both females and males in research on molecular targets for chronic pain treatment, deepening our knowledge of pain chronification.

P2-B-185 - Cellular and ultrastructural correlates of Cisplatin neurotoxicity

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Cisplatin is a chemotherapeutic agent commonly used to treat solid tumors. Besides its kidney toxicity, it has neurotoxic side effects that include peripheral neuropathy, cognitive impairment, and fatigue, commonly known as â€˜chemo-brainâ€™. We hypothesized that cisplatin induces cellular stress and

metabolic alterations detectible at the (ultra)structural level in the brain, specifically on neurons and resident macrophages known as microglia. Adult male mice were exposed to one or two 5-day cycles of cisplatin treatment (2.83 mg/kg/day, n=4 per treatment) that induced fatigue, measured by reductions in wheel running activity and body weight loss. 2D and 3D scanning electron microscopy was used to investigate the ultrastructural impact of cisplatin on microglia and neurons in the striatum, a region hypothesized to be vulnerable to cisplatin with functional implications in fatigue symptomatology. Immunohistochemical analysis of IBA1+ cells revealed that cisplatin increases density in the striatum in a dose-dependent manner. Microglia displayed moderately increased markers of cellular stress and notably decreased total and healthy endoplasmic reticulum. Two cycles of cisplatin also altered microglial interactions with synaptic elements. While currently undergoing analysis, cisplatin is hypothesized to impact morphological characteristics of IBA1+ cells, decrease dendritic spine density, and alter mitochondrial ultrastructure in the striatum. The development of interventions to prevent microglial reactivity and metabolic balance may help alleviate the neurotoxicity of cisplatin.

P2-B-186 - The role of thioredoxin-interacting protein in corticosterone-induced damage in astrocytes

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Chronic treatment with corticosterone (CORT) can induce oxidative stress and neuroinflammation. Thioredoxin-interacting protein (Txnip) inhibits antioxidant protein thioredoxin (Trx), interrupting protein thiol reduction and inducing oxidative stress. Txnip can also bind to NLRP3, facilitating NLRP3 inflammasome forming, activating caspase-1 and releasing IL-1Î². Our lab found that chronic CORT treatment increased Txnip levels in cultured mouse neurons and microglia. Astrocytes can release proinflammatory cytokines and reactive oxygen species, promoting inflammation and neuronal damage. The present study is to determine whether CORT treatment regulates Txnip and promotes oxidative stress and inflammation in astrocytes. Although treatment with CORT for 24 hours had no effect on Trx levels, this treatment increased Txnip levels in primary cultured mouse astrocytes. We also found that CORT treatment decreased Trx activity in astrocytes, but had no effect on total reduced thiol levels, protein sulfenylation and protein carbonylation, suggesting that CORT-increased Txnip may not be enough to cause oxidative damage due to high concentrations of antioxidants in astrocytes. We found that although CORT treatment had no effect on NLRP3 levels, this treatment increased Txnip/NLRP3 binding in astrocytes. We also found that CORT treatment increased caspase-1 activity and IL-1Î² release in astrocytes, suggesting that CORT treatment increases Txnip level in astrocytes which could promote NLRP3 activity, activate caspase-1 and release IL-1Î² to induce neuroinflammation.

P2-B-187 - Investigating the roles of dark/Clec7a+ microglia during early postnatal development in a mouse model of maternal immune activation

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Microglia, resident brain immune cells, play critical roles during development and in many neurodevelopmental disorders. Maternal immune activation (MIA) is any inflammatory response that can alter fetal neurodevelopment, placing offspring at increased risk of neurodevelopmental disorders. A microglial subtype of specific interest for this project is dark microglia (DM) which are different from other microglia when viewed with electron microscopy and are suggested to play a key role in vascular and synaptic remodeling. DM are abundant during normal brain development but rare in healthy adults, and they increase in number with environmental challenges. We will be looking at the hippocampus, where DM are abundant, as this region contributes to many cognitive functions altered in neurodevelopmental disorders. To test this, C57BL/6J female mice were injected with polyinosinic:polycytidylic acid 9.5 days into pregnancy to induce MIA. We examined male and female offspring at different time points during early postnatal development. We will quantify microglia expressing Clec7a (potential DM marker), comparing healthy and MIA exposed offspring by performing a double immunohistochemistry for Iba1 and Clec7a to be analyzed by brightfield microscopy and scanning electron microscopy (Celc7a staining only). This will allow to us to quantify the density, distribution, and ultrastructural features of DM/Clec7a+ cells and pave the way to determining the role of microglial Clec7a during neurodevelopment and following MIA, potentially leading to identifying a future therapeutic target.

P2-B-188 - Astrocyte dysfunction mediates cognitive impairment induced by early life stress

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Stress experienced during childhood is a robust risk factor for psychiatric disorder development in adulthood. Using a rodent model of early life stress (ELS), we have shown alterations in Lateral Amygdala

(LA) dependent fear memory with increased fear generalisation in an auditory discriminative fear conditioning (ADFC) task. We identified astrocyte dysfunction as a key mediator of stress-induced fear generalisation.

Recent evidence suggests that lasting memories are encoded in sparse ensembles of neurons known as engrams. Here we set out to determine a relationship between astrocyte function and neuronal fear engram allocation using the activity marker c-fos. **We hypothesize that c-fos staining in LA will be more widespread after fear conditioning as a result of stress during infancy.** Our preliminary data shows that ELS indeed results in increased c-fos staining in the LA, which aligns with fear generalisation following ADFC.

To test the implication of astrocytes in engram allocation, we first virally targeted astrocyte network function by overexpressing a dominant negative connexin 43 (dnCx43) and second, we impaired astrocyte calcium signalling with a calcium extruder pump (CalEx), directly into the LA. Following ADFC, we observed fear generalisation that was associated with larger c-fos staining in both dnCx43 and CalEx mice. These results suggest that astrocytes fine tune the process of engram allocation during learning and that their dysfunction impacts memory formation by increasing the number of cells allocated to a fear engram.

P2-B-189 - Revealing sex-dependent differences in cytokine release in butyrate-treated microglia cultures

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Microglia, immune cells of the central nervous system, respond to brain inflammation by releasing cytokines such as interleukin 6 (IL6), tumor necrosis factor (TNF), interleukin 1 beta (IL1Î²) (pro-inflammatory cytokines), and interleukin 10 (IL10) (anti-inflammatory). In gut dysbiosis, inflammation can increase pro-inflammatory cytokines systemically, leading to microglial immune responses in the brain. Short-chain carboxylic acids like butyrate have been investigated as signaling factors connecting gut dysbiosis and inflammation. Elevated butyrate has been reported to decrease microglial pro-inflammatory cytokine release and reduce depression-like behavior in mice. Female humans have higher rates of depression and anxiety than males. Since sex differences in microglial function are understudied, we investigated cytokine release in response to butyrate treatment in microglia cultured from postnatal day 2 female and male mouse pups. We hypothesized that microglia derived from females would show greater release of proinflammatory cytokines. Microglia expressing green fluorescent protein were cultured for 14 days and isolated with dilute trypsin in DMEM/F12. The microglia were treated with butyrate (40uM, 200uM, 1000uM) and 100 ng/ml of lipopolysaccharide or interferon-gamma for 24 hours. Then cell culture media were collected for cytokine analysis. Researchers were blinded to sex groupings to prevent bias. Two-way ANOVA demonstrated

statistically significant differences between the sexes in the release of TNF, IL6, IL1Î², and IL10. These results highlight the importance of studying sex differences on inflammation in the gut-brain axis.

P2-B-190 - The impacts of prenatal delta-9-tetrahydrocannabinol exposure on phagocytic activity of microglia and their involvement with neural remodeling in the developing mouse hippocampus

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The increasing accessibility of cannabis has heightened the need to study its neurological impacts. This is particularly true regarding use during pregnancy, as the effects of the compounds in cannabis, such as delta-9-tetrahydrocannabinol (THC), on prenatally developing offspring are not well understood. Microglia are immune cells involved in neural development through key roles like eliminating neural precursor cells (NPCs) and pruning synapses. Microglial activity can also be altered by THC. We aim to examine changes in microglial function after prenatal THC exposure in both embryos and neonates, with a focus on changes in neural remodeling resulting from phagocytic activity. We will look at the hippocampus, which demonstrates altered neural connectivity and microglial function in response to prenatal stressors. To test this, pregnant female C57BL/6J mice were given subcutaneous daily injections of THC (5 mg/kg) or vehicle during embryonic day (E)3-10. Pups were collected at E17 or postnatal day 14. Samples will be stained for immunohistochemistry with markers of microglia (P2RY12 in embryos, IBA1 in neonates) and phagocytic activity (CD68). The NPC marker Tbr2 will be examined in embryos, and co-labeling with P2RY12 positive(+)/CD68+ cells will allow for the measurement of microglial phagocytosis of NPCs. The postsynaptic density marker PSD-95 will be assessed in neonates, and co-labeling with IBA1+CD68+ cells will indicate microglial synaptic pruning. We hope to elucidate the effects of prenatal cannabis use on neural development through altered microglial function.

P2-B-191 - NMDA receptors in the neocortex: A tale of two compartments

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Widely understood to play a key role in Hebbian coincidence detection, the postsynaptic NMDAR (postNMDAR) has become a major focus in neuroscience research. However, the contribution of presynaptic NMDAR (preNMDAR) signalling in synaptic release and plasticity is less clear.

Quantitative immunogold electron microscopy in P21 C57BL/6 mice showed GluN1, GluN2A and GluN2B could be detected both pre- and postsynaptically at primary visual cortex (V1) layer 5 (L5) excitatory synapses. We then developed a sparse genetic NMDAR deletion approach using neonatal injections of AAV-eSYN-iCre into V1 L5 of $NR1^{flox/flox}$ mice to perform quadruple patch in P10-19 acute slices, recording from pyramidal cell (PC)â†'PC pairs with either pre- or postNMDAR deletion (pre- vs. postKO).

MNI-NMDA uncaging confirmed successful NMDAR removal (postKO: 0.3 pA Â± 0.4 pA, n=16 vs. control -26.7 pA Â± 4.7 pA, n=28, p<0.001). Next, we showed that AP5 did not alter EPSP amplitude or paired-pulse ratio in preKO PCâ†'PC pairs (EPSP 118% Â± 12%, n = 6; Î"PPR 0.04 Â± 0.13, n = 5) unlike postKO or controls (pooled EPSP 49% Â± 8%, n = 10, p < 0.001; Î"PPR 0.82 Â± 0.26, n = 9, p < 0.05), suggesting that pre- but not postNMDARs regulate release. Similarly, tLTD was abolished in preKO or pre- and postKO pairs (97% Â± 2%, n = 8) but not in postKO pairs or control (77% Â± 3%, n = 13, p < 0.001). In contrast, tLTP might rely more on postNMDARs (postKO: 117% Â± 6%, n = 5; control: 175% Â± 33%, n =3, p = 0.2). We also saw a reduction in L5 axonal and dendritic length in cells carrying the deletion (axon: p < 0.05; dendrite: p < 0.005).

In conclusion, we find that pre- and postNMDARs have distinct synaptic functions, suggesting the current textbook view of NMDARS needs revision.

P2-B-192 - Adaptation of magnified analysis of the Proteome for Excitatory Synaptic proteins in varied samples and evaluation of cell type-specific distributions

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Visualizing molecular diversity at individual synapses within intact brain circuits presents a significant challenge. Expansion microscopy provides a promising approach: proteins are anchored in situ to a gel which is isotropically expanded to add physical magnification to the optical magnification of

microscopes. However, its use to study synapse-related questions outside of the labs developing the techniques has been limited.

Here we independently adapted a version of Magnified Analysis of the Proteome (MAP; Ku et al., 2016. Nat. Biotechnol. 34:873-81). We present a step-by-step protocol for non-specialists for visualizing over 40 synaptic proteins in brain circuits.

Surprisingly, our findings show that the advantage of MAP over conventional immunolabeling is primarily due to improved antigen recognition, and secondarily physical expansion. Application of MAP to brains perfused with paraformaldehyde or fresh-fixed with formalin, and to formalin-fixed paraffin-embedded tissue, expands its potential to combinations with slice electrophysiology or clinical pathology specimens. Applying MAP to mice expressing YFP-ChR2 exclusively in interneurons, quantitative single synapse analyses revealed a distinct composition of AMPA and NMDA receptors and Shank family members at synapses on hippocampal interneurons versus on pyramidal neurons.

These findings exemplify the value of the versatile adapted MAP procedure presented here as an accessible tool for the broad neuroscience community to unravel the complexity of the â€˜synaptomeâ€™ across brain circuits and disease states.

P2-B-193 - The diversified astrocyte developmental programs are modulated by primary ciliary signaling

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Astrocyte diversity is greatly influenced by local environmental modulation. Here, we report that the vast majority of brain astrocytes across the entire brain possess a singular primary cilium, a specialized signaling antenna localized to cell soma. Comparative single-cell transcriptomics reveals that primary cilia mediate canonical Shh signaling to modulate astrocyte subtype-specific core features in synaptic regulation, intracellular transport, energy and metabolism. Independent of canonical Shh signaling, primary cilia are important regulators for astrocyte morphology and intracellular signaling balance. Dendritic spine analysis and transcriptomics reveal that perturbation of astrocytic cilia leads to disruption of neuronal development and global intercellular connectomes in the brain. Ultimately, mice with primary ciliary deficient astrocytes show behavioral deficits in sensorimotor function, sociability, learning and memory. Our results uncover a critical role for primary cilia in transmitting local cues that drive the region-specific diversification of astrocytes within the developing brain.

P2-B-194 - Transcriptional and spatial characterization of distinct layer-associated populations of mesenchymal cells in the mouse cortical meninges

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Introduction: The meninges provide a protective multilayer barrier surrounding the brain and spinal cord and serve as an interface structure to the brain for blood vessels, nerves, peripheral immune cells, etc. While it is known that the meninges are comprised of mesenchymal cells (MCs) which form distinct layers (the dura, arachnoid, and pia mater), the cellular identity and composition are still under investigation.

Methods: Using single-cell RNA sequencing, spatial transcriptomics, and microscopy we investigated the cellular gene and protein signatures to identify the different MC types. We also investigated border-associated astrocyte communication with the leptomeningeal cells using spatial transcriptomics.

Results: The MCs of the dura versus the leptomeninges had distinct gene signatures, with the former expressing Clec11a and Matn4, and the latter Gjb2, Coch, Slc22a6 and Lama1. Leptomeningeal cells more highly expressed anion transport and gap-junction genes, while dural cells expressed high levels of ECM and ossification genes. Both cell types differ from other mesenchymal cells such as vascular-associated mesenchymal cells and pericytes and these mesenchymal cell differences were conserved spatially in our Xenium data. In addition, the border astrocytes were identifiable in our spatial transcriptomics and microscopy data and displayed unique transcriptional signatures and morphology. Using our transcriptional data (both single-cell and spatial), we also discovered preliminary ligand-receptor interactions between the border astrocyte populations and leptomeningeal cells.

Conclusion: Our work demonstrates the heterogeneity of the mesenchymal cells of the meninges and provides foundational information for our continuing studies on how these cell populations contribute to homeostasis and brain injury.

P2-B-195 - Uncovering the role of primary cilia in astrocyte reactivity following adolescent mild traumatic brain injury

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Concussions, caused by forces to the head resulting in transient disturbances in brain function, can lead to long-term, severe neurobehavioral deficits. In Canada, due to frequent involvement in contact sports, adolescents are especially prone to repetitive concussions, which can interrupt healing and induce cumulative damage in the brain. In response to insult/injury, astrocytes are activated in a process termed reactive astrogliosis to protect neurons and promote healing. However, when prolonged/dysregulated, astrogliosis induces secondary damage. Currently, the molecular regulation of astrogliosis is poorly understood. Recently, we found that primary cilia, antenna-like sensory signalling organelles, are involved in astrogliosis regulation. Specifically, using an adolescent mouse model with inducible, astrocyte-specific cilia dysfunction, we will model concussions and (1) characterize how impaired astrocytic cilia impact behavioural outcomes and recovery post-concussion and (2) define how cilia modulate astrocyte reactivity to concussions. Our preliminary results suggest that impaired ciliary function results in reduced astrocyte reactivity following concussion in adolescent mouse brains. This study will reveal a novel function of primary cilia in regulating astrogliosis and enhance mechanistic understandings of astrocyte reactivity following brain injury.

P2-B-196 - Age-dependent induction of immune response in a zebrafish model of neurodevelopmental disorders

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Maternal immune activation, and ensuing neuroinflammation, is implicated in the etiology of neurodevelopmental disorders such as autism spectrum disorder and schizophrenia. Neuroinflammation experienced at later points in life has different functional consequences, which suggests the existence of one or more critical periods for neurodevelopmental susceptibility to inflammation.
A previous study by Solek et al. (2021) noted a significant effect of bath application of lipopolysaccharide (LPS) on both microglial morphology and cytokine profile in 3 days post fertilization (dpf) zebrafish. LPS treatment caused a rapid elevation in proinflammatory cytokine mRNA levels and led microglia to adopt a more rounded ameboid morphology. Inflammation induced during a brief 2-hour period on 3dpf affected the subsequent morphological maturation of retinal ganglion cell axon arbors in the optic tectum and had long-term functional consequences on the visual acuity of animals. Interestingly, the cytokine profile was the most upregulated in the skin of the animals, suggesting an environmental upregulation of inflammation can lead to functional consequences in neural development.
Extending these results, we have studied the consequences of externally induced inflammation at later stages of development. Bath application of LPS in older zebrafish led to a significantly altered response, with cytokine levels and microglial morphology unlike in the 3 dpf animals. The results suggest a possible critical period where environmental influences can affect the inflammatory profile of the animal with potential functional consequences in the nervous system.

P2-B-197 - Specialized influence of acetylcholine on cellular activity in the deep subiculum

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Acetylcholine (ACh) plays an instrumental role in modulating hippocampal network gain. Canonically, during periods of high ACh release, ACh mediated enhancement of intrahippocampal synapses favors hippocampal dependent encoding of new information, while hippocampal output to downstream structures is decreased. The subiculum (SUB), the major output region of the hippocampus, therefore, sits at a critical site for controlling such network activity. Transcriptomic work from our laboratory has illustrated the SUB contains a diverse set of excitatory neuron subtypes, including classical pyramidal neurons (â€œclassical cellsâ€) and a unique group of cells that occupy the deepest layer of the SUB (â€œdeep cellsâ€).

To investigate cell type specific differences in response to AChR activation, we performed whole cell patch clamp electrophysiology in acute ex vivo slices. Upon chronic application of carbachol (CCh), a nicotinic and muscarinic ACh receptor agonist, we interestingly found deep cells: (1) immediately elevated and (2) sustained a depolarized membrane potential in contrast to neighboring classical neurons. Transient application of CCh again resulted in a more sustained depolarization in deep cells. Next, we chronically applied a specific nicotinic ACh receptor agonist and found deep cells displayed similar responses as CCh. Then, to observe ACh mediated modulation of upstream glutamatergic input we expressed channelrhodopsin into upstream brain regions and measured changes to input strength before and during application of CCh. We found deep and classical cells had differential responses and modulation to each brain region.

In total, our results illustrate a SUB cell type that increases its output during periods of high ACh receptor activation. With this, deep cells may comprise a specialized circuit node for ACh-mediated memory encoding.

P2-B-198 - Optogenetic investigation of endogenous serotonin transmission in mouse prefrontal cortex: Acute impact and consequences of chronic perturbation

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The medial prefrontal cortex is essential for cognition, executive function, and emotional behaviour. Serotonergic afferents densely innervate the prefrontal cortex and pharmacological interventions targeting the serotonin system influence cognitive and emotional processing. While exogenous serotonin and related agonists regulate neuronal activity in this region, the neurophysiological consequences of synaptically-released endogenous serotonin have only begun to be characterized. Here, we use optogenetics and whole cell recording in brain slices from transgenic mice to probe the impact of endogenous serotonergic transmission in medial prefrontal cortex. First, we probe the frequency dependence of endogenous synaptic serotonin signalling in the major output neurons in prefrontal cortex. Next, we examine the sensitivity of these responses to desensitization. Then, we intervene pharmacologically to explore the interplay of serotonergic 5-HT_{1A} receptor-mediated inhibition and 5-HT_{2A} receptor-mediated disinhibition. Finally, we test the impact of acute and chronic SSRI treatment. Optogenetics gives us a critical opportunity to test the acute impact of endogenous serotonin in prefrontal cortex and its response to chronic perturbation.

P2-B-199 - Age Matters: Examining the hippocampal-based synaptic struggle following repetitive mild traumatic brain injury

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Adolescence represents a period of significant growth and maturation where adverse environmental experiences have the ability to shape and alter long-term development. Mild traumatic brain injuries (mTBI) are a significant health issue worldwide and adolescence represents a period of heightened risk for these injuries. Particularly, exposure to repeated forms of mTBI (R-mTBI) is major health concern in the pediatric patients and has the potential to have long-lasting consequences. The present study administered repeated injuries to female and male early adolescent (P30) and young adult (P70) animals using the lateral impact model. Animals received three mTBI or sham injuries spaced 72-hours apart in order to determine the influence of age at injury on bi-directional synaptic plasticity in the dentate gyrus. On post-injury days 1-6 (PID1-6) animals underwent a behavioural test battery to assess motor deficits, anxiety-like behaviour, and cognitive functioning. On PID7, hippocampal slices were prepared for *in vitro* electrophysiological recordings, and the capacity for long-term depression (LTD), as well as long-term potentiation (LTP) was examined in the dentate gyrus. Our results suggest that the capacity for LTP synaptic plasticity is impaired with exposure to r-mTBI and these effects are dependent on age and sex. These findings have important implications for understanding the long-term consequences of r-mTBI in adolescents and young adults, and may inform future interventions to mitigate cognitive deficits.

P2-B-200 - Functional analysis of APL-1/amyloid precursor protein (APP) signaling in C. elegans

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Dementia affects more than 55 million people, and Alzheimerâ€™s disease (AD) accounts for most cases. Accumulation of amyloid beta (Ab) peptide, a cleavage product of Amyloid Precursor Protein (APP), in the nervous system is a leading hypothesis for the cause of AD. However, most anti-Ab drugs have failed in clinical trials. Mutations in APP are linked to AD, highlighting the need to understand the normal physiological roles of APP. APP intracellular domain (AICD), another cleavage product of APP, translocates into the nucleus to control gene expression, like Notch signaling. However, the activity of APP signaling and its function in the nervous system are unclear. We investigate the role of APP signaling in the nervous system using Caenorhabditis elegans, which has a sole APP ortholog, APL-1.
To monitor the APL-1 signaling activity in vivo, we adapted a genetically encoded biosensor for detecting Notch signaling and observed high APL-1/APP signaling activity in neurons. Consistently, we observe that APL-1 mutants lacking the AICD exhibit defective neurotransmission. Preliminary observations show that the localization of synaptic vesicle proteins is perturbed in GABAergic motor neurons in APL-1 mutants, which may underlie the neurotransmission defect. Currently, we are investigating the cell types in which APL-1/APP functions to control neurotransmission.

P2-B-201 - Investigating the role of the cAMP signalling pathway in time-sensitive habituation mechanisms

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The brain is very sensitive to the timing of the stimuli it receives. The timing of stimulus also influences habituation, the simplest form of learning. Habituation is a learned decrement in an animalâ€™s response to repeated stimulus presentations. When stimuli are repeated at a high frequency with shorter time intervals between stimulus presentations, animals habituate faster and to a deeper extent than when longer time intervals induce the decrement. Previous mutant studies in *Caenorhabditis elegans* have provided evidence that multiple mechanisms may be underlying habituation at short (10s) or long (60s) time intervals, as some mutant animals have altered habituation phenotypes specifically at

10s or 60s intervals. *acy-1*, a homolog of adenylyl cyclase 9, is the main enzyme that catalyzes cAMP synthesis in *C. elegans*. Because *acy-1* mutants exhibit a shallower habituation phenotype than wildtype worms at short 10s intervals and habituated like wildtype animals at 60s intervals, we initially hypothesized that this gene may function in a habituation process that acts only at short intervals. However, after running an experiment at an even longer 150s interval, surprisingly, the acy-1 mutant animals showed deeper habituation than wildtype worms. The opposing effects of this mutation on habituation at 10s and 150s intervals suggest that cAMP plays a more complicated role in habituation at different time intervals. Therefore, we also investigated other candidate genes in the cAMP pathway to elucidate how this signaling pathway impacts habituation at different time intervals.

P2-B-202 - Unmasking Diaschisis: AAV-mediated transsynaptic labeling sheds light on Dendritic spine alterations after stroke

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The brain is highly interconnected, and this is crucial to understanding a wide variety of neurological diseases. In instances such as ischemic stroke, the brain connectome can be used to explain deficits observed in both the peri-infarct and distally connected regions to the stroke site. This phenomenon is referred to as â€˜diaschisisâ€™. Currently, there is a lack of understanding of the structural changes that occur at the neuronal level in both proximal and distal regions from the infarct. In this study we used 2-photon laser scanning microscopy to examine both acute and long-term changes in dendritic spine densities downstream of a photothrombotic stroke induced in the forelimb primary somatosensory cortex (FLS1). To do this, adult mice were injected with an trans-synaptic anterograde adeno-associated virus (AAV.Syn.Cre) which labelled postsynaptic neurons by activating the TdTomato and GCaMP6s fluorescent proteins allowing for visualization of neurons directly linked to the infarct site. Initial findings show that dendritic spine density is significantly decreased in the peri-infarct region and primary motor cortex after stroke as compared to controls. These findings suggest that the structural changes that occur in neurons after stroke may not only be localized to the peri-infarct region, but also to regions heavily reliant on those neurons. In conclusion, this data suggests that changes in dendritic spine densities could play a part in the global dysfunctions observed after stroke and may provide valuable information for therapeutic interventions in the future.

P2-B-203 - Potential role for the secreted glycoprotein MFG-E8 in astrocyte-mediated phagocytosis of neurons

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Dysregulated synapse elimination is a hallmark of Alzheimerâ€™s disease (AD). Microglia, the brainâ€™s resident professional phagocytes, have been the focus in this field for upwards of a decade. However, astrocytes are also capable of phagocytosing synapses and participate actively in developmental synaptic pruning. Interestingly, astrocytes express the opsonin milk fat globule epidermal growth factor-like factor 8 (MFG-E8), along with its receptor integrin Î±VÎ²5. MFG-E8 is known to mediate tissue reorganization, being a key player in mammary gland involution. Additionally, integrin Î±VÎ²5 can crosstalk with the phagocytic receptor MERTK which has been shown to initiate astrocytic phagocytosis of synapses.

MFG-E8 has recently been shown to be overexpressed in AD, and to increase the engulfment of synaptic material by astrocytes and microglia. Yet, the mechanism through and conditions in which this occurs is not fully elucidated. This work aims to explore the function of MFG-E8 regarding astrocytic phagocytosis of synaptic material in particular, as astrocytes express MFG-E8 at higher levels than microglia and are well placed for synaptic monitoring. An assay was developed to measure phagocytosis of apoptotic neuronal model cells SH-SY5Y by the astrocyte model cell line U138-MG in the presence of MFG-E8. The effects of MFG-E8 concentration, AD-like biochemical conditions, and phagocytic receptor blockers on the phagocytic capacity of U-138MG cells was measured, illuminating the result of interactions between MFG-E8 and integrin Î±VÎ²5 in astrocyte-mediated phagocytosis of neurons.

P2-B-204 - Opioids differentially regulate transmission from subclasses of prefrontal GABAergic interneurons

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Opioids regulate circuits associated with motivation and reward across the brain. In prefrontal cortex (PFC), opioid receptor activation suppresses GABA release, but the mechanisms by which opioids regulate release are unclear. Here we describe differential modulation of PFC GABAergic inputs by presynaptic delta opioid receptors (DORs), which are thought to act primarily through GÎ±i-signaling pathways. A DOR agonist, DPDPE, suppressed inhibitory currents on layer 5 pyramidal neurons, but had variable effects on short-term synaptic plasticity (STP), suggesting heterogeneity in the mechanisms of DOR regulation of transmitter release. Optogenetic targeting of parvalbumin- (PV+) and somatostatin-expressing (SOM+) subpopulations revealed that DPDPE suppressed release from both types, but with notable differences. All PV+, but only a subset of SOM+, neurons exhibited canonical presynaptic depression accompanied by an increase in short-term facilitation, whereas the remaining SOM+ responses were suppressed without

changing STP. This latter form of regulation persisted in the presence of the GÎ±i antagonist pertussis toxin and was instead mediated by a PKA-dependent pathway. 2-photon imaging of presynaptic calcium in boutons showed that both forms of presynaptic regulation suppressed action potential-evoked calcium influx, suggesting that calcium channels are targets for both forms of DOR-dependent regulation. Thus, these results demonstrate that the same opioid receptor can regulate inhibitory synapses via multiple mechanisms, even within the same cortical microcircuit.

P2-B-205 - Characterization of α7 and α4β2 nicotinic cholinergic responses in layer1 of medial prefrontal cortical neurons

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The medial prefrontal cortex (mPFC) is a brain region responsible for a variety of cognitive functions including attention and working memory. Cholinergic neurons, which release acetylcholine (ACh), are known to enhance attention and their pathophysiology is associated with disorders such as Alzheimerâ€™s disease and epilepsy. Nicotinic acetylcholine receptors (nAChRs) are activated by the cholinergic system and modulate neuronal excitability. Therefore, understanding nAChR mediated synaptic neurotransmission will allow us to better understand how the activity of neurons are precisely controlled.

Using whole cell recordings of layer 1 neurons of mouse mPFC and optogenetic stimulation of ACh release resulted in two nAChR mediated currents -- one having a rapid rise and decay kinetics and sensitive to inhibition by the Î±7 nAChR antagonist MLA. The second nAChR current was long lasting and inhibited by the Î±4Î²2 nAChR antagonist DHÎ²E. The Î±4Î²2 current was significantly inhibited by the calcium chelator EGTA-AM, while there was no effect on Î±7 currents. This suggests that ACh release eliciting Î±7 nAChR responses is mediated by tight coupling of the presynaptic calcium source and the calcium sensor, while that of Î±4Î²2 responses is a more distant coupling. Following stimulation of ACh release, there were delayed asynchronous mEPSC responses. DHÎ²E eliminated both the Î±4Î²2 currents and the asynchronous current events. However, MLA eliminated Î±7 responses but did not impact the asynchronous events, thus confirming that the asynchronous responses were mediated by Î±4Î²2 nAChRs.

P2-B-206 - Neuroanatomical relationship between cannabinoid type-1 receptors and dopamine pathways in adult mouse brains

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A close relationship exists between the endocannabinoid system and dopaminergic pathways of the brain. Previous evidence has shown cannabinoid type-1 receptor (CB1R) expression within dopamine-rich brain regions, and that CB1Rs can modulate dopamine transmission and dopamine-related behaviour and disease states through signaling cascades. Immunolabelling and high-resolution imaging were used to investigate the co-localization of CB1R and tyrosine hydroxylase (TH) staining across dopamine pathway regions in adult mice. Perfusion-fixed serial sections of brains were labelled for TH and CB1R in coronal and sagittal planes and imaged by epifluorescence and confocal microscopy. Our results show prominent TH-positive staining of the cell bodies and neurites throughout the substantia nigra, ventral tegmental area, locus coeruleus and dorsomedial hypothalamic nucleus, and diffuse labeling in caudate putamen, bed nuclei of stria terminalis, nucleus accumbens and cortical areas. CB1R labelling was concentrated at granule cell bodies of the dentate gyrus, pyramidal cell bodies of the CA1-CA3 of the hippocampus, the molecular and Purkinje cell layers of the cerebellum, substantia nigra, ventral tegmental area (VTA), subiculum, cortical regions, lateral and basolateral amygdala, and weak staining in the caudate putamen and globus pallidus. Significant colocalization of TH and CB1R was seen in the VTA and caudate putamen. We are currently assessing the full extent of the overlap between CB1R and dopamine neurons at higher resolution by immunoelectron microscopy.

P2-B-207 - Markov chain neurite trafficking model

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A damaged neuronal cytoskeleton is a hallmark of several neurodegenerative disease pathologies. The impact of a decaying cytoskeleton (over a period of decades) on intracellular trafficking has been difficult to characterize.

With the feasability and availability of obtaining high quality experimental tracking data for neurite cargo, we propose a generalized neurite trafficking model using a Markov Chain (MC). This MC has states representing underlying microtubule-motor mechanisms of kinesin and dynein transport, with associated motor velocity distributions. The transition probabilities between states can be trained with supervised learning using experimental data of tracks. These tracks are obtained by extracting kymographs from live-imaged movies and processing them through KymoButler, which automates tracing.

When using tracking data of brain-derived neurotrophic factor (BDNF) for training, the MC is able to recapitulate similar transport metrics distributions for run length, duration, and velocity in both anterograde and retrograde directions, as well as dwell time. We intend to train the MC on different types of cargos and diseased conditions of BDNF to validate its generalizability. Having a computational model for neurite transport is a step towards simulating disease progression over timescales that are not experimentally feasible.

P2-B-208 - Purkinje cell-to-CN neuron synapse stability is decreased in a mouse model of ARSACS

Brenda Toscano [1], **Zoe Dubin** [1], **Alyssa Abou-Chakra** [1], **Alanna Watt** [1]

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Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is an early-onset neurodegenerative disease characterized by progressive loss of motor coordination and cerebellar degeneration. In an ARSACS mouse model (*Sacs* knockout mice), we have previously shown a progressive decrease in the synaptic innervation from Purkinje cells onto target neurons in the cerebellar nuclei (CN), as well as glial activation in the CN. In the cerebellum, large glutamatergic CN neurons are surrounded by perineural nets (PNN). PNNs are specialized extracellular matrix that interacts with synapses and glia and are thought to stabilize and modulate synaptic plasticity and regulate oxidative stress and inflammation in several brain regions. Studies have shown that PNN are reduced in neurodegenerative diseases such as AD and PD. In the cerebellum, dissolving PNN enhances GABA release from Purkinje cell terminals and affects synaptic plasticity. Here we explored whether CN neuron PNNs were altered in ARSACS mice. We stained and quantified the area and intensity of CN PNNs and found a decrease in the area and intensity of PNN of CN neuron in ARSACS mice compared to litter-matched wildtype controls. This change was accompanied by an increase of Purkinje cell puncta size but a decrease in puncta number. These findings support a model where the stability of the synapses made by Purkinje cells onto CN neurons in ARSACS mice is impaired, likely contributing to the pathophysiology of the disease.

P2-B-209 - Selective prevention of MDGA-Neuroligin-2 interactions increases inhibitory synaptic transmission, fear memory, and anxiety

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Mutations in Neuroligin-2 (NL2) or MDGAs (MAM domain containing glycosylphosphatidylinositol anchors 1 and 2) contribute to neurodevelopmental disorders. However, how the interaction between these two synapse organizing molecules specifically regulates synapse properties and behavioral outputs remains unclear. NL2 is an inhibitory synapse-specific synapse organizer, whereas MDGAs act as synapse suppressors through blocking the interaction of NL2 and presynaptic neurexins (NRXNs). To specifically examine the roles of NL2-MDGA interactions in regulating excitatory (glutamatergic) and inhibitory (GABAergic) balance (E/I balance) in synaptic connectivity, we designed a novel and specific NL2$^{Î”Site II}$ transgenic mouse model with the NL2 site II mutated to selectively disrupt the binding of NL2 and MDGAs, without affecting other protein interactions. Using a combination of immunohistochemistry, diverse behavior paradigms, and electrophysiology techniques, we sought to determine how synapse development, neural transmission and behaviors are altered in male NL2$^{Î”Site II}$ transgenic mice. We found that NL2$^{Î”site II}$ mice showed increased anxiety, fear memory and impaired social memory. These mice also exhibited increased expression of inhibitory synapse proteins and upregulated GABAergic transmission whereas excitatory synapse proteins and synaptic transmission appeared normal. Moreover, NL2$^{Î”site II}$ did not affect long-term potentiation (LTP) recorded at hippocampal Schaffer collateral-CA1 synapses. Overall, our results suggest that NL2$^{Î”site II}$ specifically modulates inhibitory synapses and leads to behavioral abnormalities, which could have clinical implications for treatment of neurodevelopmental disorders including autism spectrum disorder (ASD), schizophrenia and bipolar disorders.

P2-B-210 - Morphological diversity of subiculum projection neurons contributes to differential cellular outputs and circuit recruitment

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As the major output region of the hippocampus, the subiculum is responsible for conveying the majority of information to extrahippocampal regions. Accumulating evidence demonstrates that heterogeneity of molecules, cells, and circuits in the subiculum may support differential subiculum computation. However, heterogeneity of dendritic morphology amongst subiculum projection neurons is not well-studied, despite this potentially lending to the subiculumâ€™s computational versatility. We hypothesized that specializations in dendritic morphology contribute to the ability of neurons to route subiculum inputs in a projection-specific manner. We analyzed the morphology of 70 subiculum neuron reconstructions and identified subgroups of morphology phenotypes that correlated with specific downstream projection targets. Next, we built computational models of the reconstructed neurons and

showed that differences in dendritic morphology predict differences in intrinsic cellular drive. To assess our predictions *in vivo*, we employed functional circuit mapping via optogenetics and multiplexed fluorescent *in situ* hybridization to quantify indicators of cellular activity. We examined local and distal inputs onto subiculum neurons and show differences in cellular drive amongst predicted morphological phenotypes and their downstream targets. Together, we show that heterogeneity in neuron morphology may support functionally dissociable outputs of the subiculum. This offers further insight into how heterogeneity within the hippocampal circuit contributes to its host of computational functions.

P2-B-211 - Microglial dopamine receptors: from neuroinflammation to animal behavior

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Background: Microglia, the brainâ€™s resident immune cells, play prime roles in physiology and pathology of the CNS. Adult microglia are key players in homeostasis, but also in induction and resolution of neuroinflammation, due to their capacity to adjust to various signals from their environment. One major signal is dopamine (DA). DA modulates fundamental functions, including coordinated movements, reward-related, emotional and cognitive processes. Study of the DA functions has traditionally relied on the expression of DA receptors on neurons. Emerging evidence suggests immunomodulatory roles of DA in the CNS and periphery, with action on microglial functions, including their inflammatory activity, migration, and cell adhesion *in vitro*. We aim to conduct in-depth characterization of the roles played by DA D1 and D2 receptors (respectively, D1R and D2R) expressed in microglia in immunomodulatory responses, behavioral phenotypes and neuronal function.

Methods: We genetically ablated D1R or D2R specifically in microglia. We employ FISH, histology, antibody array-based approaches, a battery of behavioral tests, stereotaxic injections and electrophysiology.

Results: Microglia express D1R and D2R in the striatum, and the expression increases following chronic psychostimulant administration; microglial D1 ablation leads to a form of hypo-anxiety state and abolishes baseline repetitive, compulsive-like behavior, while microglial D2 ablation results in altered striatal cytokines profile.

Conclusion: Microglia sense and respond to fluctuations in striatal DA release. Microglial D1R is associated to physiologic regulation of compulsive-like behavior as well as anxiety, whereas microglial D2 plays a major role in the homeostatic striatal cytokine profile.

P2-B-212 - Excitability and excitotoxicity susceptibility in male and female mice following pediatric mild traumatic brain injury (pmTBI)

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Mild traumatic brain injuries (mTBI) are common mechanical traumas to the head that result in either no or transient (<30 min) loss of consciousness representing approximately 80% of TBIs reported worldwide. Increasing incidence of mTBI make them a growing public health concern1. Approximately ~30% of mTBI patients experience long-term functional deficits. In particular, the effects of pediatric mTBI (pmTBI) are understudied compared to the adult population. In a closed, non-head fixed cortical control impactor (CCI) murine model of pmTBI we are using whole-cell patch-clamp electrophysiology to investigate neuronal excitability changes at acute and chronic timepoints. mTBI symptoms and pathophysiology is sexually dimorphic at both clinical and preclinical levels, thus experiments were performed in male and female cohorts of C57B6 mice. Preliminary data suggests decreased ionic dysregulation in both sexes sub-acutely, 5-30 min post-pmTBI compared to sham animals, which may persist in females up to 30 days, but reverses in male mice such they have increased ionic dysregulation compared to shams. Excitability alterations following pmTBI may identify novel therapeutic targets such as the N-methyl-D-aspartate Receptor pathways, including its downstream target pannexin-1 (Panx1)5. We are presently using Panx1 knockout mouse lines to determine if these channels contribute to sex differences after pmTBI. This work aims to elucidate the cellular targets that contribute to sex differences following mTBI.

P2-B-213 - UNC-43/CaMKII regulates presynaptic assembly in C. elegans

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Neurons communicate via interfaces known as synapses comprised of pre- and postsynaptic specializations. At the presynaptic specialization, neurotransmitter release is controlled by highly conserved proteins that reside at a protein-dense region called the active zone (AZ). AZ proteins control the recruitment and exocytosis of neurotransmitter-containing synaptic vesicles for synaptic transmission. While the components of the AZ are well characterized, the mechanisms of their assembly remain elusive. Here we found that unc-43, the sole ortholog of Ca2+/calmodulin-dependent protein kinase II (CaMKII) in Caenorhabditis elegans, is integral for presynaptic assembly. In unc-43 loss-of-

function mutants, we observed disorganized presynaptic structures determined by the presence of smaller and numerous clusters of active zones. Conversely, unc-43 gain-of-function mutants have â€˜overdevelopedâ€™ synapses with larger active zones. To examine the conservation between UNC-43 and human CaMKII in presynaptic assembly, we generated a C. elegans strain in which the unc-43 locus was replaced with human CaMKIIA (hCaMKIIA). We found that hCaMKIIA sufficiently replaces the functions of unc-43 and exhibit wild type locomotion and presynaptic structures. We introduced recessive and dominant mutations that are found in the CaMKII genes of patients with intellectual disabilities. These disease-associated mutations in hCaMKIIA resulted in similar presynaptic organization defects to unc-43 mutants. This observation suggests the functional conservation between UNC-43 and CaMKII in presynaptic assembly.

P2-B-214 - All roads lead to nep1: identifying the downstream transcriptional targets that mediate stromalin's effects on synaptic vesicle pool size

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While we know much more about genes that promote learning, little is known about genes that limit memory formation. Recently, *stromalin* has been shown to be a learning suppressor that functions by restricting the synaptic vesicle (SV) pool size in dopamine neurons (DANs) in *Drosophila melanogaster*. While we have previously showed SV numbers to be critical for learning and memory, how SV numbers are regulated remains unexplained. To advance our understanding of this enigma, a DAN-specific RNA-Seq with silenced *stromalin* was performed to identify the genes downstream of it that mediate its effects on SV numbers. RNAi lines to significantly differentially expressed genes were obtained to perform a primary aversive olfactory memory screen and a secondary SV marker (Syt:eGFP) screen in DANs. After validation experiments, two genes were outlined as primary candidates: *nep1* and *su(z)12*. Examining mRNA level after *su(z)12* knockdown suggests *nep1* levels are not regulated by *su(z)12*. Thus, we now hypothesize that *stromalin* (as part of the cohesin complex) regulates the transcription of *nep1* to suppress learning via limiting SV numbers. We are doing this by testing whether overexpression of *nep1* can rescue *stromalin* knockdown effects on memory. Additionally, using *in vivo* functional imaging with the dopamine sensor $GRAB_{DA}$, we intend to look at the dopamine release from DANs with silenced *stromalin* and/or *nep1* to test our hypothesis. Our future efforts will aim to unravel the mechanism of how *nep1* alters neuronal SV pool size.

P2-B-215 - Impact of the gut microbiome on Dendritic spine formation during postnatal development in mouse

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BACKGROUND AND AIM: Dendritic spines mature from thin, dynamic spiny [LS1] protrusions to become the sites of excitatory synaptic input on neuronal dendrites. These spines undergo significant remodelling and structural plasticity during development and learning. In adult mice, the presence of the gut microbiome has a significant impact on dendritic spine density and morphological properties. However, the relationship between the gut microbiome and initial spine formation during the first four weeks after birth has not been explored. Here we investigate the impact of disrupting (antibiotic-depleting) the gut microbiome on spine density during postnatal development in the somatosensory and motor cortices, amygdala, and hippocampus in mice. METHODS: We will treat C57BL/6 mice with antibiotics (or water for control mice) for three weeks and collect brains at P21. Fresh brain tissue will be vibratome sectioned, stained using the Golgi-Cox method, and imaged with confocal microscopy. We will measure dendritic spine density of neurons in several brain regions using established protocols. In parallel, we are generating synaptosome preparations for synaptic protein quantification to complement our imaging approach. RESULTS Golgi-Cox staining and synaptosome preparations have been optimized. We are now collecting data from our experimental groups. CONCLUSIONS: Exploring the impact of the microbiome on dendritic spines during their peak period of formation will inform upon the impact of the microbiome on the development of brain circuitry and behaviour.

P2-B-216 - Discovery and interpretation of a novel hybrid cell type in the brain.

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Cell types can be viewed as a “periodic table” for elements of the brain, wherein cells are organized and distinguished from one another based on differences in their structure, function, and connectivity. Of these cell types, it is broadly believed that neuronal and non-neuronal cells are markedly distinct and exclusive from one another.

Here, we discover and characterize a novel cell type expressing genes specific for both neurons and oligodendrocytes. First, we show the existence of these “hybrid” cells using single-cell RNA sequencing analysis and present the first such evidence that these specific neuronal and non-neuronal genes can co-exist within the same cell. Furthermore, we show that this rare group of hybrid cells appears to exist across several brain regions and has transcriptomic signatures of newborn neurons. To investigate the

abundance and distribution of these cells, we used single-cell spatial transcriptomics technology on both human and mouse brain tissue to resolve the spatial distribution of over 300 target genes. To examine their morphology and organization, we leveraged a viral targeting strategy in transgenic mice, allowing us specific access to these cells so we could fluorescently label them within the brain. This project provides insight into the location, marker genes and cell morphology of these hybrid cells, which will help in elucidating their functional role. This research will also add to our basic knowledge of cell types in the brain and could potentially influence our understanding of how new neurons are formed in adulthood.

P2-B-217 - Characterizing the role of N-Methyl-D-Aspartate receptors in long-term potentiation decay

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The field of neuroscience has historically focused on how memories are encoded and consolidated. However, how memories may be erased or forgotten at a cellular level remains an open question. An emerging theory suggests that cellular forgetting is an active process governed by dedicated molecular mechanisms. One model of active forgetting is long-term potentiation (LTP) decay. Previous studies have demonstrated that LTP decay requires ongoing synaptic activity and depends upon activation of N-Methyl-D-aspartate receptors (NMDARs). NMDARs have a diverse range of physiological properties determined by the subunit composition of each receptor. Using a form of decaying LTP, we probed the roles of different NMDAR subunits during LTP decay. We further sought to determine if blocking LTP decay converts LTP to an enduring form of LTP. Accordingly, we tested if preventing LTP decay rendered LTP susceptible to translation inhibitors which suppress canonical forms of enduring LTP. Our results suggest that blocking LTP decay converted transient, translation-independent LTP into an enduring form that requires protein synthesis. These results suggest that pharmacological prevention of LTP decay allows the conversion of LTP in a form that recruits molecular events associated with long-lasting LTP.

P2-B-218 - Computational modeling of reactive astrogliosis and its effects on dynamics in brain microcircuits

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Reactive astrogliosis, consisting of morphological and physiological alterations in glial cells, especially in astrocytes, has been observed in many central nervous system diseases. Characterizing the network dynamics that emerge from astrocyte-neuron interactions is critical to understanding the effects of reactive astrogliosis on disease, and the computational models help identify biophysical mechanisms involved in the dynamics of brain circuits. However, few or no computational models with gliotransmission can simulate brain dynamics at the mesoscale or whole-brain level. In this work, we implement a network model with neuron-astrocyte interactions and gliotransmission to simulate astrocytic synaptic modulation, the network dynamics, and its local field potential (LFP). We designed a brain microcircuit with a balanced network of 8000 excitatory neurons and 2000 inhibitory neurons, using an adaptive exponential integration-and-fire model, and 7500 astrocytes modeled calcium dynamics as leaky integration-and-fire. We explored the parameter space of astrocytic activation and gliotransmission and then analyzed the simulated signals to measure the autocorrelation, intrinsic timescales, and the power spectral density (PSD) in the raster and LFP of simulations. Preliminary results show that alterations in astrocytic activation and gliotransmission produce changes in the power spectral of LFP, suggesting that some levels of reactive astrogliosis may modulate the brain oscillations.

P2-B-219 - KCC2 as a novel biomarker and therapeutic target for motoneuron degenerative disease

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Hyperexcitability in cells throughout the corticospinal tract is a presymptomatic feature of amyotrophic lateral sclerosis (ALS) associated with lethal motor degeneration. Disinhibition is a possible cause of this hyperexcitability, potentially implicating the central nervous system-specific potassium-chloride cotransporter, KCC2, a core regulator of the strength of GABAergic neurotransmission linked to several neurological disorders. Here, we show that KCC2 is downregulated in the membrane of motor cortex neurons from post-mortem SOD1-, C9orf72- and sporadic ALS patients. Increased protein levels of KCC2 were found in plasma and cerebral spinal fluid of ALS patients and mice harbouring the SOD1*G93A mutation. Longitudinal analysis of disease progression in both SOD1*G93A and Prp-TDP43*A315T mice revealed a decrease of KCC2 membrane levels in cortical and spinal motor neurons which were already present at the presymptomatic phase. Using KCC2-enhancing compounds, CLP290 and prochlorperazine

(PCPZ) restored KCC2 membrane expression and function, delayed motor deficit onset, and extended lifespan by up to two months in mutant mice. Human-derived neurons differentiated from iPSC harbouring the SOD1*G93A mutation displayed KCC2 deficits which PCPZ treatment rescued. Acute administration of KCC2 enhancers restored chloride transport in presymptomatic and symptomatic mice and reversed motor neuron hyperexcitability in awake behaving mutant mice. These findings identify KCC2 as both an early biomarker and a disease-modifying therapeutic target for ALS.

P2-B-220 - GluN2D antagonists preferentially inhibit induction of STP over LTP

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Two forms of NMDAR-dependent synaptic potentiation are co-induced by theta-burst stimulation (TBS) of the Schaffer collaterals. The initial phase of potentiation (short-term potentiation, STP) declines in response to low frequency synaptic activation leading to a stable enhancement of synaptic transmission (long-term potentiation, LTP). STP and LTP are differentially sensitive to GluN2-preferring NMDAR antagonists and STP has been subdivided further into STP1 and STP2 [PMID: 23230236]. STP1 and LTP are inhibited more potently than STP2 by AP5 and NVP-AAM077 (GluN2A-prefering antagonists). In contrast, STP2 is more sensitive to inhibition by Ro25-698 and UBP145, which are most potent at blocking GluN2B- and GluN2D-containing receptors, respectively.
Recently two UBP145 derivatives, UBP791 and UBP1700 have been developed, which display a greater potency at GluN2D-containing receptors [PMID: 31969570]. UBP791 and UBP1700 were tested to determine if these newer antagonists have greater STP2 selectivity. Our results show UBP791 inhibited STP in a biphasic sigmoidal fashion with low (0.15 Î¼M) and high (1.8 Î¼M) IC50 values that differed 12-fold whereas LTP was inhibited in a single sigmoidal manner (IC50 = 5.6 Î¼M). The more potent UBP1700, also inhibited STP in a biphasic manner but showed a greater separation between the low (0.0033 Î¼M) and high (0.36 Î¼M) IC50 values (109-fold) and with an IC50 of 1.6 Î¼M for LTP inhibition. Both UBP791 and UBP1700 were more selective for STP inhibition compared to UBP145, making them attractive compounds to test the physiological roles of STP.

P2-C-221 - Amyloidosis and sleep disturbance following repetitive-mild traumatic brain injury in an APP knock-in mouse model of alzheimer's disease

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Mild traumatic brain injury (mTBI) is the most common type of head trauma. Patients exposed to repetitive mTBI (rmTBI) often develop pathologies and pathophysiologies, such as amyloidosis in defined regions of the brain and sleep disturbance. However, the mechanisms underlying the progression of amyloidosis induced by rmTBI remains unclear. To enhance our understanding of the chronic pathogenicity of rmTBI, we conducted a concussive TBI study using the Closed-Head Impact Model of Engineered Rotational Acceleration (CHIMERA). The transgenic APP^{NL-F} mouse model of Alzheimerâ€™s disease (AD) (n=42) received three mild-TBI within a week at 12 months of age. After 3 months of pathological maturation, the electroencephalogram (EEG) was recorded, and blood plasma and brain tissue were collected for biochemical analysis. To date, no group differences were found in the total amount of sleep, time spent in each state (wake, non-REM and REM), or sleep state transitions. However, quantitative EEG analysis revealed significant increases in the power of delta, beta, and theta frequencies during non-REM sleep in the rmTBI group. To confirm neurological injury in the brain, neurofilament-light in blood plasma, indicative of chronic neurological injury, was quantified using Meso Scale Discovery assay. Immunohistochemical analysis of amyloid plaques was performed to determine chronic amyloidosis in the injured brain. Our data suggest sustained neuropathological impacts post-TBI that may warrant intervention to delay the onset of AD-associated pathologies.

P2-C-222 - Modeling epileptic Dravet syndrome using patient-derived brain organoids

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The first line treatment for Dravet syndrome is a three-drug combination that offers partial seizure relief in ~50% of the patients, leaving half of Dravet children without treatment options. We propose that other combinations of anti-seizure medications might be effective but with >450 potential combinations, it is unclear which ones. We created iPSCs from a Dravet individual and her parents as isogenic controls for use in growing brain organoids. To accurately model epilepsy, we created individual dorsal and ventral forebrain organoids and fused them together to generate brain â€˜assembloidsâ€™. The Dravet

assembloids were smaller in size, showed a decrease in neuronal progenitor populations, along with an increase in excitatory neuron populations compared to controls. Interestingly, the bioenergetics profiles were disturbed in the Dravet assembloids and our electrophysiological recordings showed that Dravet assembloids display seizure-like hyperexcitable events. Collectively, our data suggest that neural progenitors in Dravet brains tend to exit the cell cycle faster, leading to misspecification of excitatory neurons and neurometabolic compromise. So far, the scRNA-seq dataset analysis from patient and parentsâ€™ assembloids has identified dysregulated excitatory neuron sub-populations and further investigation is ongoing for better understanding of disease etiology. Next steps are to screen drug combinations to identify those that restore bioenergetics to baseline levels in the patient assembloids and then test these combinations back in the donor child.

P2-C-223 - Investigating the therapeutic effects of a ketogenic diet in a mouse model of amyotrophic lateral sclerosis (ALS)

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease caused by the degeneration of upper and lower motor neurons, leading to loss of motor function, paralysis and death. An early clinical feature of ALS is an imbalance in energy homeostasis, where an individualâ€™s energy expenditure exceeds their energy uptake, contributing to a lower body-mass index and decreased fat stores. The factors driving this hypermetabolic state in ALS are not clear, but the consequences of an energy deficit are acceleration of the disease. To address the energy crisis in ALS, a ketogenic diet rich in fat and low carbohydrates, has been explored as a potential therapeutic approach in the treatment of the disease. However, the mechanism(s) by which a ketogenic diet exerts its therapeutic effects in ALS are not clear. In our study, we investigated the effects of a ketogenic diet in a validated mouse model of familial ALS expressing the FUSR521G mutation (ALS-FUS). Our preliminary data show that ketogenic intervention in ALS-FUS mice restores cognitive and motor function through improved mitochondrial and mRNA translation status. Analysis of whole transcriptomes from treated mice reveals mitochondrial adaptability to ketogenic interventions, suggesting that providing ketones as an alternative energy source can restore energy homeostasis in our model of ALS. These findings have helped elucidate the mechanism of action of a ketogenic diet in a mouse model of ALS and revealed potential strategies to treating the energy imbalance that occurs in ALS.

P2-C-224 - Investigating the contribution of the zebrafish rostral migratory stream to neural repair

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The zebrafish model is renowned for its remarkable regenerative ability. Following injury to the brain, neural stem cells (NSC) increase proliferation and differentiation to replace lost neurons. However, brain injuries are often performed near the stem cell niche, thus we have little understanding of how distal NSCs might contribute to long-distance brain repair. To address this question, we capitalize on the presence of a Rostral Migratory Stream (RMS)-like structure in the adult zebrafish brain, that like mammals, continuously sends new neurons to the olfactory bulbs (OB) from the forebrain stem cell niche. By developing a novel OB injury model, here we investigate whether a distal OB injury can recruit NSCs along the RMS for repair.

Results to date show that following OB injury, local immune cells are recruited to the injury site, followed by an increase in NSC proliferation in the forebrain. By 14-days post-injury an increase in neural progenitors is observed at the injury site, many of which differentiate into neurons, demonstrating that cellular migration of NSCs along the RMS can be stimulated by distal injury. Since zebrafish also have local NSCs within the OB, distinguishing between RMS-recruited and local NSC contributions upon OB injury becomes difficult. To overcome this challenge, we have recently applied cerebroventricular microinjection of thymidine analogue to uniquely label the RMS-derived NSC population in future experiments. This novel approach will help better study long-distance recruitment of NSCs along the RMS following brain injury.

P2-C-225 - Impact assessment of micro- and nanoplastics: Influence of shape and surface contaminants on neurotoxicity

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Emerging environmental pollutants become ubiquitous over the past centuries; in particular, micro- and nanoplastics (MNPs) are among the most pervasive contributors to permanent pollution of our

ecosystems and food chains. To understand the complexity and severity of MNPs exposure to living organism, hazard and risk assessments must focus on environmentally relevant concentrations and compositions. We demonstrated that MNPs penetrated murine brain, warranting further investigation into their neurotoxic effects in humans. This study represents a step forward, as we systematically compared MNPs with diverse shapes, forms and concentrations focusing on subtle and functional endpoints to identify adverse effects and characteristics that drive neurotoxicity in human cortical and nociceptive neurons. Our results showed that MNPs induced oxidative stress, cytotoxicity, and neurodegeneration in human neurons, with cortical neurons being more susceptible than nociceptors. The degree of neurotoxicity was dependent on the concentration, size, or shape of MNPs. Furthermore, we also examined the role of biofilms on MNPs, discovering that biofilm-coated MNPs significantly exacerbated these neurotoxic effects. Collectively, these combined data indicate that accumulated MNPs induces oxidative stress and neurodegeneration in human neurons and highlights the need to understand the neurological consequences of MNPs. Overall, our developed *in vitro* testing battery has significance in elucidating the effects of environmental factors and their associated pathological mechanisms in human neurons.

P2-C-226 - Insights into the effects of peripherally restricted CB1-R modulators in models of hyperdopaminergia

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Negatively modulating the cannabinoid receptor 1 (CB1-R) is being studied as a potential therapeutic approach in many indications. We have previously demonstrated the efficacy of the CB1-R negative allosteric modulator (NAM) ABM300 in alleviating psychosis-like phenotypes in two genetic mouse models of hyperdopaminergia.[1] However, ABM300â€™s limited penetration into the mouse brain (0.77 Kp, brain) raises questions about the mechanism underlying its behavioural effects. This study aims to further investigate the effects of ABM300 on psychosis-like phenotypes, determining if CB1-R or alternative mechanisms are involved, and if the effects are mediated centrally or peripherally. We have recapitulated the effect shown previously in the genetic models in a pharmacological amphetamine (AMPH) model of psychosis-like behaviours. At 10mg/kg ABM300 significantly reduces the hyperlocomotion induced by 2mg/kg AMPH. Interestingly, ABM300 has no cannabimimetic effect in the cannabinoid tetrad alone and does not block CB1 agonist effects in the tetrad. Similarly, the peripherally-restricted CB1-R orthosteric antagonist, AM6545 showed a reduction in hyperlocomotion in both genetic (Dopamine Transporter Knockout, DATKO) and pharmacological (AMPH) models. Previous studies have also shown that AM6545 has no effect on cannabinoid agonist effects in the tetrad.[2] Together, this data suggests that peripherally restricted CB1-R negative modulators and orthosteric

blockers may indirectly modulate hyperdopaminergic related behaviors; however, the underlying mechanisms remain to be established.

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P2-C-227 - Early life stress leads to long-term sex-dependent disruption in serotonin connectivity

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Chronic stress during early life strongly impacts brain development and increase susceptibility to affective disorders. Pharmacotherapies in children and youth are limited to drugs that target the serotonin system. Thus, it is imperative to understand the mechanisms underlying serotonin-induced emotional regulation and dysregulation to develop more effective treatment approaches. Here, we studied how early life stress (ELS) impacts brain-wide serotonin activity and behaviour using a mouse model of chronic developmental stress. During adulthood, *in vivo* calcium imaging of dorsal raphe nucleus (DRN) serotonin neurons of female and male ELS mice revealed a threat-induced disruption in serotonin activity. Brain-wide functional connectivity analysis demonstrated an increase in anti-correlated activity of the raphe nucleus. *In vivo* calcium imaging revealed a disruption in serotonin release in the medial orbitofrontal cortex (mOFC). Optogenetic stimulation of serotonin terminals in the mOFC elicited an anxiolytic phenotype in ELS mice in a sex-dependent manner. Overall, our findings revealed that ELS disrupts the connectivity of the serotonin system which could have implications for the treatment of affective disorders that arise from early life adversities. These data emphasize the importance of understanding the mechanisms of sex-dependent deficits underlying emotional dysregulation for the development of effective treatment strategies.

P2-C-228 - The influence of pre-injury stress in a new rat model of intimate partner violence-related brain injury

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Intimate partner violence (IPV) primarily affects women, and upwards of 90% of IPV survivors experience brain injury (BI). IPV-BI is unique as survivors often experience a combination of mild TBI (mTBI), non-fatal strangulation (NFS), and stress which may modify pathobiology and functional deficits compared to the injuries in isolation. Using a novel rat model of IPV-BI, we previously found that mTBI+NFS exacerbated behavioural deficits and neuroinflammation compared to isolated injuries. The purpose of this study was to investigate how stress may further influence IPV-BI outcomes. It was hypothesized that rats exposed to stress + IPV-BI would have worse behavioural deficits and an altered pathophysiological profile compared to IPV-BI in isolation. Young-adult female rats were either exposed to a predator odour stress model (i.e., fox odour 2,5- dihydro-2,4,5-Trimethylthiazoline; TMT) or control condition for 15 minutes then immediately administered an IPV-BI (i.e., mTBI + NFS) or sham injury. Rats were then assigned to either a 2-hour or 7-day recovery to provide insights into the temporal profile of neuropathophysiology and blood biomarkers. The 7-day recovery rats also underwent behaviour tests to assess motor function, cognition, social behaviour, along with anxiety- and depressive-like behaviours. Analysis of brain and blood are ongoing; however, preliminary results indicate that exposure to the predator odour stress is in an important modifying factor in IPV-BI altering blood biomarkers related to inflammation and behavioural profile.

P2-C-229 - Interfering with Src-Panx1 interaction suppresses NMDA-induced excitotoxicity in hippocampal neurons

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During an ischemic stroke, focal loss of energy triggers ionic dysregulation, and release of extracellular glutamate, triggering excitotoxicity in the ischemic core. Excitotoxic neuronal death is facilitated by the overactivation of *N*-methyl-D-aspartate receptors (NMDAR) leading to influx of calcium. Our lab has shown that NMDARs trigger pannexin1 (Panx1) channel opening via phosphorylation by sarcoma (Src) kinases at Panx1 tyrosine 308 furthering the pathological calcium influx. In its active conformation, substrate recognition sites on Src are available to bind targets such as Panx1, leading to their phosphorylation. We hypothesized that interfering with Src will suppress the NMDA-induced excitotoxic response. In CA1 hippocampal neurons, we interfered with Src function using inhibitors and in-house designed mimetic peptides for Src interaction motifs on Panx1, and observed ionic dysregulation using whole-cell patch-clamp recordings. PHPS1 blocks src homology region 2 containing phosphatase, which may affect the phosphorylation state of Src. Two novel peptides were designed to interact with Src

directly at different putative homology domains and are predicted to impact the kinaseâ€™s ability to interact with and phosphorylate targets. Preliminary evidence suggests interfering with Src interaction sites decrease the size of the excitotoxic response. Preliminary PHPS1 data suggest it may influence the excitotoxic response. Interfering with the Src-Panx1 interaction, upstream of Panx1 phosphorylation, may disrupt NMDA-induced excitotoxicity and be neuroprotective during stroke.

P2-C-230 - Elucidating the role of adult PDGFRβ-expressing neural stem cells in stroke

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Stroke is a major cause of death and disability worldwide. Injury following stroke stimulates endogenous neurogenesis and gliogenesis associated with the activation of quiescent neural stem cells (NSCs) and the recruitment of NSC-derived progeny to affected brain regions. Recently, a new subtype of NSC expressing PDGFRÎ² was discovered in the Sub-Ventricular-Zone (SVZ). It has been demonstrated that this lineage of NSCs has a gliogenic fate and give rise to new astrocytes and oligodendrocytes. However, the involvement of PDGFRÎ²-derived NSCs in stroke pathobiology remains unknown.
Our study aims to investigate the dynamics of PDGFRÎ²-expressing NSCs and the fate of their progeny after stroke. To this end, we use the PDGFRÎ²-TdTomato mouse line that allow us to track the lineage of PDGFRÎ²-expressing NSCs. Mice are subjected to ischemic stroke via middle cerebral artery occlusion (MCAo).

Our results demonstrated an increased number of PDGFRÎ²-TdT$^+$ cells in the SVZ of the ipsilateral hemisphere at 7, 14 and 30 days after stroke. Interestingly, these PDGFRÎ²-TdT$^+$ cells were CD13-negative, suggesting that these cells were not pericytes. Furthermore, after stroke, these cells became active and differentiate into gliogenic and in part into neurogenic cells.
Here we discovered that PDGFRÎ²-expressing NSCs are activated after stroke and generate glial and few neuronal cells. Finally, since the heterogeneity of adult NSCs is now largely recognized, we plan to study how other NSCs lineages contribute to post-stroke recovery.

P2-C-231 - Chemogenetic neuronal excitation unveils IP3R-dependent calcium dynamics dysregulation linked to metabolic stress of early retinal ganglion cell damage in glaucoma

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We hypothesized that Inositol 1,4,5-trisphosphate receptor (IP3R)-dependent retinal ganglion cell (RGC) calcium (Ca^{2+})dynamics and subsequent ATP production are affected in the early stages of ocular hypertension (OHT). To modulate RGC selective neuronal excitation, an adeno-associated virus vector encoding Gq-coupled DREADD (AAV.hM3Dq) was delivered intraocularly. Two-photon laser scanning microscopy (TPLSM) was used to record hM3Dq-evoked i) single-RGC Ca^{2+} dynamics in transgenic mice carrying the Ca^{2+} sensor GCaMP6f, or ii) single-RGC ATP changes in mice received with an AAV vector encoding the ATP sensor Ateam. OHT was induced by intracameral injection of magnetic microbeads, and Ca^{2+} and ATP signals were recorded two weeks later, prior to RGC loss. TPLSM imaging was performed in living mice by trans-scleral imaging before and after the clozapine N-oxide (CNO) administration. Live trans-scleral Ca^{2+} and ATP imaging under CNO administration showed Ca^{2+} dynamics and energy balance instability in glaucomatous RGCs. For example, RGCs subjected to OHT displayed an increase in spike frequency and drop in ATP changes relative to sham controls, suggesting hyperexcitability and subsequent energy consumption in neurons during glaucomatous damage. Analysis of molecular pathways revealed an RGC-specific reduction in gene and protein expression of the IP3R1, which is responsible for pumping Ca^{2+} from the endoplasmic reticulum to the cytoplasm and mitochondria. Our study reveals AAV.hM3Dq as a useful toolbox to investigate the involvement of G-coupled protein receptor signaling pathways in early RGC damage, and suggests that dysregulation of IP3R1 has a profoundly detrimental effect on the ability of these neurons to regulate intracellular Ca^{2+} and energy homeostasis.

P2-C-232 - Genomic unbiased characterization of human traits associated with polyglutamine disease genes

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Polyglutamine (polyQ) diseases, such as Huntington disease and certain spinocerebellar ataxia, are a group of severe neurological disorders caused by repeat expansions of the glutamine codon. With no effective treatments, we aimed to profile their genes and unravel shared biology to guide future studies and therapeutic interventions (e.g. gene knockdown approaches). We performed an unbiased phenome-wide study to identify traits linked to polyQ disease genes by analyzing data from the Open Targets Genetics Database to infer the consequences of therapeutically targeting these genes and identify new biology. Leveraging the locus-to-gene (L2G) fine-mapping tool, we assessed trait-gene associations, utilizing a scoring metric to quantify evidence strength (0 to 1). Higher scores indicated stronger gene-trait associations. We further employed network analysis with *ggraph* R package to explore shared

biology among these genes. Quality control measures involved excluding count-based traits linked to widespread genome signals, and the analyses were restricted to peer-reviewed PubMed studies. We curated 3,095 study traits associated with these genes across 20 categories. Applying an L2G filtering threshold of 0.5, we retained 215 diverse phenotype/gene associations, encompassing both pathogenic and non-pathogenic traits, including neurological outcomes. Notably, network analysis revealed shared traits like depression, suggesting a theoretical link and shared underlying mechanisms. These analyses emphasize the pleiotropic nature of polyQ genes, providing detailed therapeutic insights.

P2-C-233 - Investigating the impact of COVID-19 on Neuroinflammation using an optimized western blotting protocol

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BACKGROUND AND AIM: Neurological manifestations are commonly associated with COVID-19. Fatigue, cognitive impairments, and depression in COVID-19 have been linked to heightened peripheral inflammation through a mechanism that may involve impairment of blood brain barrier integrity leading to neuroinflammation. Emerging work supports a link between neuroinflammation and COVID-19; however, the cellular and molecular mechanisms remain poorly understood. In imaging studies conducted within the Swayne and Tremblay Labs, we use male and female Syrian hamster COVID-19 model brains collected at 1-, 3-, 5-, 7-, and 31-days following intranasal inoculation with SARS-CoV-2 to investigate the impact on astrocytes and microglia, respectively. This model exhibits key aspects of most human cases. To complement the imaging studies, here we investigate changes in the levels of protein markers often used to study neuroinflammation. METHODS: To this end, we optimized a protocol to generate lysates from specific regions (e.g., cortex, hippocampus) of these brains, which underwent extensive fixation prior to removal from the high-containment facility. We then used Western blotting to quantify protein levels. RESULTS: We have successfully optimized analysis of glial acidic fibrillary protein (GFAP), an astrocytic filament protein upregulated by neuroinflammation, in our samples. We are now optimizing additional markers of inflammation, as well as markers for tight junctions and synapses. The outcomes of this work will complement our imaging-based investigations on neuroinflammation in COVID-19.

P2-C-234 - Randomized controlled trial on brief electrical stimulation to accelerate axon regeneration and functional recovery following cubital tunnel surgery

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Objectives: Post-surgical electrical stimulation (ES) enhances motor and sensory regeneration in animal models. In this study, we investigated the hypothesis that ES following cubital tunnel surgery in patients would result in significantly better outcomes compared to surgery alone.
Methods: Patients with severe ulnar nerve compression were randomly assigned to the treatment or control group in a 2:1 ratio. The control group received cubital tunnel surgery and sham stimulation, while patients in the treatment group received 1 hour of 20Hz ES following surgery. Patients were followed yearly for 3 years. At each visit, axonal regeneration was quantified using motor unit number estimation (MUNE) and functional recovery was evaluated using grip strength and key pinch strength. Statistical analysis was performed using non-parametric tests with statistical significance set at $p<0.05$.
Results: Twenty-four patients were enrolled: 8 received surgery alone and 16 received surgery and ES. At three years following surgery, MUNE was significantly higher in the treatment group (182Â±25) compared to controls (93Â±14). Grip strength was significantly improved in the treatment group (43Â±3kg) at 3 years post-operatively compared to controls (39Â±3kg). Key pinch strength was 5.2Â±0.5kg in the treatment group compared to 4.4Â±0.8kg in controls ($p<0.05$).
Conclusions: These results suggest that post-surgical ES enhances axonal regeneration, muscle reinnervation and functional recovery. We propose that ES may be a clinically useful adjunct to surgical release for severe ulnar nerve injuries.

P2-C-235 - The neural correlates of the porteus maze task in mild cognitive imapairment

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The Porteus Maze Test (PMT) assesses executive functioning, planning, processing speed, and foresight. While the PMT has explored prefrontal cortex activity in various neurological conditions, its neural basis

in Mild Cognitive Impairment (MCI) remains unexplored. This study aims to characterize the neural substrates of the PMT in MCI using an MR-compatible tablet. Thirty healthy controls and 12 MCI patients (68.83 8.76, 16 female) underwent fMRI using a 3T MRI system at St. Michaelâ€™s hospital. Participants used a novel MR-compatible tablet and stylus in the MRI to perform the PMT and a control task, which involved tracing a solved maze. Functional MRI captured brain activity during both tasks, and task completion times were recorded. At the subject level, voxel-wise general linear model (GLM) analyses measured brain activity for maze and control tasks relative to a fixation condition. Group-level activation maps were then obtained via GLMs, incorporating age, Montreal Cognitive Assessment (MoCA) scores, and task completion time as covariates. In the maze task, both groups exhibited widespread frontal-parietal activity, to a greater extent than seen in the control task. MCI patients demonstrated increased activation in the cingulate cortex, hippocampus, amygdala, and insula during the maze task. This study characterized the impact of MCI on PMT behavioural performance and task-related brain activity, offering novel insights into this neurocognitive assessment and its potential utility in MCI.

P2-C-236 - Iron-rich cells display morphological heterogeneity across aging and Alzheimer's disease pathology conditions

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Neurodegenerative progression of multiple brain diseases is sustained and exacerbated by aging. This includes Alzheimerâ€™s disease (AD), which involves cognitive decline and progressive memory loss. Resident brain immune cells in microglia have been suspected to play contributive roles towards progressing AD, with iron-enriched cells, including microglia and likely central nervous system (CNS) macrophages, having been found both in mouse models and post-mortem patient brains. These immune cells exist in heterogeneous states implying diverse functions in pathology, but morphological heterogeneity remains unknown. Thus, we sought to investigate iron-rich cells in 14 month male aged mouse healthy wild-type (WT) and APP-PS1 (amyloid-beta overexpressing) prefrontal cortex, a brain region affected over dementia progression. Interestingly, iron-rich cells were found to be enriched at the CNS interface and perivascular space in both mouse models, novelly suggesting that putative iron-rich cells with cerebrovascular pathology in the aging and dementia continuum. Scanning electron microscopy confirmed different staining patterns of iron aggregates in microglia/CNS macrophages and variable enrichment of secretory granules in iron-rich cells at the CNS-meninges border. Our results imply that iron-enriched cells, including microglia and CNS macrophages, have morphological heterogeneity and possibly functions across aging and AD pathology. We encourage further work characterizing these cells to better understand their roles in aging and neurodegenerative diseases.

P2-C-237 - Determining the role of IRF2BPL in neurological disease

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De novo, heterozygous truncating mutations in the single-exon gene, *IRF2BPL* cause a serious pediatric brain condition. Children are born developing typically, but at around age five, they develop a progressive ataxia, lose milestones such as speech, and develop seizures. This disorder gradually worsens until they are immobile in their teens. Some missense variants in this gene are also linked to autism spectrum disorders and Parkinson's disease. We have created the first *Irf2bpl* knockout mice. These KO mice are runted and have motor problems at three months. They also display cortical thinning. Our preliminary characterization of Irf2bpl KO mice reveal phenotypes across the brain with key areas being affected: cortex, cerebellum, and potentially the basal ganglia, all areas relevant to ataxia, autism spectrum disorder, and Parkinsonâ€™s disease. Additionally, we aim to learn more about Irf2bpl function by identifying where it binds in the genome and what other genes it controls. Although this is a rare disease, understanding this gene can help those with this condition and provide insights into common neurological disorders like autism and Parkinson's disease.

P2-C-238 - Impaired neurogenesis in Parkinson's disease: Insights from altered Hippocampal Doublecortin expression

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Introduction: Parkinson's disease (PD) is a complex neurodegenerative disorder characterized by motor and non-motor symptoms. Motor symptoms include bradykinesia, resting tremors, muscular rigidity, and postural instability, while non-motor symptoms include cognitive impairments, mood disturbances, sleep disturbances, autonomic dysfunction, and sensory abnormalities. Some of these symptoms may be influenced by the proper hippocampus functioning, including adult neurogenesis. Doublecortin (DCX) is a microtubule-associated protein that plays a pivotal role in the development and differentiation of migrating neurons. **Objective:** To investigate the expression of DCX in the subgranular zone (SGZ) of the hippocampus in postmortem brain tissue obtained from both control subjects and individuals diagnosed with PD. **Methods:** This study utilized postmortem human brain tissue of PD and age-matched control individuals to investigate DCX expression in the context of adult hippocampal neurogenesis. **Results:** Our findings reveal a reduction in the quantity of DCX-positive cells in the SGZ of the dentate gyrus in

postmortem brain tissue from PD patients. Additionally, there is a decrease in the nuclear area of these DCX-positive cells, suggesting an impairment in the process of adult hippocampal neurogenesis. **Discussion and Conclusion:** We provide evidence supporting that the process of hippocampal adult neurogenesis is likely to be compromised in PD patients before cognitive dysfunction.

P2-C-239 - Dickkopf-1 exacerbates neurovascular deregulation and cognitive deficits upon cerebral small vessel disease (cSVD) associated with microinfarctions

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Cerebral small vessel disease (cSVD) constitutes a major risk factor for vascular dementia (VaD). cSVD are associated with sporadic multifocal cerebral microinfarctions (CMIs), which are small ischemic lesions caused by the obstruction of penetrating arterioles. CMIs deregulates neurovascular functions leading to cerebral blood flow (CBF) impairments, neuroinflammation, neuronal damage and cognitive deficits. Our lab has demonstrated that the canonical Wnt pathway is required to maintain neurovascular functions upon ischemic lesions. Expression of Dickkopf-1 (DKK1), an endogenous inhibitor of the canonical Wnt pathway, is induced under pathological conditions. DKK1 circulating levels are elevated in patients with cerebrovascular diseases, and its increased expression is associated with dementia. Our study postulates that DKK1 worsens structural and functional brain damage after CMIs. To test this hypothesis, transgenic mice which allow the conditional induction of DKK1 were subjected to CMIs using a novel sporadic cerebral micro-occlusion model. Our findings indicate that DKK1 increases monocyte frequency and promotes their pro-inflammatory polarization upon CMIs. Moreover, DKK1 induction hampers the recovery of brain perfusion, outlining CBF dysfunction. DKK1 induction aggravates neuronal damage more particularly in the hippocampus and triggers anxiety-like behavior in mice. Our study suggests that DKK1 is a potent modulator of CMIs pathobiology, and thus highlight its potential as a novel therapeutic target to attenuate the cognitive deficits associated with CMIs.

P2-C-240 - Impaired Cerebrospinal fluid circulation and cerebral lymphatic drainage in a rat model of chronic Hydrocephalus

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The cerebrospinal fluid (CSF) not only protects the brain but also maintains homeostasis by removing metabolic waste produced by brain activity. This study hypothesizes that chronic CSF circulatory dysfunction, such as normal pressure hydrocephalus (NPH), may be a critical condition in neurodegenerative diseases associated with metabolic waste accumulation. To investigate the glymphatic system and cerebral lymphatic drainage in a rat model of chronic hydrocephalus induced by kaolin injection, we performed time-dependent evaluations of intraparenchymal injection of tracers or intra-cisterna magna, as well as intraventricular injection of Evans blue. The study systemically evaluated the dysfunction of CSF circulation and lymphatic drainage in the brain from various perspectives, including the glymphatic system, transependymal CSF flow, subarachnoid CSF flow, meningeal lymphatic drainage, and peripheral lymphatic drainage to deep cervical lymph nodes. The results indicated delayed glymphatic and cerebral lymphatic drainage in the kaolin-induced hydrocephalus model. Based on these findings, our research indicated that dysfunction of CSF circulation, as observed in conditions such as NPH, may act as an initiating or exacerbating factor in neurodegenerative diseases. This can lead to the accumulation of metabolic waste, as seen in Alzheimer's disease. Our research can help identify risk factors and provide insight into the underlying pathophysiology of neurodegenerative diseases, which may lead to the development of novel therapeutic strategies.

P2-C-241 - Cellular characterization of novel GPM6A (Glycoprotein M6a) point mutation in hiPSC-derived neurons and cerebral organoids

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Glycoprotein M6a (GPM6A) is a transmembrane protein fundamental to the processes of neuronal differentiation and axonal elongation. Commonly localized in lipid rafts of neuronal growth cone lamellipodia, GPM6A transcript and protein is observed throughout the central nervous system with peak expression noted in the cerebral cortex, cerebellum, and amygdala. In humans, GPM6A mutation has been linked to several neurological deficits including neurodevelopmental disabilities. Importantly, the molecular mechanisms contributing to these abnormalities have yet to be explored. Here, we investigated the functional significance of a de novo, heterozygous, tyrosine-to-serine (Y37S) point mutation in GPM6A associated with global developmental delay, hypotonia, and autism spectrum disorder. Using human induced pluripotent stem cell (hiPSC) and CRISPR/Cas9 gene editing, GPM6A-Y37S hiPSC-derived neural progenitor cells, forebrain neurons, and cerebral organoids have been established for the first time to investigate the impact of the mutation on neuronal development and function. Canonical markers of cellular differentiation and morphology were used to confirm cell-specific expression as well as protein localization via fluorescent and brightfield imaging. Morphological, electrophysiological, and biochemical assays were performed to investigate abnormalities in neural progenitor proliferation, neurite outgrowth, and neuronal activity. We anticipate the developed models,

together with the phenotypic assessments, to shed light on the neurodevelopmental significance of GPM6A mutation.

P2-C-242 - Failure of action potential propagation along Purkinje cell axons in a mouse model of ataxia

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Autosomal recessive spastic ataxia of the Charlevoix-Saguenay (ARSACS) is an inherited ataxia highly prevalent in the Charlevoix-Saguenay region of Quebec. Cerebellar Purkinje cells are affected early in ARSACS, including changes in axonal morphology. We used a mouse model of ARSACS in which the gene *Sacs* had been knocked out (ARSACS mice) and their wild-type littermates (WT) to understand the impact of morphological changes on axonal function in ARSACS. Using simultaneous dual electrophysiological recordings from the soma and axons of individual Purkinje cells, we measured the propagation of action potentials in Purkinje cell axons. These recordings showed that action potential propagation in ARSACS axons is profoundly impaired early in disease (disease onset, ~p40) when motor deficits are mild. In many ARSACS Purkinje cell axons, action potential propagation failure increased steeply as we recorded at further distances from the soma, suggesting that axonal propagation may be passive rather than active. We investigated factors that are likely to contribute to axonal impairment and found an increase in small, focal axonal swellings near the soma in ARSACS Purkinje cells. We have not, however, found evidence that these swellings impact action potential propagation in ARSACS Purkinje cells. Given the severity with which action potential propagation is affected early in disease progression, it seems likely that axonal dysfunction is an important contributor to ARSACS pathology. Understanding its underlying causes could be vital for treating this and related diseases.

P2-C-243 - Sex differences in the expression and connectivity of cortical parvalbumin-interneurons

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Parvalbumin inhibitory interneurons (PV-INS) are the largest subclass of interneurons in the cortex and are important for maintaining excitatory/inhibitory balance. Previous work has indicated that PV-INs are particularly vulnerable to numerous types of insult. Our lab has identified sex differences in the vulnerability of retrosplenial cortex (RSC) PV-INs in various conditions including Alzheimerâ€™s Disease, traumatic brain injury and early life stress. Here, we investigated whether we could identify any baseline sex differences in PV-INs number, connectivity and function in the RSC of healthy mice that might underly the differential vulnerability between males and females. First, we show that females have fewer PV-INs than males and, the PV-INs in females are less likely to be surrounded by protective perineuronal nets than in males. In females, we also found that PV-INs had a greater number of presynaptic contacts per PV cell than in males. This pattern suggests that the loss of an individual PV-IN may have a greater functional impact in females compared to males. To test this idea, we used chemogenetic inhibition of RSC PV-INs. We found that females were more impacted in terms of RSC hyperexcitability and behavioural changes in a trace fear conditioning task. Together, these results confirm that sex differences exist in PV-IN expression and connectivity which, makes this neuron population more vulnerable to insult in females compared to males.

P2-C-244 - Olfactory function in prodromal Parkinson's Disease: Alpha-synucleinopathy triggered in the anterior olfactory nucleus

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Parkinsonâ€™s disease (PD) is typically diagnosed at stages 3/4 when motor deficits and severe neuropathology are evident. Approximately 90% of PD patients display olfactory deficits, preceding motor symptoms by at least 4 years. Alpha-synuclein (a-syn) aggregates, the pathological hallmark of PD, first appear in the anterior olfactory nucleus (AON). The AON is a hub integrating top-down contextual input from the hippocampus and bottom-up sensory input from the olfactory bulb, subserving both simple odour detection and discrimination, as well as higher-order olfactory memory. Albeit a-syn accumulates in the AON early in PD, its role in pathology and symptom manifestations remains unclear. This study addresses this gap by examining the progressive spread of a-syn following intracerebral injections of a-syn preformed fibrils (PFFs) in the AON of transgenic (Tg) A53T mice. Olfactory function is assessed over time using a robust go/no-go paradigm we developed. As anticipated, baseline olfactory performance remains unaffected in Tg mice without PFF injections. Olfactory sensitivity appears to increase in PFF-injected mice from 1 to 3 months post-injection, aligning with prior findings of enhanced olfactory detection upon AON inhibition. We hypothesize that olfactory memory and motor deficits will emerge in PFF-injected mice with histology analysis revealing a-syn pathology and neuroinflammation. This study will contribute to understanding the mechanisms underlying olfactory deficits in PD and developing a reliable and cost-effective diagnostic tool for early PD identification.

P2-C-245 - Characterization of EEG epileptiform activity after repetitive mild Traumatic Brain Injuries (rmTBIs) in APP/PS1 mouse models of Alzheimer's disease

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Traumatic brain injuries (TBIs) are a major public health issue, with an approximate annual rate of 165,000 TBIs per year in Canada. Epileptiform abnormalities can surface after TBIs which can lead to post-traumatic epilepsy or long-term pathophysiological changes in the brain. In animal studies epileptiform activity has been observed acutely after TBIs and is thought to exacerbate Alzheimer's disease pathology. We aim to characterize intracranial electroencephalographic (EEG) activity one month after repeated mild TBIs (rmTBI) in APP/PS1 mice. We also assess neurological injury by measuring neuroinflammatory biomarkers in blood plasma, amyloid-beta neuropathology, and sleep. We used the closed-head impact model of engineered rotational acceleration (CHIMERA) method to deliver rmTBIs to female and male APP/PS1 mice (n=18), at six months of age. One month after rmTBIs, we implanted EEG cortical electrodes and recorded 72 continuous hours of EEG, followed by brain and plasma sample collection. Using the Seizure Pro Sirenia software, we quantified seizures and epileptiform activity. We found that only 2 mice exhibited seizures one-month post-TBI. There were no group differences in the quantification of epileptiform activity or AÎ² plaque in the cortex and hippocampus. However, we detected increased neurofilament light (NF-L) in the plasma of the rmTBI mice, indicating sustained neurological injury at this timepoint. Our study suggests that rmTBIs do not cause enduring seizures in the APP/PS1 mouse model.

P2-C-246 - Effects of fatty acid amide hydrolase inhibition on functional connectivity of the amygdala in adults with post-traumatic stress disorder

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Background: The endocannabinoid (eCB) system is a neuromodulatory system that regulates stress and fear, two processes that are dysregulated in post-traumatic stress disorder (PTSD). The eCB ligand

anandamide (AEA), which is degraded by fatty acid amide hydrolase (FAAH), is believed to play a role in these behaviors, particularly in mediating amygdala reactivity to threat. Potentiating AEA signalling through FAAH inhibition may be a promising mechanism to enhance amygdala function and promote adaptive fear and stress regulation in PTSD.
Methods: We tested the hypothesis that FAAH inhibition alters the functional connectivity of the amygdala in PTSD patients. In this randomized controlled trial, patients (n = 101) received 25 mg of a FAAH inhibitor (JNJ-42165279) or placebo twice a day for 4 weeks, and then underwent a resting-state functional MRI scan.
Results: After controlling for motion and sex, we found a significant main effect of treatment group in one cluster (14 voxels; voxel-wise p = 0.002; cluster Î± = 0.05), such that the FAAH inhibitor group exhibited greater functional connectivity between the bilateral amygdala and left intracalcarine cortex as compared to the placebo group.
Conclusions: These findings suggest that pharmacologically increasing AEA in PTSD may alter the functional connectivity between the amygdala and primary visual areas. Future research is needed to explore if this is associated with changes in symptom severity.

P2-C-247 - Chronic neuropathologies in a transgenic mouse model of tauopathy, using CHIMERA interfaced and direct impacts

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Objective

To induce two paradigms of Closed Head Injury Model of Engineered Rotational Acceleration (CHIMERA) TBI (a single interfaced head impact, or repetitive direct impacts) to rTg4510, and to investigate neuropathologies at 1-d, 1-mo, and 6-mo post-injury.

Methods

Two TBI paradigms: in the high-energy single TBI arm (sh), 2-mo male rTg4510 received either interfaced head impact (3.4J), or sham (with anesthesia and body restraint, but no head impact). In the low-energy repetitive TBI arm (rl), mice received either 6 direct head impacts (6x0.7J) within 12 days, or 6 sham injuries. Brain tissues and cardiac plasma samples were collected at 1-d, 1-mo, or 6-mo post-final injury. Longitudinal saphenous plasma samples were collected every 2 weeks until sample harvest.

Results

In sh-TBI, 27.5% of TBI mice (19/69) died immediately after TBI or reached humane endpoint. Surviving mice had significantly prolonged LRR duration compared to sham ($p<0.001$). In rl-TBI, there was 0% mortality and no significant increase in LRR ($p=0.5902$). Iba-1 immunostaining was increased in the optic tract at 1-mo post-injury in both TBI paradigms. Interestingly, at 6-mo post-TBI, total tau (DA9) was decreased in frontal cortex of sh (0.0093) but increased in entorhinal cortex of rl ($p=0.0170$).

Conclusions

In 2-mo rTg4510 mice, a single high-energy CHIMERA interfaced head impact (1x3.4J) induced mortality and increased LRR. This was not observed in repetitive, low-energy direct head impacts (6x0.7J). Current data suggests that long-term brain tau burden may be influenced by TBI paradigm.

P2-C-248 - Phenomic characterization of C. elegans orthologs of Parkinson's disease-associated genes

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Our current understanding of the genetic contributions to Parkinsonâ€™s Disease (PD) has been expanded by advances in genome wide association studies in the past decade, but efforts in the functional characterization of newly identified risk loci have lagged behind the rate of new discoveries. To address this issue, I established a pipeline for *in vivo* characterization of orthologs of newly identified PD risk loci. *C. elegans* is an effective system for such studies because *C. elegans* have orthologs to many PD-associated and biologically relevant genes, and our lab developed the Multi-Worm Tracker (MWT) for high-throughput characterization of behavioural and morphological phenotypes in populations of freely behaving animals in real time. Phenotyping 180 mutant strains with mutations in orthologs of PD-linked will yield unique phenotypic profiles spanning up to 30 features for 83 disease-linked gene orthologs. From the data generated, I will build a machine learning model to classify likely PD-linked genes from genes with unassigned significance to PD. I will also perform hierarchical clustering of phenotypic profiles to predict novel gene interactions and uncover potential molecular pathways involved in PD. This research will establish high-throughput genotype-to-phenotype characterization of newly identified risk genes for PD, and identify new functional interactions and gene networks to inform future disease modelling efforts and further our understanding of the biological processes underlying PD.

P2-C-249 - Embodiment in virtual reality for chronic pain patients: A resting-state EEG pilot study

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BACKGROUND AND AIM: In recent years, virtual reality (VR) has emerged as a promising alternative for alleviating chronic pain (CP), with VR-embodiment being a key theorized mechanism of its effectiveness. This mechanism involves establishing a perceptual connection between the individual and their virtual representation through virtual avatars, potentially normalizing distorted body representations in CP individuals. Despite its potential, the impact of VR-embodiment on neural processes linked to CP, particularly in alpha (8-12Hz) frequency bands, remains poorly understood. METHOD: This experimental, exploratory pilot study recruited 14 CP patients (female, age-matched) who experienced a meditative virtual environment either with or without a gender-matched synchronized avatar. Pre- and post-VR resting-state EEG signals and subjective scores were collected. RESULTS: Results revealed increased alpha power post-VR in both groups, notably lower frontal alpha power in the Avatar group. Additionally, The cluster-based permutation test found a significant intergroup difference in beta oscillations (12-27 Hz) clusters over the parietal lobe, with lower beta power in the avatar group. Beta has been proposed to be linked to brain-wide activity during the predictive coding. CONCLUSION: These findings suggest that having an avatar in VR may modulate neural processes related to CP, specifically in the alpha and beta frequency bands. Further confirmatory studies are needed to better understand the underlying mechanisms of VR-embodiment in CP management

P2-C-250 - The effects of increased heroin availability on punishment-induced abstinence and stress-induced relapse in rats

Erin Page [1], **Augustin Pawlak** [1], **Catarina Borges** [1], **Emily Ah-Yen** [1], **Danna Morales** [1], **Samantha Dorrance** [1], **Uri Shalev** [1]

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A defining characteristic of addiction is the persistent drug use, despite the negative consequences. In addition, the likelihood of relapse remains one of the main challenges regarding the treatment of substance use disorder. Research focusing on the underlying mechanisms involved in relapse is critical for the development of effective treatments. Our research has demonstrated that environmental stressors, such as caloric restriction, during periods of abstinence, reliably increase the probability of relapse. It is not known whether increasing the availability of the drug will impact the effects of caloric restriction on relapse following punishment-imposed abstinence. This study aims to determine if a continuous 5-minute drug access under a seeking-taking chain schedule, would impact the development of punishment-imposed abstinence and acute food deprivation-induced relapse. Ten female and ten

male Long-Evan rats were trained to self-administer heroin (0.05 mg/kg/infusion). Next, punishment-induced abstinence was achieved by exposure to electric foot-shock on 30% of completed seek cycles. Two subsequent relapse tests were conducted under sated or food-deprived conditions. Preliminary results show that, in males, food deprivation led to a robust increase in drug seeking. This provides evidence that extended access, which more closely mimics the human condition, intensifies stress-induced relapse. In future experiments, we will compare the resistance to punishment-imposed abstinence following limited and extended access to heroin.

P2-C-251 - Investigating the role of Claudin-11 in Hypomyelination Leukodystrophy using a novel humanized knock-in mouse model

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Claudin-11, a tight junction protein encoded by the gene CLDN11, plays an important role in stabilizing the apposition of myelin sheath membranes in the central nervous system and potentiating their insulative properties. De novo stoploss mutations in CLDN11 were recently identified as a cause of a novel form of hypomyelinating leukodystrophy, termed HLD22. The aim of this study was to generate and characterize a humanized knock-in (KI) mouse model carrying the stoploss mutation found in patients. We observed clear signs of brain pathology in the KI mice, signified by reduced brain weight and size, hypomyelination as assessed by BlackGold staining, and altered myelin ultrastructure based on electron microscopy analysis of callosal axons. RNA-seq analysis of the white matter-rich spinal cord highlighted a number of dysregulated biological processes, including pathways related to cell adhesion and junction assembly, dendrite development and morphogenesis, and locomotory behaviour. Immunoblot analysis of brain tissues indicated that the expression or stability of mutant claudin-11 is compromised. Finally, these neuropathological changes were associated with impaired locomotion. In summary, our findings demonstrate that this KI mouse model may be a valuable tool for further understanding the disease mechanisms of claudin-11-related HLD and for preclinical therapeutic studies.

P2-C-252 - The role of corticotropin-releasing factor in acute food deprivation-induced relapse to heroin seeking after punishment-imposed abstinence in rats

Danna Morales [1], **Samantha Dorrance** [1], **Catarina Borges** [1], **Emily Ah-Yen** [1], **Erin Page** [1], **Uri Shalev** [1]

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Treatment of substance use disorder (SUD) poses a significant challenge, particularly in addressing relapse. This study investigates the role of corticotropin-releasing factor (CRF) in stress-induced relapse within a distinctive rat model, aiming to enhance ecological validity and address existing model criticisms. The research aims to understand the impact of CRF on acute food deprivation-induced relapse following punishment-imposed abstinence in Long-Evan rats, offering valuable insights into potential therapeutic interventions for SUD. Rats underwent a protocol involving heroin self-administration under a seeking-taking chain schedule, probabilistic footshock punishment-imposed abstinence, and acute food deprivation as a relapse trigger. Preceding heroin-seeking relapse tests, CRF-receptor antagonist injections were administered intracerebroventricularly. Preliminary data reveal a noteworthy reduction in seek lever responses among food-deprived rats treated with the CRF antagonist, suggesting a potential attenuation effect. Exploring the role of CRF in an ecologically relevant animal model, contributes to advancing our understanding of stress-induced relapse mechanisms. Positive results would enable the continued utilization of this model to identify relevant brain circuits associated with stress-induced relapse, while negative results would indicate improved ecological validity of the punishment-imposed abstinence procedure compared to existing stress-induced relapse models, such as the reinstatement procedure.

P2-C-253 - Noradrenergic depletion fosters resilient phenotype in a mouse model of depression

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Major depressive disorder severely impairs daily functioning and quality of life, with stress playing a significant role in its development. The locus coeruleus noradrenergic (LC-NE) system plays a significant role in adaptive stress response. To investigate the NE system's contribution to resilience to stress-related depressive disorders, knockout (KO) mice with vesicular monoamine transporter type-2 depletion in dopamine beta hydroxylase neurons were used, disabling central noradrenaline (NE) release. WT and KO mice underwent learned helplessness test, a depression-like behavior model, with footshock escape parameters (failures and escape latency) used for k-means clustering to classify mice

into resilient (RES) and susceptible (SUS) cohorts.
Using fiber photometry to track the activity change of LC-NE neurons in WTs throughout the test, we found that in RES mice area under the curve centered on shock stimulation was significantly lower. Notably, 10 days post-inescapable footshock, the number of SUS KO animals decreased by 36.4%, while SUS WT only by 7.4%, signifying more rapid extinction of SUS phenotype in KO mice. Finally, we found that DREADD-mediated inhibition of NE neurons led to a marked decrease in failures and escape latency compared to sham controls, confirming that acute manipulation of LC-NE activity results in a more pronounced RES phenotype.
Taken together, our results provide new insights into the neurobiological basis of resilience and inform the development of more effective and personalized clinical treatment for stress-related depressive disorders.

P2-C-254 - Comprehensive analysis of cytokines in ventromedial prefrontal cortex of depressed suicides: support for the hypothesis of chronic low-level neuroinflammation in depression

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Pre-clinical and clinical evidence has implicated inflammation in the pathophysiology of depression. Abnormal cytokine levels in blood, cerebrospinal fluid, and post-mortem brain samples have been associated with depression and suicidality. To our knowledge, however, a comprehensive analysis of cytokines in brain samples from patients with depression has yet to be conducted. In the current study, we performed comprehensive profiling of 80 cytokines, chemokines and growth factors in well-characterized ventromedial prefrontal cortex (vmPFC) samples (Douglas-Bell Canada Brain Bank) from 37 depressed suicides (16 cases with and 21 cases without a history of severe childhood abuse) and 17 matched sudden-death controls. Human antibody array (RayBioÂ®, chemiluminescent detection) was used to measure all markers. Only IL-6 and CCL13 displayed a significant incrasecompared to controls, and exclusively in cases with no history of child abuse. We also conducted a morphological analysis of Iba1-immunostained microglia in vmPFC grey matter in the same three subject groups (n=15/group). Distribution of the various morphological phenotypes assessed (ramified, primed, activated and, ameboid) was similar between groups, indicating that microglia/macrophages do not display particular signs of priming/activation in the vmPFC of depressed suicides with or without child abuse history. These results support the increasingly prevalent view that chronic low-level neuroinflammation accompanies depressive symptoms in a subset of patients.

P2-C-255 - Z944, a novel T-type calcium channel inhibitor, reverses hyperalgesia in acute and extended morphine withdrawal

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Opioid use disorder (OUD) is a major contributor to drug-related deaths worldwide. Cessation of opioid use for detoxification causes severe withdrawal symptoms, including prominent hyperalgesia â€“ a contributor to the negative reinforcement of drug taking. Here we examine the effects of Z944, a first-in-class selective T-type calcium channel blocker, that is a prominent candidate drug for pain disorders. We used a rat model of morphine withdrawal-induced hyperalgesia to evaluate the efficacy of Z944 on pain tolerance in the von Frey test for mechanical hyperalgesia. Morphine (15 mg/kg, i.p.) was given once a day, 5 days/week, modelling intermittent access during human opioid use scenarios. Von Frey tests were conducted 2-3 times a week ~23 h after a morphine injection. Animals subjected to 3 weeks (15 treatments) of morphine experienced a ~35% increase in pain sensitivity which was improved by acute administration of Z944 (p.o.) at 10 mg/kg; whereas subtherapeutic doses of 2.5 and 5 mg/kg had an insignificant effect. Seven-day treatment with Z944 (10 mg/kg) in morphine-dependent animals undergoing extended withdrawal resulted in a significant increase in paw retraction thresholds, indicative of a diminution of morphine withdrawal-induced hyperalgesia. Importantly, this improvement persisted after treatment, with 5 times faster recovery to pre-morphine pain tolerance compared to controls. These findings suggest that intervention with Z944 during detoxification improves treatment outcomes, implicating T-type calcium channels in the pathogenesis of withdrawal-associated pain.

P2-C-256 - Flp-dependent α-syn (A53T) overexpression in a mouse model to study selective vulnerability in Parkinson's Disease

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[1] McGill University

Parkinsonâ€™s disease (PD) is the second most common neurodegenerative disease and is defined by motor symptoms, Lewy body (LB) propagation, and the loss of dopaminergic (DA) neurons. Although L-DOPA has been used to manage symptoms since the 1960s, there is still no medication to slow down or prevent PD. Several lines of evidence show that substantia nigra pars compacta (SNc) neurons expressing Aldh1a1 are more vulnerable to neurodegeneration than other types of DA neurons. In order to study selective vulnerability in mice, we generated a Flp-dependent viral vector overexpressing human alpha-synuclein (A53T) (Î±-Syn), a protein present in LB and associated with familial cases of PD. After injecting

this virus in Dat-2A-Flpo mice, we observed a progressive loss of TH+ DA neurons at starting 4 weeks after injection. The loss of TH+ neurons showed some correlation with locomotor behaviour as assessed in the openfield test but not in rotarod and cylinder tests. To determine if our model reflects the selective vulnerability of Aldh1a1+ neurons observed in the PD brains, we have characterized the loss of Aldh1a1-expressing DA neurons compared to Calbindin-1-expressing DA neurons exposed to Î±-syn stress. Our model in conjunction with other intersectional genetic tools will allow us to observe changes in cellular features of different DA neuron subtypes leading up to degeneration.

P2-C-257 - Repurposing cellulose ethers as emerging therapeutics to treat Alzheimer's disease

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Alzheimerâ€™s disease (AD) is a progressive neurodegenerative disease, and the major cause of dementia, with no curative therapy available. Currently, over 750,000 Canadians and 50 million individuals globally are affected by AD, and cases numbers are expected to triple by 2050, because of aging populations. Hence, novel therapeutics are urgently needed to treat AD. Our objectives were to test the therapeutic effect of TC-5RW, a representative of FDA-approved food/pharmaceutical additives, cellulose ethers (CEs), in AD using *in vitro* and *in vivo* models. TC-5RW (10 Âµg/ml) inhibited amyloid beta (AÎ²) aggregation and neurotoxicity in AÎ²-exposed neuronal cells. We determined that single and weekly treatment (4g/kg subcutaneously) with TC-5RW rescued memory impairment of transgenic 5XFAD mice. We confirmed that TC-5RW treatment significantly inhibited AÎ² oligomer and plaque burden and its associated neuroinflammation via reducing activated astrogliosis, microgliosis, and proinflammatory mediator glial maturation factor beta (GMFÎ²). Notably, we also found that TC-5RW reduced lipopolysaccharide (LPS)-induced activated gliosis and GMFÎ² as well as other key neuroinflammation mediators including NLRP3 inflammasome and its associated markers (Caspase-1, Asc1). Our intriguing results show that TC-5RW has a significant therapeutic effect against AÎ² pathologies and cognitive impairments, and potent anti-inflammatory activity to rescue neuroinflammation. In summary, for the first time, we tested and validated FDA-approved TC-5RW as a potential, emerging, and effective compound to halt and treat AD.

P2-C-258 - Evaluating the protective roles of microglia against chronic amyloid beta treatment in human iPSC-derived neurospheres

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Over the past decades Alzheimerâ€™s Disease (AD) research utilized murine AD models to study disease mechanisms to identify potential therapeutic targets. However, the translation from murine AD models to human AD treatments has not been successful. In addition, cell culture models using human cells have been restricted to 2D *in vitro* cultures that do not recapitulate major disease hallmarks, such as the formation of AÎ² plaques, and provide limited options to study the effect of microglia, prime targets in the search of novel treatment strategies. Here we present a novel 3D neurosphere model, consisting of mature neurons and astrocytes, grown from human iPSC-derived cells in which we successfully induced plaque-like AÎ² aggregation after chronic AÎ² treatment. Our model allows for the timed introduction of iPSC-derived microglia into the mature neurosphere to study AÎ²-microglia interaction in order to unravel new disease pathways. We found that these spheres display high levels of spontaneous neuronal activity which is perturbed by prolonged AÎ² exposure leading to oxidative stress and ultimately the death of neurons. We transferred microglia at two different timepoints during chronic AÎ² exposure to test their impact on neuronal function. Infiltrating microglia display high phagocytic activity towards AÎ² and reduced extracellular AÎ² deposits, loss of neuronal activity and oxidative stress when added early. Microglia transferred late during AÎ² exposure ameliorated neuronal death but failed to recover neuronal function or reduce oxidative stress at this time point.

P2-C-259 - Sensory and habituation profiles of ASD risk gene orthologs highly expressed in C. elegans glia

Catharine Rankin [1], **Lexis Kepler** [1]

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Alterations in sensory processing and habituation, a form of non-associative learning where one learns to stop responding to repetitive stimuli, are reported in up to 90 percent of Autism Spectrum Disorder (ASD) cases. Recent studies have found that impairing select ASD risk genes in glia directly contributes to neurophysiological and behavioural phenotypes associated with ASD. However, for the majority of ASD risk genes, there is limited knowledge on whether altered gene function in glia contributes to phenotypic impairments. Here, we used our labâ€™s recently published phenomic screen of ASD-risk gene orthologs, and a *C. elegans* single-cell gene expression database, to identify the sensory and habituation profiles of orthologs of ASD-risk genes highly expressed in *C. elegans* glia. For each glia cell, we identified subsets of risk genes that affected multiple phenotypes similarly. These findings suggest that select ASD risk gene orthologs may act in common pathways in glia which contribute to sensory responsivity and habituation. Taking advantage of the cell-specificity of the Auxin-Inducible Degradation system, we are currently working to create glia-specific degradation lines to confirm the glia-specific role

of ASD risk gene orthologs in the affected phenotypes and assess the temporal requirements of gene function across the lifespan.

P2-C-260 - Reelin reverses alterations in microglial morphology induced by chronic corticosterone

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Depression is a leading cause of disability worldwide, and existing treatments do not effectively resolve symptoms for all those affected. Inflammation has been linked to treatment resistance, and immune dysfunction is common to depression. Reelin, an endogenous protein, is depleted in depression, but reverses despair-like behaviour in chronically stressed animals. The morphology of microglia changes with inflammation, and reelin has been linked to inflammatory functions, but relationships between reelin and microglia are poorly understood. To evaluate whether reelin alters microglia morphology in chronic stress, rats were administered daily corticosterone (CORT, 40 mg/kg) or vehicle injections for 21 days and reelin (3 Î¼g) or vehicle tail vein injections on days 11 and 21. Immunohistochemistry for CD11b was conducted on free floating sections to visualize hippocampal microglia. Microglial cell outlines (30 cells/subject), soma (30 cells/subject), and processes (6 cells/subject), were traced to evaluate microglial morphology. CORT increased soma sizes, which were normalized with reelin treatment. Vehicle treated rats receiving reelin also had increased soma sizes. Data shows that processes were retracted in reelin-treated and CORT-treated rats, but partially recovered in rats receiving CORT and reelin. Results suggest that both CORT or reelin alone were pro-inflammatory, but reelin reversed the inflammatory-like morphology in CORT-treated rats. Considering these findings, we suggest that reelin homeostasis is important in the regulation of neuroinflammation.

P2-C-261 - Impaired habituation of cortical sensory responses to unreinforced repetitive stimuli in huntington disease model-mice

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Huntington disease (HD) is a fatal inherited neurodegenerative disorder, characterized by progressively disordered cognition and movement, largely due to early cortical and striatal neuron dysfunction and subsequent degeneration. Our group showed that equivalent sensory input, from various modalities, activates greater cortical surface area in HD model-mice (sensory spread). We now seek to elucidate mechanisms underlying sensory spread and determine how it impacts behavior and cognition, using zQ175 HD mice selectively expressing GCaMP sensors in cortical pyramidal neurons or parvalbumin interneurons, in conjunction with chronic cortical mesoscale imaging. We show that sensory spread is evident in zQ175 by 8 months (m) of age but is not seen in younger (4m-old) animals. Young zQ175 mice do show reduced cortical functional connectivity, possibly due to loss of intracortical pyramidal neuron synapses â€“ as suggested by our recently published in vitro study. We hypothesize sensory spread is a consequence of reduced cortical inhibition, which is itself compensatory for this reduced functional excitatory connectivity. We are examining how sensory spread affects non-associative habituation learning, whereby responses to repetitive closely timed sensory stimuli diminish upon repetition. Cortical responses to repeated, unreinforced visual stimuli indeed decrement less over time in zQ175 mice. We hypothesize deficient cortical inhibition underlies impaired habituation of cortical responses and are exploring this directly and examining impacts on associated startle behaviors.

P2-C-262 - Investigating metabolic signatures of Microglia during Multiple Sclerosis progression

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Multiple sclerosis (MS) manifests as a demyelinating and degenerative condition within the central nervous system. While effective treatments for relapsing-remitting MS have been developed, drugs that effectively impede MS progression are still elusive. Microglia, the brain-resident macrophages, efficiently phagocytose damaged myelin, creating a permissive environment for remyelination. However, during chronic activation, microglia lose phagocytic capabilities, and likely contribute to the progression of MS. Our preliminary findings indicate that microglia within the spinal cords of experimental autoimmune encephalomyelitis (EAE) mice, a model of MS, express lactate dehydrogenase A, an enzyme that converts pyruvate to lactate, during peak EAE severity (day 16-18). Additionally, we observed an increase in monocarboxylate transporter-4 (a lactate exporter), and its chaperone, extracellular matrix metalloproteinase inducer (EMMPRIN). Disrupting this pathway through siRNA-mediated EMMPRIN knockdown diminished lactate export, inhibited glycolysis and reduced TNF-Î± production in LPS-stimulated microglia. Further, this led to enhanced myelin phagocytosis in these cells. To find whether chronic activation of microglia leads to mitochondrial dysfunction, we are studying the metabolic differences in microglia between peak and chronic EAE (>day 30) using techniques like met-flow, confocal microscopy, and single-cell RNA sequencing. We anticipate that this work will uncover novel metabolic players and pathways that can be targeted therapeutically to address the progression of MS.

P2-C-263 - IRE1 activation in spinal cord development and repair in the Zebrafish model

Kayla Talabis [1], **Susan Logue** [1], **Benjamin Lindsey** [1]

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The Unfolded Protein Response (UPR) is a cellular pathway that functions in the maintenance of proteostasis in response to endoplasmic reticulum stress. Recent studies implicate the IRE1 branch of the UPR in the development of the CNS and in response to spinal cord injury (SCI). To date, studies have focused mainly on mammalian models and thus we know little about the role of the UPR in the CNS of other vertebrates. In this study, we take advantage of the many attributes of the zebrafish model, including its capacity for neurorepair, to investigate the activation of IRE1 during spinal cord development and following SCI.

Employing the *Tg(xbp1s:eGFP)* transgenic reporter fish that expresses GFP upon IRE1 activation, we show that IRE1 is strongly upregulated in early larvae compared to juveniles and adults. High levels of IRE1 expression are displayed at 4-days post fertilization (dpf) before decreasing at 16-dpf and declining into adulthood. Interestingly, when exposed to the ER stress inducing drug Dithiothreitol (DTT), larvae at 4-dpf also show elevated levels of IRE1 activation compared to later stages of development, suggesting IRE1 is less inducible with age. We next established a larval SCI model to ask whether IRE1 signaling was upregulated after SCI and returned to physiological levels upon regeneration. Based on our preliminary observations, we predict that upon SCI, IRE1 activation peaks early at 1-hour post-SCI before starting to attenuate. Collectively, our study demonstrates a role for IRE1 in early spinal cord development and the initiation of spinal cord repair.

P2-C-264 - Investigating the ischemic cellular responses of human iPSC-derived neurons and rodent cortical neurons: A comparative study

Marzieh Sedighipour Chafjiri [1], **Jacqueline Vanderluit** [1]

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Stroke is the second leading cause of death globally and the leading cause of long-term disability. In the days post-stroke, infarct size can expand due to delayed cell death of injured neurons in the penumbra surrounding the infarct core. Reperfusion treatments in the clinic can rescue some of these neurons; however, there are no treatments targeting neuron survival. Although novel strategies to rescue injured neurons have proven effective in preclinical stroke studies they have failed to translate into effective clinical treatments. Until recently, most of the preclinical stroke studies were performed on rodents. The development of human induced pluripotent stem cells (iPSC) has provided an opportunity to study how

human neurons respond to an ischemic stroke in real-time. The goal of my study is to compare the cellular responses to ischemic stroke between cultures of human iPSC-derived neurons and mouse neurons. To replicate the ischemic conditions of the infarct penumbra, oxygen-glucose deprivation (OGD) was performed using 3% oxygen for 3 hours. Cultures were collected at 24 hours and the rates of cell death and axon degeneration were compared. Hypoxia was confirmed with the translocation of hypoxia-inducible factor-1 to the nucleus following OGD. Notable differences were observed in the cellular composition of control cultures of human and mouse neurons. Following OGD, differences in cell death were observed. Our findings will aid in understanding the differences and similarities between the responses of human and mouse neurons to stroke-like conditions.

P2-C-265 - Rapid and efficient generation of oligodendrocytes from patient-derived iPSCs to study ALS

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INTRODUCTION: In the central nervous system (CNS), oligodendrocytes are responsible for the myelination of axons and are involved in various neurological diseases. The limited access to human oligodendrocytes from patients is a major obstacle for disease modeling. Induced pluripotent stem cells (iPSCs) are genetically reprogrammed stem cells that can self-renew indefinitely in vitro and differentiate into all cell types. **OBJECTIVES:** The objectives of the project are to generate iPSC-derived oligodendrocytes from patients with Amyotrophic Lateral Sclerosis (ALS) and develop a 3D in vitro model using iPSC-derived oligodendrocytes and motor neurons. **METHODS:** iPSC lines derived from patient cells carrying specific known mutations will be produced and characterized. The iPSCs will then be redifferentiated into oligodendrocytes. Immunofluorescence (IF), RNA-seq and qPCR analyses will be used to evaluate oligodendrocytes' terminal differentiation potential and characterize their gene expression profiles. **RESULTS:** A protocol allowing the differentiation of iPSCs into oligodendrocytes was established. Our results showed that the generated cells express oligodendrocyte markers after 28 days of culture and promote motor neuron myelination in a 3D co-culture model. **CONCLUSION:** A rapid and efficient protocol for generating oligodendrocytes is key for disease modeling and to better understand neuropathological mechanisms associated with ALS.

P2-C-266 - Molecular and synaptic adaptations promote resilience in the ataxic cerebellum

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Regional heterogeneity is important for normal function in the cerebellum and can be disturbed in disease. Atrophy in spinocerebellar ataxia type 6 (SCA6) has been reported to be restricted to the anterior cerebellum while the posterior is spared. Does this mean that the posterior cerebellum is untouched by disease in SCA6? Answering this question will shed light on mechanisms of adaptation in the cerebellum and may lead to novel treatments. Here we showed that in a mouse model of SCA6 (SCA6 mice), degeneration resembles that reported in patients: anterior zone Purkinje cell degenerated while nodular zone Purkinje cells survived. Long before degeneration takes place, anterior Purkinje cells in SCA6 mice fired with reduced frequency and regularity compared to litter-matched WT mice, while nodular Purkinje cells firing was unaffected. Surprisingly, using RNA sequencing, we discovered the majority of differentially expressed genes in SCA6 were in the posterior cerebellum that does not degenerate. Intriguingly, many genes gained regional expression patterns, suggesting that SCA6 enhances regional heterogeneity. Using gene ontology, we identified that genes at parallel fiber to Purkinje cell synapses were significantly altered in posterior cerebellum. To corroborate this, we measured the density of parallel fiber synapses and found that they were unchanged in anterior zone but reduced in nodular zone. Taken together, we report unexpected molecular and connectivity adaptations in posterior cerebellum in SCA6 that may protect Purkinje cells from degeneration.

P2-D-267 - Individual neural representations during overt speech through electroencephalogram-based classification analysis

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Neural representation of speech has classically been known to reside in Broca's area. However, recent researches that decoded speech information from implanted electrodes have demonstrated the effectiveness of utilizing signals from the motor cortex. In this study, we investigated how the neural representation of speech varies among individuals using electroencephalography (EEG), which allows for the measurement of brain activity across the entire brain.

Five native Japanese speakers were instructed to read aloud 68 Japanese phonetic characters displayed on a screen as quickly as possible. During the task, continuous recording of EEG with 64 channels took place.

To investigate the activity differences in anatomical brain regions, cortical current source signals were estimated from the acquired EEG. Eight statistical values of the time-domain signals (i.e., maximum, minimum, mean, variance, etc.) were computed for each source and used as feature values for the input of the support vector machine for the binary classification of the five vowels corresponding to the characters.

The classification accuracies varied by class, participant, and current source. Two out of five participants showed higher accuracy in the right hemisphere than in the left hemisphere. The regions of high accuracy were mainly in the language area including the Brocaâ€™s area, but different area, such as motor-related area and auditory area, could also significantly classified depending on the participant.

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P2-D-268 - How different tDCS electrode placements affect the corticospinal excitability: A TMS-MRI study

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Transcranial Direct Current Stimulation (tDCS) applied to the primary motor cortex (M1) has been shown to modulate corticospinal excitability (CSE). This study investigates the impact of two common tDCS electrode setups: conventional, with the active electrode placed directly on M1, and Posterior-Anterior (PA), with the active electrode on the posterior and the return electrode on the anterior sides of M1. Current flow modeling (CFM) studies suggest that these setups may result in varied current distributions, potentially influencing CSE differently. PA tDCS may offer deeper and more effective M1 stimulation than conventional tDCS. This study investigates if these setups differently impact CSE, hypothesizing that PA tDCS yields more profound and consistent modulation, while conventional tDCS may lead to localized and less reliable neuromodulation. In a within-subject study, 10 participants attended 3 days of testing with a 72-hour washout period between sessions. tDCS (1mA, 7 minutes, 25cm2 electrodes) was applied in 3 setups (Conventional, PA, sham). Single-pulse transcranial Magnetic Stimulation was used to generate motor-evoked potentials (MEPs) before and after tDCS application. MEP amplitude assessed setup impact on CSE. Preliminary statistical analysis, using a paired-sample t-test, revealed no significant differences in MEPs between conventional and PA tDCS (t=2.24, p=0.06). However, a statistically significant increase in MEPs was observed after conventional tDCS compared to baseline (t=4.18, p=0.004), while no significant change occurred after PA tDCS (t=0.27, p=0.41). Despite preliminary results differing from CFM predictions, further discussion will explore neurophysiological mechanisms and electrode placement's specific influence on CSE.

P2-D-269 - Noradrenaline modulation of interneuron subtypes of the primary motor cortex during sensorimotor learning

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The primary motor cortex is a key region for learning fine motor skills. Noradrenergic (NA) inputs from the locus coeruleus to the motor cortex have been shown to play a role in sensorimotor learning, notably in enhancing motor execution and signaling unexpected rewards. However, the impact of these NA signals on cortical circuits remains poorly understood. Our lab has recently mapped the expression of NA receptor onto the different neuronal subtypes of the cortex, revealing high levels of NA receptor transcripts in GABAergic interneurons (INs) of the superficial layers of the primary motor cortex contains high levels of adrenergic receptor transcripts. Here, we aim to characterize the influence of NA signals on the activity of VIP- and NDNF-expressing INs during sensorimotor learning. To achieve this, we will train head-fixed mice in an auditory detection task, where they learn to press a lever in response to an auditory tone to obtain a reward. Using in-vivo two-photon calcium imaging, we will compare the activity of VIP- and NDNF- expressing INs in the primary motor cortex between task-naÃ¯ve and expert mice. Our preliminary results indicate that NDNF neuron activity increases during reward delivery and this response is dependent on expectation levels, suggesting that NDNF neuron activity correlates to NA signaling during behavior. Along with repeating these experiments in VIP neurons, we are currently validating the potential contribution of NA release to these findings by testing pharmacological and gene-editing methods to alter NA receptor functions.

P2-D-270 - Restless legs syndrome drug screen in C. elegans model

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Restless legs syndrome (RLS) is a chronic sleep-related sensorimotor disorder characterized by a strong impulse to move the legs and relieve uncomfortable sensations. In 2007, a genome-wide association study identified significant associations between RLS and three genomic regions, one of which comprises a highly significant intronic variant in the homeobox gene *MEIS1*. Carriers of this particular *MEIS1* variant have a 50% increased risk of developing RLS. Using a simple and strong genetic model organism *Caenorhabditis elegant,* our team previously reported reduced expression of *UNC-62* (*C. elegans* ortholog of *MEIS1*) to be associated with iron homeostasis via an increased expression of ferritin ortholog (FTN-1). The *unc-62* orthologue is expressed in different tissues (hypodermis, intestine, and

nervous system), and its downregulation leads to a strong movement phenotype. Different *unc-62* alleles showed defects in *cat-2/tyrosine hydroxylase* (involved in dopamine synthesis). In our work, we took advantage of the mobility difference between the *unc-62(e644)* strain and the control strain (*N2*) and performed an unbiased drug screen. We identified 21 compounds (from a library of ~4,000 compounds) showing promise in their ability to rescue the mobility of *unc-62(e644)*. The benefits and impact of these compounds are under validation. To correlate the dopaminergic system and iron expression, we generated *unc-62* strains expressing GFP::DAT-1 and GFP::FTN-1 to monitor their expression. Our work highlights the advantages of using the unc-62 model to evaluate other genes and pathways involved in RLS.

P2-D-271 - Characterising changes to inhibitory neurotransmission in the cerebellum using a female adolescent rat model of chronic neuropathic pain

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OBJECTIVE Chronic pain is prevalent but understudied in adolescents and females. Neuroimaging evidence suggests a role for the cerebellum in pain, but its specific involvement is seldom explored. Therefore, this study characterised changes in nociception and cerebellar GABAergic function using the sciatic nerve chronic constriction injury (CCI) model of neuropathic pain in female adolescent rats.

METHODS Female rats received a sham injury or CCI at 42-days-old. Over 28 days post-injury, the elevated plus maze (EPM) measured anxiety-like behaviour while Von Frey and Hot/Cold Plate respectively measured mechanical and thermal nociception. At 75-days-old, cerebella were collected for immunofluorescence of calbindin, GAD-65/67, and $GABA_Aa1$.

RESULTS Rats spent less time in the open arms of the EPM post-injury, compared to baseline ($p<0.05$), but there was no effect of injury ($p>0.05$). On the von Frey, the uninjured hind paw of CCI rats reacted to larger filaments than sham ratsâ€™, from post-injury day (PID) 14 ($p<0.05$). The injured hind paw of CCI rats reacted to smaller filament weights than sham ratsâ€™, between PID 5-14 ($p<0.05$). CCI rats displayed shorter latencies than sham rats to show a hind paw withdrawal to cold stimuli. Immunofluorescence analysis in the cerebellum is ongoing, but we expect a reduction to GAD-65/67 and $GABA_Aa1$ immunoreactivity post-CCI.

CONCLUSIONS We have detailed a model of chronic neuropathic pain in female adolescent rats characterised by changes in mechanical and cold nociception. This will facilitate preclinical investigations into the role of the cerebellum in chronic pain processing.

P2-D-272 - DeepLabCut-based behavioral features extraction for recognizing movement patterns in the common marmoset

Jiayue Yang [1], **Justine Clery** [1]

[1] McGill University

In systems neuroscience, movement is a fundamental feature of behavioral outcomes, which might be indicative of the animalâ€™s well-being and a particular disease. Traditional behavioral tests usually require the experimenter to observe and collect behavioral data. However, human observation is not always reproducible among experimenters and may introduce analysis errors. Thus, to mitigate the impact of human intervention, our objective is to identify behavioral components within marmosetsâ€™ home cages, devoid of any human presence. In this study, we utilized DeepLabCut, a deep-learning program for non-invasive animal behavior tracking. Marmosets were housed in family units within home cages, with their behaviors recorded via a Webcam-based system. DeepLabCut was then employed to construct a skeleton model with spatially labeled body parts. Using custom Python scripts, we analyzed the labeled animal assembly, extracting speed, inter-individual distance, and head angles of the marmosets. Our results show that DeepLabCut-based behavioral extraction enables precise quantitative predictions of various movement patterns in healthy marmosets, reflecting movement intensity, social interaction, and postural abnormality. This project acts as a reference dataset for comparing the movements exhibited by the marmoset model of synucleinopathy (Parkinsonâ€™s disease, dementia). It serves as a potential tool to detect early biomarkers, such as behavioral abnormalities of synucleinopathy, which can be eventually translated into use in human diagnosis and future clinical care.

P2-D-273 - Subversive compensation during chronic sodium channel blockade reduces the efficacy of subtype-selective sodium channel inhibitors

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Voltage-gated sodium (Nav) channels play an important role in neuronal excitability. Nav subtypes 1.3, 1.7 and 1.8 are particularly important for the excitability nociceptive sensory neurons, yet drugs that selectively block Nav1.7 or Nav1.8 have proven clinically ineffective. We investigated whether their poor efficacy is because chronic blockade of one type of Nav channel triggers compensatory upregulation of other Nav channels. Such compensation is possible since excitability is degenerate, meaning different

channel combinations can produce equivalent excitability. Cultured nociceptors normally rely on Nav1.7 and Nav1.3 for spike generation, as evident from acutely blocking either channel pharmacologically. But nociceptor excitability was not altered after blocking either channel chronically (for 24 hours or for 4-7 days). This is due to compensatory upregulation of Nav1.3 when Nav1.7 is blocked, or vice versa. When both channels were blocked with TTX, nociceptor excitability became more reliant on the TTX-resistant channel Nav1.8. Changes in channel expression were verified with immunocytochemistry. In vivo, tactile and thermal hypersensitivity caused by chronic inflammation were reversed by the first dose of a Nav1.7-selective inhibitor, but this effect was absent by the second day of testing after twice daily dosing of the drug. This loss of efficacy is consistent with the time course of compensatory changes observed in vitro. Notably, preclinical pain testing traditionally involves a single drug dose despite patients needing to take drugs chronically. Our results show that subversive compensation can reduce drug efficacy during chronic treatment.

P2-D-274 - Fully annotated connectome of Drosophila melanogaster's optic lobe

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Despite their small brains, Drosophila melanogaster flies are adept flyers, able to perform fast and intricate visually guided maneuvers. The flyâ€™s visual system has been studied for decades from the cell types that comprise it, through its computational capabilities, to the wealth of behaviours it engenders. Janeliaâ€™s FlyEM team has imaged the complete nervous system of an adult male fly using Focused Ion Beam and Scanning Electron Microscopy. Subsequently, the entire volume was reassembled and Google Researchâ€™s connectomics group carried out automatic segmentation of the volume into neuron fragments, which were then proofread by Janelia annotators.

Here, we catalogue all neurons in the right optic lobe using their morphology, followed by repeated iterations of connectivity analysis. We classified over 51,000 neurons into ~700 cell types generating a curated connectome of the complete visual system. This dataset allows for quantitative analysis of the flow of information between brain regions, which, due to the repeated columnar structure, includes variability assessment. To facilitate access to this wealth of information and encourage future use by the community, we have developed methods that register single neurons to a specific address based on their morphology, location, and connectivity. These methods position the neuron in an â€˜address spaceâ€™ that facilitates predictions about the computational function of the cell type, instructs testing of prior physiological and behavioural results, and generates new experimental hypotheses.

P2-D-275 - Mesoscale two-photon imaging of mouse cerebellar neurons during motor learning

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The cerebellum is recently found to be involved in not only supervised motor learning tasks, but also a wide range of cognitive learning tasks. The mouse cerebellum is organized into sagittal functional microzones not easily segmented by anatomical landmarks, and these microzones were tuned to different sensory stimuli and cognitive variables. However, how the coordination of them sculpts complex animal behavior remains to be elucidated. Here, we attempt to address this by employing mesoscale two photon imaging of large areas of the cerebellum simultaneously. This will be paired with the International Brain Lab (IBL) task where the mouse has to learn to turn a wheel in response to visual cues to get rewards. We show that we can record Purkinje cell complex spikes activity of many cerebellar regions and they encode different behavioral variables of the task. We hope this line of research will pave the way for a more complete understanding of cerebellumâ€™s role in motor learning.

P2-D-276 - The Role of Drosophila Calcium Binding Proteins Frequenin1 and Frequenin2 in Taxol-Induced Nociceptive Hypersensitivity

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Chronic pain can develop with the chemotherapy medication Taxol, in which prolonged use can result in nociceptive hypersensitivity. Neuronal Calcium Sensor 1, the human orthologue for *Drosophila* calcium binding proteins Frequenin1 (Frq1) and Frequenin2 (Frq2), has been shown *in vitro* to be reduced upon Taxol treatment through cleavage by the calcium-dependent protease Calpain (Calp). We hypothesize that Frq1 and Frq2 are required for nociception and that changes in Frq1 and Frq2 expression is an underlying mechanism of Taxol induced-nociceptive hypersensitivity. To determine if Frq1 and Frq2 are required for nociception, we examined frq1 and frq2 mutants and found that these mutant larvae displayed nociceptive hypersensitivity to thermal stimuli in comparison to their genetic controls. Next, we used CRISPR-Cas9 to tag the endogenous frq1 and frq2 genes with a flag epitope. We found that Frq1 and Frq2 are widely expressed in the *Drosophila* central nervous system and this localization

overlaps with the nociceptive circuit. We additionally found that Taxol-treated control larvae phenocopied frq1 and frq2 loss-of-function mutants, and overexpression of Frq prevented nociceptive hypersensitivity. Lastly, we used CRISPR to mutate the Calp cleavage site on Frq1 and Frq2 from Isoleucine-35 to Alanine-35, which was shown *in vitro* to prevent Calp cleavage, and are currently testing if this protects against Taxol-induced nociceptive hypersensitivity. Collectively, our data demonstrates a novel role for Frq1 and Frq2 in nociception and Taxol induced-nociceptive hypersensitivity.

P2-D-277 - Selective ablation of glutamatergic neurons in the lateral cerebellar nuclei causes significant motor impairment in mice

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Background:

Cerebellar nuclei (CN) constitute the sole cerebellar output and play a central role in cerebellar circuits. Accumulating evidence implicates a central role of CN connectivity in neurological diseases, including ataxia. However, because of the compact topography and close functional connection between CN and the cerebellar cortex, identifying cerebellar deficits exclusively linked to CN is challenging. In this study, we have experimentally ablated large glutamatergic neuronal (LGN) and evaluated the impairment of motor coordination in mice.

Methods:

We injected adeno-associated virus (AAV) in lateral CN of Vglut2-Cre+mice through stereotaxic surgery, followed by IP injection of diphtheria toxin (DT) to ablate LGN. Motor coordination was evaluated by rotarod and beam walking tests. Immunolabeling with anti-SMI32 and -GFP antibodies was performed in cerebellar sections.

Results:

Double staining with anti-SMI32 and -GFP antibodies confirmed expression of GFP and degenerated SMI32 positive neurons at the site of AAV injection in the lateral CN of Vglut2-Cre+ in comparison with Vglut2-Cre- mice. Analysis of motor coordination by rotarod test indicated that the latency to fall was significantly different before and after AAV/DT injection in the Vglut2-Cre+ group. Elapsed time and number of steps in the beam walking test were significantly higher in AAV/DT injected Vglut2-Cre+ AAV/DT mice compared to controls.

Conclusion:

We demonstrate for the first time that degeneration of the large glutamatergic neurons in lateral CN plays a major role in ataxic phenotype development

P2-D-278 - Harmonized cross-species cell atlases of trigeminal and dorsal root ganglia

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Peripheral sensory neurons in the dorsal root ganglion (DRG) and trigeminal ganglion (TG) are specialized to detect and transduce diverse environmental stimuli including touch, temperature, and pain to the central nervous system. Recent advances in single-cell RNA-sequencing (scRNA-seq) have provided new insights into the diversity of sensory ganglia cell types in rodents, non-human primates, and humans, but it remains difficult to compare transcriptomically defined cell types across studies and species. Here, we built cross-species harmonized atlases of DRG and TG cell types that describe 18 neuronal and 11 non-neuronal cell types across 6 species and 32 studies. We then demonstrate the utility of this harmonized reference atlas by using it to annotate newly profiled DRG nuclei/cells from both human and the highly regenerative axolotl. We observe that the transcriptomic profiles of sensory neuron subtypes are broadly similar across vertebrates, but the expression of functionally important neuropeptides and channels can vary notably. The new resources and data presented here can guide future studies in comparative transcriptomics, simplify cell type nomenclature differences across studies, and help prioritize targets for future analgesic development.

P2-D-279 - The histone methyltransferase G9a regulates expression of a cyclic GMP-dependent protein kinase and thermal nociception in Drosophila melanogaster

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[1] University of Windsor

The *Drosophila melanogaster foraging* gene (*for*) encodes a cyclic GMP-dependent protein kinase (PKG) that regulates nociception. Furthermore, PKG expression is increased in nerve injury-induced nociceptive hypersensitivity mouse models. It is unknown if *for* expression changes in response to injury in *Drosophila*. Additionally, the mechanisms by which PKG expression are regulated in response to injury remains unknown. The histone methyltransferase *G9a* has previously been shown to regulate *for* mRNA expression, and we previously found higher *for* levels resulted in nociceptive hypersensitivity, whereas loss of *for* reduced nociceptive sensitivity. However, a direct link between *for* and *G9a* with respect to nociception has not been established. We used a thermal nociception assay and found that *G9a* null mutants displayed nociceptive hypersensitivity in comparison to their genetic control. Next, we examined FOR protein levels using Western blots and an antibody that recognizes all FOR isoforms. We found that *G9a* null mutants had a higher amount of FOR protein in comparison to the control. We then used an antibody specific for the FOR P1 isoform, which was previously shown to be important for nociception, and found that FOR P1 expression was upregulated in *G9a* null mutants. Current experiments involve examining the *G9a for* null double mutants to determine if this increase in FOR expression is required for the nociceptive hypersensitivity seen in *G9a* null mutants. Collectively, our data demonstrates that *G9a* negatively regulates nociception and FOR P1protein expression.

P2-D-280 - Nociceptor neurons control vaccine-induced immunity

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Nociceptors, the sensory neurons that detect noxious stimuli and trigger pain, interact with immune cells to modulate immune responses. The nociceptor-released neuropeptide Substance P promotes B cell polarization, antibody class switching to IgE, and IgE release in models of allergic inflammation. In this study, we investigated whether nociceptors respond to vaccine adjuvants and control IgG

production and clonal selection in the context of vaccination. We activated and sensitized sensory neurons from mice with vaccines and adjuvants against influenza virus and pneumococcal and meningococcal bacteria in vitro and evaluated influenza vaccine-specific IgG antibody levels in mice with ablated nociceptors. Our results showed that sensory neurons respond to vaccines and exhibit differential activation by various noxious ligands. In mice with ablated nociceptors, IgG2c titers were reduced, while capsaicin-treated mice showed increased IgG titers. These findings suggest a role for nociceptors in maintaining humoral immunity after vaccination. We will further explore how sensory neuron ablation or overactivation affects B-cell trafficking and antibody production in response to vaccination and pathogen challenges in mice. This research provides insights into the role of nociceptor neurons in humoral immune responses during vaccination and has implications for the development of more effective vaccines.

P2-E-281 - The gut bacterial indole receptor, aryl-hydrocarbon receptor, is required in the mediobasal hypothalamus for metabolic homeostasis

Hallie Wachsmuth [1], **Nathan Connell** [1], **Rachel Meyer** [1], **Frank Duca** [1]

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Obesity prevalence is rapidly increasing, coinciding with increased consumption of a western-style diet, high in fat and sugar and low in fiber. Diet shifts the composition of the gut microbiota, which contributes to host metabolic health partially via generating metabolites, some of which can act directly at the brain. Consumption of a high-fat diet (HFD) induces hypothalamic inflammation, resulting in hyperphagia and obesity. Gut bacterial tryptophan metabolites, indoles, cross the blood brain barrier and reduce brain inflammation via the aryl hydrocarbon receptor (AhR). We found that HFD-fed rodents have drastically reduced gut indole levels compared to healthy controls. We hypothesized that reductions in hypothalamic-AhR signaling cause hypothalamic inflammation and obesity. Therefore, we injected a control or AhR shRNA AAV into the mediobasal hypothalamus (MBH) in chow-fed and HFD-fed mice. Knockdown of MBH AhR robustly increased body weight and adiposity in both diet groups via increases in weekly food intake. IHC and RNAscope revealed increased MBH astrogliosis and TNF-alpha expression in AhR knockdown groups. Lastly, we used cell-specific AAVs to knockdown AhR in neurons or astrocytes in the MBH and found that AhR knockdown in either cell type increased bodyweight and adiposity compared to controls. These data demonstrate MBH AhR is necessary for energy homeostasis and that reduced AhR signaling via decreased production of indoles may play a role in diet induced obesity, highlighting AhR as a potential therapeutic target for obesity.

P2-E-282 - Lighting the way: sex differences in circadian disruption of Degu behaviour

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The circadian system regulates a range of physiological and behavioural functions in living organisms. Disrupted circadian rhythm affects mood, learning, and memory. Yet, ramifications of circadian disruption are still not yet fully understood. Past studies have largely studied males of nocturnal species but both sexes of diurnal species may exhibit very different responses. Males of some diurnal rodents exhibit deficits in memory and increased anxiety-like behaviour following exposure to disruptive light/dark (LD) cycles, but femalesâ€™ responses are sparsely reported. We investigated the effects of circadian disruption on anxiety-like behaviour and spatial memory in both sexes of degus, a diurnal and highly social rodent. Female and male adult degus were exposed to constant light (LL) or normal 12:12 photo period for 60 days. In the last 15 days, degus were tested for spatial memory (Barnes maze, novel object placement recognition), locomotor activity (open field), and anxiety-like behaviours (open field test, and elevated plus maze). Results indicate that LL males made fewer errors and had shorter latencies to reach the escape hole across all five learning days compared to controls. In contrast, LL females made more errors compared to controls, but were still faster to reach the escape hole, suggesting that circadian disruption might impact males and females differently. Analyses of other behaviours are ongoing. We expect this work will increase our understanding of sex-specific responses to circadian disruptions in species with different daily routines and life histories.

P2-E-283 - The largest and most detailed dorsal vagal complex cell atlas

Cecilia Hes [1], **Abigail Tomlinson** [2], **Martin Myers** [2], **Paul Sabatini** [1]

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Objective: To develop a cell atlas of the mouse dorsal vagal complex (DVC).

Methods: We obtained a de novo database of DVC cells from adult C57BL/6J male mice either fasted and refed or fed ad libitum (n=10), and mice either PBS or LiCl injected (n=10). We isolated DVC nuclei and subjected them to sn-RNAseq using 10XGenomics and Illumina pipelines. Data was analyzed through CellRanger and Seurat in R.

Results: Across 59639 cells we found 16 glial, 26 neuronal and 3 vascular/connective clusters. We

assigned 42 cell identities using three layers of granularity. Some astrocytes seem to be highly excitable due to the specific expression of KCNJ3 and lack of expression of the AMPAr subunit GluA2, which also makes them Ca+permeable. Moreover, we are first to describe expression of HCRT in the DVC and a distinct GLP1 neuronal cluster likely activated by prolactin since PRLR is differentially expressed. We also found overlapping expression of TH and CCK in some neurons, previously thought to be distinct cell types. These cells display high K+channel H subunit KCNH8 and the chromatin-related CASZ1 expression in comparison to both CCK+ and TH+ populations. In addition, glial cells have stress-related transcriptional programs in the LiCl treated mice versus those refed (rewarding stimulus).

Conclusion: Our DVC cell atlas includes 42 cell identities, of which 26 are neuronal, and it first describes expression HCRT and co-expression TH and CCK in DVC neurons.

P2-E-285 - Bistable hypothalamic network regulating the neuroendocrine stress response

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The stress response necessitates an immediate boost in vital physiological functions from their homeostatic operation to elevated emergency response. However, neural mechanisms underlying this state shift remain largely unknown. Using a combination of *in vivo* electrophysiology and computational modeling, we report that corticotropin releasing hormone (CRH) neurons in the paraventricular nucleus of the hypothalamus (PVN), the effector neurons of the hormonal stress response, rapidly transition between distinct activity states through recurrent inhibition. Specifically, *in vivo* optrode recordings show that under non-stress conditions, CRH neurons often fire with rhythmic brief bursts (RB), constraining the firing rate due to long inter-burst intervals. In anesthetized mice, sciatic nerve stimulation, a pain-mimicking stressful stimuli, rapidly switched RB to continuous single spiking (SS), permitting a large increase in firing rate. Similarly, prostaglandin E2, an inflammation stress signal, increased CRH firing via shift from RB to SS. In awake head-fixed mice, we demonstrate rapid and sustained state changes after tactile, visual, and auditory startle stressors. A spiking network model shows that recurrent inhibition can control this activity-state switch, and consequently the gain of spiking responses to excitatory inputs. Moreover, *in vivo* neuropharmacological and chemogenetic manipulations of GABAergic inputs to CRH neurons produced model-predicted switches in activity states. Together, we present a network mechanism for state-dependent activity dynamics in CRH neurons.

P2-E-286 - Investigating the impact of acute Trazodone administration on sleep in mice

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[1] Simon Fraser University

Trazodone is a triazolopyridine derivative developed as an anti-depressant. Due to its sleep-promoting properties, trazodone is currently being evaluated as a therapeutic agent in conditions associated with sleep disturbances, notably Alzheimerâ€™s disease. Our study delineates a methodology for facilitating administration of trazodone to mice. The acidic nature of trazodone makes injection at a physiological pH level challenging and sleep studies necessitating intracranial EEG/EMG, make oral gavage challenging. We propose administering trazodone with highly palatable food, offering a non-invasive and translational route of administration. We identified the type of food to mix with the compound to facilitate self-administration of the drug reliably and reproducibly with minimal impacts on circadian rhythms and sleep in mice (C57BL/6, aged 10-12 months; n=9). Our results suggest a yogurt-based treat was most effective. Subsequently, the established method was applied to assess the effects of trazodone on sleep in another cohort of mice (C57BL/6, aged 10-11 months; n=15). Analysis is ongoing to examine the alterations in sleep architecture and power spectra through EEG/EMG recordings. The preliminary findings suggest an increase in the duration of NREM sleep after the first dose of trazodone, accompanied by a corresponding reduction in wakefulness periods. This study illustrates a translational trazodone administration technique in mice and highlights the potential therapeutic use of trazodone for ameliorating sleep disturbances associated with Alzheimerâ€™s disease pathology.

P2-E-287 - Role of dorsomedial hypothalamic orexin receptor 2 expressing neuron in energy homeostasis

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The hypothalamus is a critical site regulating energy homeostasis by responding to metabolic signals and accordingly regulates energy intake and expenditure. While many cell types contribute to maintaining energy balance, Orexin(OX)-expressing cells in the lateral hypothalamus are critically important. Previous literature suggests OX neurons exert anti-obesogenic properties through the orexin receptor-2(OXR2). To better target OXR2 neurons, we developed and validated an Oxr2Cre mouse strain to accurately manipulate these cells and confirmed insertion of Cre recombinase into the Oxr2 locus does not impact body weight. Using a Cre-dependent GFP allele, we find Oxr2-labelled neurons across hypothalamic nuclei but at particularly high density with the dorsomedial hypothalamus(DMH). We

further find these DMH-OXR2 neurons are activated by refeeding following an overnight fast, suggesting they are satiety promoting. Using monosynaptic rabies retrograde tracing we find DMH-OXR2 neurons get input from many sites critical for appetite regulation including the nucleus of the solitary tract and parabrachial nucleus. Furthermore, anterograde projection tracing reveals DMH-OXR2 neurons project to the arcuate nucleus and paraventricular hypothalamus, two sites with critical roles regulating appetite and body weight. Our future studies will use opto- and chemogenetics to activate or inactivate DMH-OXR2 cells and define their function. As DMH-OXR2 cells are an unstudied hypothalamic population, these studies will further our understanding of how the OX system maintains energy homeostasis.

P2-E-288 - Effects of prenatal delta-9-tetrahydrocannabinol (THC) vapour exposure on body weight, glucose metabolism, and feeding behaviors in chow and high-fat diet fed rats

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It is reported that 4-20% of people use cannabis during pregnancy. Evidence suggests that prenatal cannabis exposure (PCE) increases risk for developing obesity and diabetes later in life, but this association has not been fully explored. Therefore, the aim of this study was to characterise PCE effects on adiposity, glucose metabolism and feeding behaviours in adulthood, and assess if diet or sex impacts this.

Pregnant Sprague Dawley rats were exposed to delta-9-tetrahydrocannabinol (THC; 100mg/ml) or control vapour (polyethylene glycol) across gestation. Then adult offspring were subjected to adiposity, glucose metabolism and feeding measures when chow fed and following 4-months of 60% high fat diet (HFD) or 10% low fat diet (LFD) access.

PCE did not initially influence bodyweight or adiposity but did reduce bodyweight gain when on HFD/LFD, irrespective of diet. Further, PCE had bidirectional effects on glucose metabolism that were diet and sex dependent; when chow-fed, PCE improved glucose metabolism in both sexes, but when on HFD/LFD, PCE improved glucose metabolism in females and impaired glucose metabolism in males, irrespective of diet. Finally, PCE had diet-dependent effects on feeding behaviors: when chow-fed, PCE increased daily energy intake and acutely increased high-carbohydrate food intake when given a choice; but when on HFD/LFD, PCE had no effect on daily energy intake and decreased sucrose preference.

This work enhances current understanding of the potential risks of PCE, knowledge that may be used to develop more accurate public health guidelines.

P2-E-289 - Oh syn-ap! The role of synaptotagmin 11 in osmoregulation

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The osmosensitive neurons of the hypothalamus (ONs) possess mechanosensitive dN-TRPV1 channels that cause depolarization in response to cell shrinkage. Increases in dN-TRPV1 activity can increase vasopressin (VP) release from ONs, which enhances water reabsorption at the kidneys to prevent further increases in osmolality. We recently showed that dN-TRPV1 translocates to the plasma membrane following sustained high osmolality exposure (e.g., longer than 1 hour) and that it could play an important role in maintaining ON activity and VP release. dN-TRPV1 translocation requires SNARE-mediated exocytosis and Ca^{2+} influx through L-type channels. We sought to further investigate SNARE-dependent exocytosis and hypothesized that synaptotagmin 11 (Syt11) is essential for translocation of multiple types of ion channels in ONs, specifically dN-TRPV1 and $Ca_V1.3$. We used stereotactic surgery techniques to inject AAV-shRNA into the supraoptic nucleus (SON) to knock down Syt11 expression and then used immunocytochemistry to determine whether Syt11 is implicated in translocation of dN-TRPV1 and $Ca_V1.3$ as well as in hypertrophy. We now show that reduced expression of Syt11 significantly reduces both translocation of dN-TRPV1 and $Ca_V1.3$. These data suggest that Syt11 plays a significant role in and helps identify further the mechanisms surrounding osmoregulation and VP release.

P2-E-290 - Triglyceride metabolism in adipokinetic hormone-producing cells regulates sex differences in fat breakdown

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In all animals, triglyceride is the main form of stored fat, and is found within a specialized organelle called a lipid droplet. Lipid droplets have been studied extensively in non-neuronal cells; however, less is known about neuronal lipid droplets under normal physiological conditions. We visualized *Drosophila* neuronal lipid droplets via neuron-specific expression of a lipid droplet-targeted GFP. We

found lipid droplets in neurons under normal physiological conditions and showed age- and diet-dependent changes to neuronal lipid droplets in both sexes. We identified multiple genes, including triglyceride lipase *brummer* (*bmm*), that regulate the number of neuronal lipid droplets. Pan-neuronal loss of triglyceride- and lipid droplet-associated genes caused whole-body sex-specific metabolic defects in animals. In particular, we show that loss of *bmm* function in one subgroup of ~18 neurons called the adipokinetic hormone-producing cells reproduces sex-specific phenotypes associated with a loss of neuron function. Profound sex-specific changes to the brain lipidome caused by loss of neuronal *bmm* suggest triglyceride- and lipid droplet-associated genes normally play a key role in supporting neuron function via regulation of intracellular lipid metabolism.

P2-F-291 - Rewiring fear: Blocking M1 muscarinic receptors prevents contextual fear memory updating

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Memory updating is a dynamic process essential for integrating new information into existing memories. However, maladaptive memory updating, such as the generalization of fear memories, is a hallmark characteristic of disorders like PTSD. Previously, our lab has shown that the destabilization of resistant strongly-encoded contextual fear memories can be induced through the activation of M1 mAChRs in the dorsal CA1. In this current study, we built on this previous work to investigate whether the generalization of contextual fear memories to other contexts can be behaviorally demonstrated using a novel fear memory updating paradigm. Here, we exposed male rats to alternate contexts following the reactivation of a previously acquired contextual fear memory. First, we show that exposure to an alternate context following the reactivation of a contextual fear memory allows for the updating of such memory to other contexts. Following that, we confirm that this process is reactivation-dependent by showing that the effect does not occur if rats do not undergo a reactivation session. Building on that, we also show that an updated fear memory produced in this manner persists for at least 4 weeks after updating. Consistent with past memory reconsolidation work, we also show that fear memory updating requires exposure to alternate contextual information within the 6h reconsolidation window. Finally, based on our previous work, we show that fear memory is dependent on M1 muscarinic receptors. These findings strongly suggest that generalization of fear memories requires reactivation and destabilization of the original fear memory, suggesting a possible approach for the treatment of disorders characterized by maladaptive memories.

P2-F-292 - Entrainment of medial prefrontal cortex activity using non-invasive sensory stimulation

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In rodents, repeated exposure to a fear-associated cue reduces fear behaviour via the process of fear extinction. Neural circuits underlying fear extinction, in particular connections between the basolateral amygdala and medial prefrontal cortex (mPFC), are known to produce neural oscillations at 6-12 Hz during extinction. Recently, optogenetic entrainment of this amygdala-prefrontal circuitry at these frequencies reduced fear behaviour. For translational purposes, an important question is whether extinction-related neural oscillations in the mPFC can be similarly generated using a non-invasive method. To this end, we developed an apparatus to administer rhythmic sensory stimulation (RSS)â€”light flickers and auditory pipsâ€”to mice. Electrodes were surgically implanted in the mPFC to record in vivo electrophysiological activity while administering RSS at multiple frequencies within the 6-12 Hz range. Spectral analysis showed that visual stimulation, but not auditory, significantly increased the local field potential power at the respective RSS frequency delivered. These initial findings that indicate RSS entrains mPFC local field potentials warrant further investigation as a potential non-invasive approach to accelerating fear extinction.

P2-F-293 - The effect of electroconvulsive therapy on retrograde and anterograde memory

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Electroconvulsive therapy (ECT) is recognized as the most efficacious treatment for medication-resistant depression, however, its broad use is limited due to side effects on cognitive abilities, especially amnesia. The human clinical data reported a majority of amnesia being anterograde, while retrograde amnesia remains under-researched in mechanism studies using animal models. In rodents receiving electroconvulsive shock (ECS), the animal analog of ECT, a significant increase of adult neurogenesis in the hippocampus has been observed. ECT-induced amnesia typically emerges two weeks after the first session, which temporally matches the timing of adult-born neurons in the hippocampus to form connections with existing neurons; thus, elevated adult neurogenesis following ECT may account for amnesia as the integration of new-born neurons disrupts established memory circuits. The current investigation on ECS-induced amnesia remains rudimental with limited methodological diversity and so far no parallel comparison between anterograde and retrograde effects. Therefore, this study aims to provide validation of ECS-induced anterograde and retrograde amnesia under identical experimental

conditions. AsclCreER mice are receiving 20-day chronic ECS mimicking ECT schedule while trained on contextual fear conditioning before or after the chronic ECS, and tested on fear memory for retrograde and anterograde amnesia respectively. We hypothesize both amnesia to be observed in ECS-treated mice, potentially with robust adult-neurogenesis elevation in the hippocampus.

P2-F-294 - Opioid generalization of the stimulus effects of morphine in rats

Jessica Karlovcec [1], **Ella Claridge** [1], **Davin Peart** [1], **Jennifer Murray** [1]

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Background: Interoceptive effects elicited by opioids can guide behaviours through Pavlovian associations with drug-related stimuli. Following the acquisition of appropriate conditioned sucrose-seeking guided by a morphine (M) stimulus, stimulus specificity can be determined by assessing different opioid receptor agonists and comparing results to baseline discrimination behaviour. **Methods:** Male and female rats received daily injections of either M or saline (S) before chamber placement. Training sessions consisted of 8 presentations of a white noise (WN) conditioned stimulus each followed by sucrose delivery on M, but not on intermixed S sessions. Following stable discrimination, rats completed generalization cycles consisting of two qualification (Q) sessions identical to training, followed by a test session if sufficient discrimination between the Q sessions was demonstrated. On tests, responding to a single WN presentation was recorded after rats were pre-treated with one of four doses of either M, oxycodone (O), hydromorphone (H), fentanyl (F), naloxone (N), or S. All rats were tested on all drugs. **Results:** Male and female rats acquired the discrimination. As hypothesized, preliminary results indicate the M generalization curve appears to plateau at doses higher than that of training. Curves for O, H, and F appear to follow an inverted-u pattern. N blocks agonist-appropriate responding. **Conclusions:** Our findings demonstrate that the stimulus effects of morphine can generalize to alternative doses of other opioid agonists and the antagonist blocks the effect of morphine.

P2-F-295 - Expression of the positive-feature morphine occasion setter does not differ across estrous phase or between sexes

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Introduction: Interoceptive drug stimuli may acquire the conditioned modulatory properties used to induce conditioned appetitive behaviours by exteroceptive reward cues. These behaviours may be modelled using a Pavlovian drug discrimination task in which a positive-feature (FP) occasion setter (OS), the drug stimulus, is trained to disambiguate when an exteroceptive white noise (WN) conditioned stimulus is followed by sucrose, the unconditioned stimulus. Prior work suggested females may be less sensitive to FP morphine generalization than males, so this study investigated the impact of endogenous gonadal hormones. *Methods*: Female and male rats received daily intermixed injections of 3.2mg/kg morphine or saline before training sessions. Females were monitored daily for changes in estrous cyclicity using vaginal lavage and cytology. Training sessions consisted of 8 presentations of the WN. On morphine, but not saline, sessions, each presentation was followed by brief access to sucrose. After stable discrimination, rats were tested for generalization of the morphine training dose to 0, 1.0, 3.2, and 5.4mg/kg morphine. Females completed generalization at each dose in vaginal diestrus, proestrus, metestrus, and estrus. *Results*: Behaviour did not differ across sex nor phase of the estrous cycle. *Conclusions*: Circulating gonadal hormones do not influence expression of morphine drug discrimination when learning is acquired while freely cycling. Learning under more hormonally-controlled conditions is warranted to assess whether expression of such associations can be state dependent.

P2-F-296 - Paralog- and sex-specific roles for GSK-3 in cognitive behaviours in adult mouse models

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Glycogen synthase kinase-3 (GSK-3), a pivotal serine/threonine kinase, is recognized for its role in a range of diseases that include neurodevelopmental and neurodegenerative disorders. In mammals, it exists as two paralogs, GSK-3Î± and GSK-3Î². Their non-redundant roles in cognitive function are yet to be fully determined. This study aims to identify the specific roles of these paralogs on various cognitive functions and includes sex as an experimental variable. Using adult mice with conditional knockout (cKO) of GSK-3Î± and GSK-3Î² genes in the forebrain, we have studied behaviours relevant to neurological conditions including anxiety, executive function, and novel object recognition memory. No changes were observed in locomotion in GSK-3Î± and GSK-3Î² cKOs compared to their littermate controls. Female GSK-3Î² cKO mice showed a decrease in anxiety in the elevated zero maze test. Spatial working memory, tested via the Y-maze, was unaffected in GSK-3Î± and GSK-3Î² cKOs, although a trend for reduced spatial reference memory was observed in GSK-3Î² cKOs. In the puzzle box test, GSK-3Î± and GSK-3Î² cKO mice showed enhanced executive function, solving tasks faster than controls. Female GSK-3Î² cKOs showed improved executive short-term memory in this test. Notably, an impairment in novel object recognition memory

was observed only in GSK-3Î² cKOs. In summary, these findings reveal complex, paralog- and sex-specific roles in cognitive function that demonstrate the need to carefully consider therapeutics targeting GSK-3.

P2-F-297 - Lost in transition: Ongoing development of a modified five choice serial reaction time task to assess adolescent attention in rats

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Behaviour disorders such as attention deficit hyperactivity disorder (ADHD) are among the most prevalent neurodevelopmental disorders in children and adolescents in Canada. There is currently a lack of translationally relevant tasks that assess attention in adolescent rodents, as the limited duration of the adolescent window presents a challenge for conditioned learning. We have begun development of a modified version of the well-established 5-choice serial reaction time task (5CSRTT) to expedite rule acquisition in adolescent rats. Two cohorts of both male and female Sprague Dawley rats were trained on the novel protocol beginning on postnatal day 33 to measure attention, response latency, and accuracy. The shortened pre-training protocol excludes punishment for incorrect responses and increases the daily training session duration to 60 minutes. After successful pre-training, a first cohort (n=16) progressed directly to the final stage of Basic Touch training of the 5CSRTT protocol (stimulus duration = 1s). For a second cohort (n=8), difficulty was incrementally increased by adjusting the stimulus duration daily according to the individual response latency from the previous training session until the criteria of the final stage of Basic Touch training was reached. The second approach enabled adolescent rats to acquire the task to an average accuracy of 50% with a stimulus duration of 1 second in a 3-week window. Future experiments will continue to refine these training protocols to enable investigation of factors influencing attention and learning in adolescent rats.

P2-F-298 - Dorsal and ventral hippocampal contributions to probabilistic reversal learning

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In the wild, the quest for rewards such as food is often fraught with uncertainty. Animals, including humans, must adapt to these uncertainties, learning to adjust their strategies for obtaining rewards. This adaptability is impaired in various neurological disorders like depression, OCD, schizophrenia, among others. The processing of uncertain reward outcomes is primarily linked to the orbitofrontal cortex (OFC), striatum, and amygdala. However, our previous research indicates a potential role of hippocampal neurogenesis in influencing reward feedback sensitivity. We proposed that the hippocampus, due to its connections to the prefrontal cortex (PFC), amygdala, and striatum, might have a significant but underexplored role in probabilistic learning. The hippocampus is a complex structure, often conceptually split into dorsal and ventral regions, with the former associated with cognitive functions like spatial navigation and the latter with emotional responses. Recent studies, however, suggest that this dichotomy is overly simplistic, with both regions contributing to various learning and memory processes. Our research aims to clarify the hippocampus's involvement in probabilistic learning and identify the specific regions engaged in this process. We employed pharmacological and chemogenetic methods to selectively inactivate the dorsal and ventral hippocampus. Pharmacological inactivation revealed distinct impacts of disabling these regions. Chemogenetic inactivation of the dorsal hippocampus showed no notable effects, but we observed significant differences between our adeno-associated virus (AAV) treated and control groups, suggesting a more complex role of the hippocampus in probabilistic learning.

P2-F-299 - Exploring the interplay of early-life trauma, endocannabinoid system Dysregulation, and problematic cannabis use among young adults

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Cannabis is a commonly used substance, particularly among young adults. Of those who use cannabis, it is estimated that 1 in 5 users will go on to develop a cannabis use disorder (CUD). Early-life trauma (ELT) is a significant predictor of problematic cannabis use, potentially linked to dysregulation of the endocannabinoid (eCB) system. This current study aimed to explore the relationship between ELT, current stress, and cannabis use, and whether a unique eCB profile may underlie the relationship between ELT and problematic cannabis use. Male and female university students (N=90; ages 18-29) who do and do not use cannabis responded to a series of questionnaires assessing ELT, current stress, mental health, and cannabis use. Blood was collected for later analysis of eCBs in plasma. Data reveal a significant relationship between ELT and problematic cannabis use. A younger age of initiation of cannabis use and current stress mediated the relationship between ELT and problematic cannabis use. Participants with ELT history were also more likely to report using cannabis to cope with stress, a motivation that contributes to problematic use. Relationships to circulating endocannabinoids will also be discussed. Exploring the relationship between ELT and problematic cannabis use among young adults

will not only facilitate more informed use within this population, but also holds the potential for informing targeted therapeutic interventions and clinical strategies.

P2-F-300 - Identifying representational structure in CA1 to benchmark theoretical models of cognitive mapping

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The hippocampus and associated regions of the neocortex are thought to support diverse learning and memory processes through cognitive maps instantiated in the activity of principal neurons. Growing evidence suggests that such maps are formed across metric spaces to support flexible, goal-directed navigation and long-term memory; functions that are impaired following hippocampal damage. These findings have recently motivated an increasing number of theories expressed in computational models to explain how the hippocampal system instantiates cognitive maps. However, there has been no consensus on how to compare predictions across models and, importantly, against empirical observation. To address this need, we recorded from large populations in hippocampal subregion CA1 in a condition-rich geometric deformation paradigm (5,413 unique neurons recorded across 207 sessions in 10 geometries, forming 69,744 rate maps). Leveraging novel similarity- and decoding-based approaches, we observed that a constrained range of allocentric, boundary vector-based models accurately predict CA1 spatial representation (within maximum theoretical limits). The success of such models to predict CA1 representation suggest that an allocentric vector-based code strongly determines population-level neuronal dynamics in CA1. These are the first results to our knowledge demonstrating that large-scale predictions from neurobiological models of cognitive mapping can be directly evaluated against population dynamics in freely behaving animals. The present dataset and framework provides a new benchmark for theoretical innovations in cognitive mapping research and, more generally, establishes a novel approach to compare representational structure across brain regions, assays, species, and theoretical models.

P2-F-301 - Investigating the role of unique hippocampal cell types in fear memory and anxiety-like behaviours

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Following a traumatic event, some individuals experience adverse and persistent psychological symptoms, resulting in post-traumatic stress disorder (PTSD). To better understand this disordered type of memory, we must first understand the cells and molecules involved in experimentally well-controlled models of fear memory, so that effective interventional strategies for PTSD can be developed. Our published research has shown that classic "textbook" cell types of many brain regions can be divided into distinct subtypes based on the genes they express. Work from our lab has identified transcriptomically unique cell types in the ventral subiculum â€“ the primary output region of the hippocampus largely involved in emotive memory.

Here, we sought to identify the recruitment, connectivity, and causal role of these cell types in fear memory. To begin, using a combination of single-cell RNA sequencing and multiplexed *in situ* hybridization, we found that two of these unique cell types may be preferentially recruited in fear memory encoding and/or retrieval. Using a mouse line that provides selective genetic access to these cells, we used viral tracing to identify the precise downstream targets of these two cell types. Finally, we employed optogenetics in these mouse lines to assess causal roles in memory and anxiety-like behaviours. These findings begin to elucidate the key cell-type- and memory-stage-specific logic underlying fear memory in the brain. In the future, such findings may provide a vital framework for investigating the cellular and molecular targets for interventions in fear-memory-associated disorders like PTSD.

P2-F-302 - Cognitive and behavioral assessment using an in-cage touchscreen apparatus in the common marmoset

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Cognition is incredibly important for adapting to the environment. Disorders like Parkinsonâ€™s disease, depression, or autism spectrum dramatically affect cognitive ability, reduce self-agency, and increase adversity through social biases and diminished autonomy. The common marmoset (*Callithrix Jacchus)* has become a novel model for brain research due to their behavioral and genetic homology to humans, quick maturation, and fast reproductive cycle; making them strong candidates as preclinical models and for translatable research between rodents, primates, and humans. To assess the viability of performing cognitive testing on marmosets, we conducted tasks in a non-invasive way using an in-cage touchscreen system (SmartChair from Rogue Research) and NIMH MonkeyLogic software. The built-in RFID system limited human interaction, enabled parallel task execution based on subjects, and allowed for autonomous, ad-libitum participation in the tasks including pair-wise visual discrimination / reversal learning, socio-semantic categorization, and visuo-motor conditional learning. Tasks were performed by 4 marmosets (2M, 2F, average age 15 months old). Reaction time, hits/misses and perseveration were collected to assess learning. We have observed that marmosets learn and perform human and rodent validated tasks, providing evidence for marmosets as a viable model for translational research. We

demonstrate a powerful tool for researching numerous aspects of cognition in the common marmoset such as sensation, perception, attention, memory, and executive function.

P2-F-303 - A touchscreen-based patch foraging paradigm for mice

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Foraging animals may encounter rewards in “patches” of finite, local concentrations of food throughout an environment, and make choices about when to leave a food patch being depleted as reward is consumed (exploit), in search of new, better reward (explore). Animals may manage this explore-exploit dilemma via sequential evaluation of current patch reward against the overall value of the environment. Methods: We developed a touchscreen-based task for patch foraging in mice. Adult male C57BL/6J mice were trained to demonstrate their choices by making touch responses to visual cues representing either a “patch”, indicating the possibility to harvest food rewards that depleted over subsequent harvests, or a “travel” cue, indicating the possibility to open a new, replenished food patch. Results: All mice successfully learned the touchscreen task, with discrimination of touch responding to operant outcomes. Patch-leaving choice behaviour and pre-choice response latencies were sensitive to declining patch reward. Across session learning was indicated by mice tending to leave patches with less reward depletion in later behaviour sessions. Pre-feeding (mice given chow 1hr before behaviour) suggested differences in metabolic/motivational state modulated patch-leaving choices early on within behavioural sessions. Choice and response latency relationships were conserved across patches with different amounts of initial reward. Future investigations will probe the impact of an acute inflammatory challenge to alter decision-making during patch foraging behaviour.

P2-F-304 - Effects of elevated Anandamide via fatty acid Amide Hydrolase inhibition on stress reactivity in patients with post-traumatic stress disorder

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Background: Post-traumatic stress disorder (PTSD) is a severe psychiatric condition with limited treatment options. Recent research in animal models and healthy humans suggests that elevating the endocannabinoid ligand anandamide (AEA) via inhibition of its main degradative enzyme, fatty acid

amide hydrolase (FAAH) can modulate stress and fear, and may thus constitute a novel intervention for PTSD. Here, we aimed to determine if FAAH inhibition could modulate stress reactivity in PTSD patients.

Methods: â€˜PTSD patients (N = 101) â€™were randomized to JNJ-42165279 (FAAH inhibitor) or a placebo for 12 weeks. In week four, they completed lab sessions with an acute stressor or control task while psychophysiological measures such as skin conductance rate (SCR) and heart rate variability (HRV) were assessed. We then compared the effect of the stressor versus control task between patients on JNJ-42165279 and placebo.

Results: The stressor task had a significant effect on SCR frequency for $F(1,58) = 45.333$, $p < 0.001$, but did not affect HRV ($p = 0.428$). There was no significant effect of JNJ-42165279 on stress reactivity versus placebo.

Conclusion: The lack of effect of FAAH inhibition PTSD symptoms and stress measures suggests that elevated AEA may not be a suitable target for this psychiatric indication. This could be crucial for refining future FAAH inhibition interventions and tailoring treatments for PTSD patients.

P2-F-305 - Modulation of select mesolimbic dopamine neurons and food-directed behavior by omega-3 fatty acids and GPR120

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Dopamine (DA) signaling in the mesolimbic pathway plays a central role in the control of motivation and emotion and is modulated by dietary fatty acids. Omega-3 polyunsaturated fatty acids (n-3 PUFAs) are increasingly recognized for the beneficial effects on mental health. GPR120 (FFAR4) is a long-chain fatty acid receptor mediating the insulin sensitizing effect of omega-3 fatty acids in the periphery and that possesses strong anti-inflammatory consequences when activated in microglia. We found that GPR120 is also expressed on midbrain DA neurons via in situ hybridization and that GPR120 activation induces DA release and enhances neuronal arborization in a primary culture. However, the role of GPR120 in DA neurons on energy balance and behavior is unknown. Here, we used a AAV approach (AAV9-TH-GFP or AAV9-TH-Cre) to selectively knockout GPR120 from DA neurons of the ventral tegmental area in adult $GPR120^{Flox/Flox}$ mice. Locomotor activity and feeding behavior were measured in CLAMS metabolic cages. Food-motivated behavior was assessed in operant cages using both fixed and progressive ratio tasks. Anxiety-like behavior, exploratory activity, social interaction, and anhedonia in these mice were also evaluated. $GPR120^{DA}$ KO mice exhibited reduced exploratory behavior in novel environments, but

overall locomotor activity, anxiety-like behavior, sociability and sucrose anhedonia were unchanged. GPR120^{DA} KO mice consumed more free-feeding meals in CLAMS chambers and exhibited greater motivation for sucrose pellets in operant setting. Together, these findings are compatible with findings that omega-3 fatty acid dietary deficiency increases the effortful responding for palatable foods and that ICV administration of omega-3 fatty acids or a GPR120 agonist suppresses food reward. GPR120 signaling in DA neurons may thereby mediate the actions of n-3 on food-directed behavior.

P2-F-306 - The relationship between sleep and performance on tests of pattern separation and the Cambridge Neuropsychological Test Automated Battery (CANTAB)

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Sleep disturbances are considered both a dementia risk factor and symptom. The present research aimed to identify cognitive tests sensitive to sleep-dependent cognition. Study 1 was a correlational exploratory study. We recruited young (N=89; aged 18-30 years) and older (N=40; aged 50-100 years) adults and monitored their sleep patterns over 7 consecutive days using actigraphy watches and sleep diaries. On day 7, participants completed cognitive testing in the laboratory. Cognitive tests included the Prodromal Alzheimer's and MCI battery from the Cambridge Neuropsychological Test Automated Battery (CANTAB), and the Mnemonic Similarity Task (MST), which taxes pattern separation. We also assessed the older adults' cognitive performance using the Montreal Cognitive Assessment (MoCA). Study 2 examined the effects of a night of total sleep deprivation on cognitive performance using the same cognitive tests. We observed a sleep deprived group (N=16; aged 18-40) overnight in the lab. The rested control group (N=32; aged 18-40) slept normally at home. In study 1, we observed a stronger, but not statistically significant relationship between sleep quantity and MST performance in the older adults, and statistically significant relationships between CANTAB DMS performance and sleep quality in the older sample. In study 2, the sleep deprived participants showed poorer MST performance and longer DMS response latencies than rested subjects, but findings were non-significant. Sleep-dependent cognitive tests could be used as clinical trial outcome measures for sleep-promoting treatments.

P2-F-307 - Effects of sleep displacement and sleep fragmentation on hippocampal neurogenesis in mice

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Deprivation or continuous fragmentation of sleep suppresses the proliferation and maturation of new neurons in the hippocampus of adult rodents. A more common type of sleep disruption occurs in humans working nightshifts, when sleep is displaced from night to day. We evaluated hippocampal neurogenesis in mice (C57BL6/j, ~3 months age) subject to sleep deprivation procedures. A sleep displacement group (n=6, 3 females) was deprived of sleep for 12 hours each day during the typical rest phase (12h lights-on) and left undisturbed during the active phase (lights off) for 7 consecutive days. A sleep fragmentation group (n=8, 4 females) were disturbed every 2 minutes during their rest phase for 7 days. The control group (n=14, 7 females) remained undisturbed in their home cage. Activity during the active phase for all mice was recorded via infrared motion sensors. Mice were injected with 200mg/kg of BrdU 2-hours prior to brain and blood sample collection at the end of the light period on day 7. Preliminary analysis suggests the week-long procedures induced an accumulating sleep deficit, as indicated by a progressive increase in rest bout duration and total rest time at night. Plasma corticosterone quantified by ELISA assay did not differ across conditions or between sexes; effects of the procedures on neurogenesis were independent of this stress hormone. To assess neurogenesis, brain sections spanning the range of the dentate gyrus were stained using immunohistochemistry. All sections have been imaged using a Nikon A1R Laser Scanning Confocal system and cell counting is ongoing.

P2-F-308 - Cerebellar decision signal shaped by the habenula

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Motor planning enables animals to prepare and take desirable actions to maximize survival. While the execution of this mental process requires coordination from a distributed, brain-wide network, recent studies have highlighted the cerebellum as a core modulating region. However, it remains unclear how the cerebellum integrates internal brain states for motor planning. To investigate this, we have combined light-field and two-photon calcium imaging, optogenetic manipulation, and an operant-conditioning task in larval zebrafish. We find the strongly correlated preparatory signals in the cerebellum and habenula. These preparatory signals are evoked by sensory stimuli and gained through learning. Disrupting the preparatory signal in the habenula affects both the signal in the cerebellum as well as the behavioral outcome. Optogenetic stimulation leads to rotation in the neural manifold in the channelrhodopsin(ChR) animals, while in control siblings light stimuli are encoded orthogonally to the motor planning signals. This result shows that the cerebellum-dependent motor planning relies on the input from the habenula, suggesting a habenula-cerebellum interplay as a conserved motivation-decision motif. Moreover, the cerebellum is capable of encoding â€˜irrelevantâ€™ information orthogonally, highlighting a cognitive cerebellum.

P2-F-309 - The role of lateral habenula transmission to dopamine center in associative learning and defensive behaviors

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Adapting behavioral responses to threats is crucial for survival. The lateral habenula (LHb) receives neural inputs from the basal ganglia and limbic system, and in turn sends neural projections to the dopaminergic ventral tegmental area (VTA). In this project, we test the hypothesis that VTA-projecting LHb neurons encode aversive signals involved in associative learning to promote escape behavior. Specifically, using an intersectional viral approach to drive expression of GCaMP6s in VTA-projecting neurons, we found that an auditory cue (tone) paired with a foot shock progressively causes cue-driven activity in VTA-projecting LHb neurons during an avoidance learning task (paired group), which is not observed when the tone is not contingent to a foot shock (unpaired). We also found that activity at VTA-projecting LHb neurons increases when mice initiate avoidance responses in the avoidance learning task, as well as at movement onset in the tail suspension test (TST). Blocking transmission at VTA-projecting LHb using the tetanus light chain (TeLC) is sufficient to reduce avoidance learning, and to reduce movement in the TST. Finally, optogenetically assisted whole cell electrophysiology recordings revealed synaptic adaptations at LHb terminals synapsing onto dopamine neurons (TH+) after a single avoidance learning session, yet onto TH- neurons after three sessions of avoidance learning (expert mice). Together, these results support the importance of the LHb-VTA pathway contribution for cue-outcome association and to engage defensive responses in aversive contexts.

P2-F-310 - Temporal difference learning theory predicts the evolution of hippocampal neuronal dynamics during learning a reward-based navigation task

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The hippocampus plays a crucial role in constructing a cognitive map of the environment, which aids in navigation and memory-based behaviors. Recent work has revealed that this spatial representation undergoes important changes when reward locations are learned. Here, we aim to understand the emergence and evolution of this reward-related representation across weeks as mice learn a

hippocampal-dependent memory task. We used one-photon miniscopes to image the activity of large ensembles of CA1 neurons during a Trial-Unique Nonmatch to Location (TUNL) touchscreen task. In the sample phase of this task, a white square is presented in one of five positions on the touchscreen. A nose poke to this square starts a delay period (increasing from 2 to 8 secs as the mice learn the task). Following this delay, two white squares are displayed, and the mouse must choose the nonmatching square to receive the reward, located on the back wall of the touchscreen chamber. Our analysis reveals the following: 1) A precise representation of location (mean decoding error ~4cm). 2) As animals learn the task, distinct neuronal responses emerge. First, place cells become directionally tuned. Second, a subset of the population shifts from encoding location to encoding either the presence of a touchscreen cue, reward, or reward prediction. â€˜Reward prediction cellsâ€™ encode the value of the reward through the amplitude of their neural responses. 3) Across weeks, as mice master the task, the proportions of these specialized cell types change in accordance with that expected by the Temporal Difference Learning theory. The proportion of reward cells decreases while the proportion of cue-selective and reward-prediction cells both increase. These results describe how CA1 populations evolve over time as animals master the TUNL task and offer support to the Temporal Difference Learning theory.

P2-F-311 - Understanding the influence of sweet additives in an oral morphine self-administration model in male and female rats

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Introduction: Morphine is predominantly consumed orally, but rarely administered via this route in preclinical literature. To increase translatability, we use a two-lever oral morphine self-administration (SA) model with a reinforcer solution containing grapefruit juice (GFJ), thought to increase morphineâ€™s bioavailability, and varying sucrose (SUC) concentrations to overcome morphineâ€™s bitter taste. However, conclusions on morphineâ€™s reinforcing properties in our model are obfuscated by these sweet additives and the lack of knowledge of GFJâ€™s pharmacokinetic impact on morphine in rats. Methods: Exp 1: Rats were trained to lever press for a morphine solution using a SUC fading procedure (20%, 10%, 5%); half with GFJ solution during training and half without. Seven 10-session phases followed, each manipulating a component of the reinforcer solution to discern additive-dependent differences in consumption. Exp 2: Rats were trained to lever press for a morphine solution containing 5% SUC with or without GFJ. Two 3-day testing cycles followed, during which a normal SA session was followed by high or low dose naloxone administration and an additional SA session. Results: Stable consumption across phases does not seem sex-dependent but likely influenced by vehicle sweetness and modulation of morphine pharmacokinetics by GFJ. Consummatory behaviour after naloxone administration indicates additive- and sex-dependent differences. Conclusions: Further

understanding of the impact of sweet additives used in oral morphine SA is imperative to elucidate morphine-seeking behaviours.

P2-F-312 - Cell-type-specific control of innate defensive responses in the anterior hypothalamic nucleus

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Animals rely on innate defensive responses to survive in natural habitats. Upon exposure to threat, animals engage in risk assessment behaviours during which they evaluate their environment and subsequently undergo a decision-making process between freezing, escape, and defensive attack behaviours. While recent studies have highlighted the neural mechanisms underlying escape behaviours, it remains unclear how the overall sequence of innate defensive behaviours is achieved in the brain. Here, we demonstrate that the anterior hypothalamic nucleus (AHN) mediates innate defensive behaviours through cell-type-specific control of risk assessment and escape responses. In this study, we paired calcium imaging and optogenetic manipulation with a wide range of behavioural studies to characterize the roles of GABAergic and glutamatergic AHN neurons in mediating innate defensive behaviours. Through fiber photometry during a predator-evoked avoidance task, we showed that GABAergic and glutamatergic AHN neurons have distinct activity patterns during innate defensive behaviours. Furthermore, optogenetic stimulation of GABAergic AHN neurons evoked risk-assessment behaviours, whereas stimulation of glutamatergic AHN neurons evoked escape responses. Lastly, we found that stimulation of each cell type carried negative valence and induced mild conditioned place aversion. Together, these results demonstrate a cell-type-specific control of innate defensive responses within the AHN, where GABAergic neurons mediate risk-assessment responses, and glutamatergic neurons mediate escape responses.

P2-F-313 - The effect of chemogenetic activation of the anterior paraventricular nucleus of the thalamus on cue-induced heroin seeking in sated and food-restricted rats, and the validation of the DREADD ligand, J60 (JHU37160)

Emily Ah-Yen [1], **Catarina Borges** [1], **Katherine Krehbiel** [1], **Julia Azuelos** [1], **Mahgol Darvishmolla** [1], **Richard Courtemanche** [1], **Uri Shalev** [1]

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Chronic food restriction increases the risk for relapse in addiction treatments. The anterior paraventricular nucleus of the thalamus (aPVT) plays a critical role in drug relapse and drug seeking. Here, we chemogenetically activated the aPVT using a low J60 dose (0.1mg/kg) to examine cue-induced heroin seeking in food restricted and sated conditions in male and female rats. Rats were trained to self-administer heroin (0.1mg/kg/infusion) for 10 days, followed by 16 days of forced abstinence. During forced abstinence, rats were either sated or food restricted. On day 15 of food restriction, rats were injected with either low dose J60 to activate the aPVT or with vehicle and underwent a heroin-seeking test under extinction conditions. We expect that food-restricted rats will have greater heroin-seeking than the sated rats, and that aPVT activation will reduce heroin seeking induced by food restriction. We validated J60 neuronal activation in two doses: 0.1mg/kg; (N=3) and 1mg/kg (N=1). We infused 4 Long Evans rats with AAV8-hSyn-hM3D(Gq)-mCherry in the PVT. After 6 weeks of expression, we recorded multi-unit and single-unit activity after injection of either dose of J60. Multi-unit and single-unit analysis indicate J60 increases neuronal activity at both doses, and this excitation is stable for at least 1hr. Future directions would include testing a high dose of J60 with this heroin-seeking behavioral design and assess a possible dose-dependent attenuation of heroin seeking.

P2-G-314 - Multiple single-unit recording of spinal cord neurons from freely-moving mice with a novel implant

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Despite the key role of the dorsal spinal cord and its neurons in processing somatosensory information, the circuits and mechanisms underlying this process remain to be fully investigated. Spinal cord neuron physiology has been investigated mainly in anesthetized animals or in vitro, using whole-cell recording in slices. Limited attempts have been made to explore the electrical activity of dorsal horn neurons in awake animals, due to constraints, including the size, movement, and accessibility of this region. Our current study addresses these challenges by introducing a novel electrode designed for recording multiple single-unit activities. It integrates two bundles of eight independent nichrome electrodes with different lengths, facilitating recording of spinal cord neuronal activity across various laminae. Our 3D-printed lumbar prosthesis allows flexible movement of the electrodes to keep them aligned with the spine during locomotion and minimize the artifacts caused by breathing. By using this new device, we obtained stable recordings of neuronal activities while testing thermal and mechanical sensitivity of the hind paws but also when animal performed several behavioral tasks. Using automated and manual sorting techniques single units could be easily isolated to reveal the correlations of their activities with specific behaviors.

The development of this novel tool allows us, for the first time, to characterize specific activity patterns of spinal cord neurons in freely moving mice. This will enable us to comprehensively understand how the dorsal horn neurons encode sensory information in awake conditions and facilitate the study the sensorimotor interactions, previously inaccessible at the spinal cord level.

P2-G-315 - Development of a novel, high-throughput flow cytometry method for evaluating synaptic protein signalling in isolated neuronal synaptosomes

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Synapse dysfunction, notably impairments in dynamic actin remodelling, is a major determinant in several neurodevelopmental and neurodegenerative diseases, often affecting synaptic plasticity and memory. Synaptosomes are an accessible model for studying synaptic signalling molecules, as they consist of detached but intact synaptic terminals that are functionally active and can be analyzed individually or in bulk. This ex vivo model retains its catalogue of synaptic proteins present in vivo, including cofilin, a key actin regulator that severs dynamic actin filaments to promote depolymerization, and its phosphorylated and inactive form, which allows for actin expansion during synaptic plasticity. Although the impact of impaired actin remodelling in synapse formation has been previously explored in neurological diseases, current tools to evaluate post-translational modifications in synaptic proteins are limited. Here, I developed a novel flow cytometry protocol to evaluate the expression of Cofilin and phosphorylated Cofilin in thousands of isolated murine synaptosomes. My results demonstrate antibody validation for pre and post-synaptic markers to verify intact synaptosomes and offer a high-throughput method to evaluate rapidly altered post-translational modifications in synaptic proteins. Future manipulations of upstream pathways of Cofilin using my novel assay will help elucidate the role of various epigenetic regulators in actin cytoskeleton dynamics and its significance in neurodevelopmental and neurodegenerative diseases.

P2-G-316 - SCADR: A single-cell platform for characterizing impacts of Gene variants on protein function

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Autism spectrum disorder (ASD) is the most common genetic neurodevelopmental disorder, yet no effective treatment options are available. Whole genome sequencing has implicated the protein and lipid phosphatase PTEN in the development of ASD, and the most common disease-associated PTEN mutations are single nucleotide missense mutations that occur throughout PTEN structure. These variants can have damaging or unknown impacts on protein function. In the canonical model, PTEN regulates abnormal growth through suppression of PI3K/Akt/mTOR activity. However, links to non-canonical signaling cascades suggest a more complicated story. Here, we present our platform for simultaneous multiplex assessment of PTEN-dependent signaling pathways to better understand variant impact on PTEN functions. In order to fully characterize these impacts, our lab has developed a standalone app called SCADR (Single-cell Analytics for Dose-Response) that can perform deep phenotypic profiling of signaling networks at a single cell level. SCADR provides insight into how signaling dysregulation and variant dosage impacts disease development, potentially identifying novel therapeutic options for PTEN associated diseases.

P2-G-317 - Developing a multiomics approach that utilizes microscopy, spatial, and single-cell technologies to decipher the governing principles that regulate GBM evolution

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Glioblastoma (GBM) is a highly aggressive adult brain cancer with less than 10% survival over five years. Our study focuses on understanding the complex interaction of different cell types and structures within GBM tissues. We initially used 17,601 phase contrast images from 15 GBM patients to demonstrate that cell organizational geometries are linked to cell-type identities. Our aim was then to decode the more intricate geometries within GBM tissues.

To achieve this, we created a custom gene panel of 414 padlock probes, targeting various GBM cell types and representing the neurodevelopmental and injury response gradient common in GBM tissues. We analyzed 12 patient-derived GBM tissues (16 sections in total) using the Xenium platform. Four sections were labeled with fluorescent antibodies to study structural elements, aiding in tissue segmentation and analysis of textural and morphometric patterns. The remaining sections were stained with H&E. We employed deep-learning and computer vision for tissue segmentation and to identify geometric patterns, integrating these findings with single-cell and spatial gene expression analyses.

Our research revealed that data from different modalities and analysis paradigms (gene and protein expression, geometric patterning) offers a complementary yet distinct perspective. By integrating these insights, we found that hierarchical pattern analysis enhances the understanding of GBM. This approach could serve as a clinical tool for profiling GBM, offering insights into tumor growth and resistance through the study of cellular community interactions.

P2-G-318 - Enhancing rodent pose estimation with DensePose CSE and stable diffusion: A novel framework for synthetic data generation and real-world application

Haozong Zeng [1], **Hao Hu** [1], **Tony Fong** [1], **Timothy Murphy** [1]

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Most existing pose estimation methods require extensive image labeling, sometimes struggling with the problem of losing 2D markers due to occlusion and can only track sparse key points on the complex surfaces of animals. To address these challenges, we introduced a novel framework that overcomes such limitations by leveraging the synergy of DensePose CSE and Stable Diffusion and developed a pipeline optimized for rodents. DensePose CSE tracks nearby key points on a rodentâ€™s 3D surface even when a 2D marker is occluded. To create training datasets without significant labor, we first integrated Stable Diffusion, guided by ControlNet, with a 3D mouse body model based on CT scans. The 3D model could already be used to create synthetic images with defined poses and key points, and our new approach can generate ultra-dense annotated synthetic images (100x) for DensePose CSE, establishing dense image-to-surface correspondences for rodents. We also extracted joint rotation data from non-rodent datasets, which are easier to obtain, to drive the 3D mouse model for specific tasks, thereby saving labor. Then, we trained our DensePose CSE model on such synthetic datasets. To validate our framework, we acquired videos of mice with multiple synchronized cameras at different angles. Specifically, We computed the average precision of our methods on manually annotated datasets that we collected or got from public sources. Our framework circumvented the labor and errors prevalent in human annotation, demonstrating its efficacy in real-world applications.

P2-G-319 - Characterization of missense variant impacts on the PTEN interaction

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The routine sequencing of human genomes has provided a wealth of new gene variant information, spanning both patient and healthy populations. This has led to the discovery of numerous missense variants, presenting a challenge as their functional assessment and potential roles in diagnosis or disease risk remain uncertain. Focusing on the human PTEN gene as a case study, we screened over 120 variants, many strongly associated with Cancer, Autism Spectrum Disorders, and PHTS. To identify PTEN's protein binding partners and to quantify variant-specific changes in its interactome, we employed protein proximity labeling with BioID2 in human cell lines, followed by isolation and characterization of associated proteins. Results promise a fuller understanding of the molecular mechanisms and signaling pathways mediating disease-associated variants.

P2-G-321 - Combining robotics with machine learning to improve pain behavior testing

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Preclinical pain research relies on behavioral testing to infer the pain sensitivity and affective state of a mouse. Many assays suffer from poor reproducibility because stimuli are applied by hand and/or output measures are subjective. Beyond this, most assays quantify single behaviors by eye, neglecting information available from subtle changes in associated behaviors. We developed a robot named RAMalgo (Reproducible Automated Multimodal Algometer) that uses machine learning to automate the acquisition and detection of evoked pain behaviors from up to 10 mice, applying optogenetic, radiant heat, or mechanical stimuli. Using keypoint detection, we track the paw position from substage video in real time and automatically drive linear actuators to aim and stimulate. Paw withdrawal is measured with millisecond resolution using reflectance from a red LED focused on the paw for optogenetic and radiant heat stimulation, or force feedback measurements during mechanical stimulation. Analysis of videos showed that stimulus-evoked withdrawals correlate with non-reflexive behaviors such as increased licking, guarding and flinching of the paw, indicating negative emotional valence. Given effects of stress on pain, we used machine learning to automatically segment behaviors to track how stress behaviors evolve over time (e.g. during acclimatization), and to assess how stress depends on mouse handling methods. By standardizing testing procedures and quantifying additional aspects of mouse behavior, RAMalgo improves the reproducibility, throughput, and comprehensiveness of pain testing.

Funded by CIHR and a Proof-of-Principle Commercialization Grant from the Hospital for Sick Children.

Christopher Dedek and Steve Prescott have filed a patent for the device described in this work.

P2-G-322 - Modelling the depressed mind: simulated dendritic spine loss better induces depression-like behaviours than does impaired reward learning in deep reinforcement learning

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Deep Reinforcement Learning is a branch of artificial intelligence that utilizes artificial neural networks to model reward-based learning in biological agents. Here, we modify a Deep Reinforcement Learning approach to stimulate the effect of dendritic spine loss, as seen in Major Depressive Disorder (MDD), by imposing a suppressive effect on the connections between neurons in the artificial network. Surprisingly, this simulated spine loss is sufficient to induce a variety of depression-like behaviors, including anhedonia, generalized avoidance, increased discounting, and an altered exploration/exploitation balance in the artificially intelligent agent. These results support a conceptual model that MDD is a consequence of the reduction of brain connectivity rather than an imbalance in monoamines. The computational model suggests that reversing the spine-loss effect aids in the rescue of rewarding behavior under certain conditions. This supports the search for treatments that increase neuroplasticity and synaptogenesis as a means of treatment, specifically for individuals with treatment-resistant depression.

P2-G-323 - Design and expression of an Ankyrin-like molecular probe for alpha-Neurexin 1 labelling in live neurons.

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Neurexins (NRXNs) are a set of polymorphic cell adhesion molecules that play a key role in formation of new synaptic connections and pre-synaptic differentiation. Multiple binding partners for both Î±- and Î²-neurexins help shape the synaptic terminal and stablish stable connections between neurons. Additionally, NRXNs participate in remodeling of synapses terminal and the establishment of new connections. Impairment of proper neurexin function has been related to several psychological, developmental disorders and have been proposed to play a role in neurodegenerative pathogenesis in Alzheimerâ€™s disease alongside their post-synaptic counterpart neuroligin 1 (NLGN1). However, we lack the tools to study molecular mechanisms involving neurexins impairments in live cells without

undergoing genetic engineering or heterologous gene expression.
Herein we describe the development of a selective molecular probe for a-NRXN 1 to allow the real-time tracking of the receptor in live neurons. To achieve this, we used Î±-latrotoxin (Î±-LTX), a neurotoxin from the Latrodectus genus. Docking simulations were carried out to determine the binding domain to Î±-NRXN. We generated a bacterial recombinant expression vector to produce an ankyrin-like fragment of Î±-LTX. This Î±-LTX fragment was then used in transfected HEK 293 cells with Î±-NRXN 1 to measure binding selectivity and cytotoxicity. The final molecular probe offers a new approach for neurexin labelling in live cells to track its dynamic re-organization at synaptic termini during synaptic plasticity.

P2-G-324 - 3D-Bioprinting hiPSC derived brain model: innovative platforms for brain disease modeling and drug screening

Stefano Sorrentino [1], **Stefan Wendt** [1], **Wenji Cai** [1], **Christopher Lee** [1], **Declan Brennan** [1], **Xiujuan Wu** [1], **Haakon Nygaard** [1]

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Our understanding of brain physiology and pathology is limited by the lack of models closely resembling the human brain. Human induced pluripotent stem cell (hiPSC) 3-dimensional (3D) models, such as organoids and neurospheres, are emerging as innovative approaches to modeling nervous tissue *in vitro*. However, they rely on hiPSC self-organization and are characterized by low reproducibility and homogeneity. 3D bioprinting is an innovative bioengineering technique that combines biomaterials and live cells to shape 3D structures in a layer-by-layer fashion. The surrounding matrix improves the exchange of nutrients, oxygen, and drugs making them closer to the physiological fluidic dynamic. Here, we generated a 3D-Bioprinted brain model suitable for long-lasting culturing of iPSCs-derived cortical neurons and astrocytes starting from neuronal precursor cells (NPCs). NPCs can be successfully bioprinted in a multilayer wood-pile structure to mimic the human cerebral cortex architecture with high spatial resolution, low pressure, and high speed maintaining cell viability and proliferation. NPCs can be efficiently expanded and differentiated into cortical neurons/astrocytes in the 3D environment establishing functional networks across layers. Moreover, when synthetic AÎ²42 is administrated to the culturing medium, hiPSC-derived microglia can efficiently infiltrate into 3D bioprinted constructs and phagocyte amyloid deposits. Overall, our data indicate the potential of personalized 3D bioprinted neuronal models as a future possible drug screening and disease modeling platform.

P2-G-325 - The Omniroute maze: a novel rodent navigation apparatus that integrates dynamically configurable routes, sensory cues and automated reward delivery

Adam Lester [1], **Afsoon Gharib Mombeini** [1], **Gurnoor Kaur** [1], **Nadira Djafri** [1], **Abhishek Dhir** [1], **Matin Narimani** [1], **Manu Madhav** [1]

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We constructed a novel maze that enables flexible, real-time control over routes and sensory cues, akin to virtual reality (VR) systems but for unconstrained real-world rodent behavior. The maze measures 90 x 90 cm and features 60 movable wall segments that can be programmatically configured to create unique routes within a 3 x 3 grid. Four projectors, arrayed around the maze's perimeter, display distinct visual cues on both sides of any subset of raised walls and the maze floor. These projectors also house speakers to provide directional auditory cues. The system incorporates high-speed 3D tracking of a rat's position and orientation, allowing for closed-loop control of the paths and environmental cues based on real-time behavior. An automated gantry system delivers food-based rewards anywhere in the maze. Both the hardware components and the electrophysiological data collection system are controlled using the Robot Operating System (ROS) framework. The Omniroute maze supports the replication of classic behavioral mazes and a variety of configurations to test hypotheses about the interaction between routes, cues, neural representations, and navigational decisions. Its automated reconfiguration, tracking, and reward delivery enable high-throughput experiments on complex navigation behaviors without the potential biases introduced by direct experimenter intervention. Designed from the ground up for robust operation, the Omniroute system utilizes affordable hardware and software to facilitate easy fabrication and assembly ass well as replicability by other researchers.

P2-G-326 - Neuro-immune interactions in long-term sliced cortical organoids

Declan Brennan [1], **Stefan Wendt** [1], **Stefano Sorrentino** [1], **Haakon Nygaard** [1]

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IPSC-derived cerebral organoids are often limited by reduced cell-type diversity that prevents translatability of findings to the clinic. We aimed to model the interaction between CD8+ T-cells and microglia in Alzheimer's disease that occurs due to Blood-brain barrier breakdown. To do this, cortical organoids were generated using a modified version of a previously established protocol, sliced at 300 Âµm and placed on a transwell membrane which allows for greater oxygen diffusion. At day 186 from initial EB formation, organoid slices were incubated with iPSC-derived microglia and primary CD8+ T-cells and co-cultured for 14 days with or without APOE4 and IFN-Î³ treatment. Both CD8+ T-cells and microglia successfully infiltrated into the organoids, visualized by immunostaining. Additionally, organoids showed the presence of neurons, astrocytes, oligodendrocyte precursors, microglia, and CD8+ T cells. Given that protein components of the blood can induce inflammation, cortical organoid slices

were treated with serum and RNA was isolated for Bulk RNA sequencing. Serum treatment increased the expression of genes associated with inflammatory astrocytes, including GFAP, VIM, CD44, and CRYAB. Additionally, increased ECM deposition was observed via both RNA sequencing and immunostaining, potentially increasing the potential for infiltration and migration of immune cells to the site of inflammation. In summary, our study utilizing iPSC-derived cortical organoids reveals a successful modeling of the interaction between CD8+ T-cells and microglia, highlighting the potential of this system to investigate the impact of BBB-breakdown on neuro-immune responses, with implications for understanding disease mechanisms and therapeutic interventions.

P2-G-327 - PokeMouse: Towards automated 3D pose estimation and unified representation in rodent models

Hao Hu [1], **Tony Fong** [1], **Helge Rhodin** [1], **Timothy Murphy** [1]

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Accurate measurement and analysis of animal behaviors are crucial for advancing our understanding of neuroscience. Previous studies heavily relied on 2D/3D keypoint pose estimators, contributing significantly to the field. However, previous tracking methods face many challenges, including sensitivity to occlusion, limited transferability, and susceptibility to illumination variations. In contrast, the rotation method with a predefined skeleton, may provide a consistent metric for behavioral analysis. The 3D model also enhances spatial understanding, enabling reliable categorization across subjects and generalization to diverse experimental scenarios.

To estimate 3D pose from video recording, we introduce Pose Optimization with a Kinematic Evidence-based mouse model (Poke-mouse), a SMPL-format 3D mouse body model rooted in 3D rotation. Preprocessing involves extracting mice with the Segment Anything Model, 3D surface reconstruction with space carving, and 2D semantic keypoints tracking using Deeplabcut. Incorporating this evidence, rodent pose is estimated within the 3D model. Performance evaluation used an open-source multi-camera dataset, assessing accuracy and robustness through metrics such as Mean Per Joint Position Error (in pixels) and Mean Squared Error for silhouette (in pixels).

This method enhances robustness, spatial understanding, transferability, and reduces sensitivity to illumination changes. Our approach aims to establish a more accurate and standardized foundation for understanding the intricate relationship between behavior and neural activity.

P2-G-328 - Streamlining in vivo neuroscience experiments: A novel file structure approach

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In vivo neural imaging experiments often face a significant challenge: the laborious analysis process can take weeks to yield even preliminary conclusions. While numerous tools are available for data processing, such as converting image stacks into neural signals, there is little to no solutions that address the complexity of subsequent analysis steps, which incorporate behavioral data and consider the experimental design. We propose a file structure for individual neural recordings and entire experiments that simplifies data management at each analysis stage. Despite the initial processing step varying between different data types, such as fiber photometry signals and mini-microscope images, the overall processing pipeline for in vivo neural recordings is remarkably similar. Our proposed file structure, utilizing the Hierarchical Data Format (HDF), comprises four main sections: acquisition, behavior, processed data, and analyzed data. Each section corresponds to a key analysis step and includes relevant metadata, such as algorithm names, parameters, linked data, etc. The experimentâ€™s file structure, also in HDF, outlines the experimental design, links all associated recordings, and groups analyzed data according to the defined experimental design. We illustrate the benefits of these file structures using a fiber photometry experiment as an example. Overall, our file organization approach streamlines the analysis process, ensures reproducibility, and enhances the efficiency of data analysis in *in vivo* neuroscience experiments.

P2-G-329 - Mapping cognitive activity from electrocorticography local field potentials in humans performing N-back task

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Advancements in data science and assistive technologies have made intracranial brain-computer interfaces (iBCIs) increasingly viable for enhancing the quality of life in physically disabled individuals. Intracortical micro-electrode implants are a common choice for such a communication system due to their fine temporal and spatial resolution. The small size of these implants makes the implantation plan critical

for the successful exfiltration of information, particularly when targeting representations of task goals that lack robust anatomical correlates. Working memory processes including encoding, retrieval, and maintenance are observed in many areas of the brain. Using human electrocorticography recordings during a working memory experiment, we were able to localize cognitive activity associated with the task and to identify key locations involved with executive memory functions. From the analysis, we could propose an optimal iBCI implant location with the desired features. The general approach is not limited to working memory but could also be used to map other goal-encoding factors such as movement intentions, decision-making, and visual-spatial attention.

P2-H-330 - Understanding the individual challenges and experiences of YAs with a diagnosis of glioblastoma and high-grade astrocytoma

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Objectives/purpose: Young adults (YA) (aged 18-39) are uniquely impacted by high-grade glioma (HGG). However, there are few resources and information available to address these unique needs. The purpose of this study is to explore the experiences and challenges faced by this population to inform the development of resources to help address their specific needs.

Methods: A simultaneous mixed methods (Quant-Qual) design was chosen to survey and interview YA HGG patients at the Princess Margaret Cancer Center. The survey explored patient demographics, symptom experience and level of satisfaction with current care at Princess Margaret. For the interviews, we asked questions pertaining to their illness experience and needs. We triangulated the data sets using descriptive statistics for the survey and thematic analysis for the interviews. A coding framework was created to determine emerging themes using qualitative data analysis software NVivo 10.

Results: To date, 7 surveys and interviews (3 men; 4 women; age range 19-37) have been completed. Triangulation of the results illustrated that living with HGG as a YA is extremely challenging because of: 1) disruptions in life goals and plans due to cognitive changes and/or seizures ; 2) a lack of YA HGG specific education, advocacy, and support resources at diagnosis and during treatment; and 3) A call for more YA HGG community building and connecting.

Conclusions and clinical implications: These results highlight a connection between the challenges of daily living of a high-grade glioma diagnosis in young adults. Next steps include creating tools to better inform and support these young adults.

P2-H-331 - A decolonial perspective in neuroscience: A thematic literature review

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Background: Racism and whiteness contribute to many aspects of the academe, from how knowledge is produced, to who is producing it. With the increased recognition for the need to carry out a decolonial agenda in teaching and research across institutions and disciplines, how does neuroscience address the need for a decolonial perspective?

Aim: To analyze literature thematically for insights into racism and marginalization in Neuroscience.

Methods: Neuroscientific literature was scanned using terms related to racism and epistemic exclusion, and analyzed to extract dominant themes and perspectives. **Results**: Ten publications were included in our preliminary analyses of the literature. The main themes discussed related to the colonial roots of the discipline, the coloniality in research, and the marginalization of scholars. Themes related to representation, faculty experiences and pipeline in the discipline were also discussed. Specifically, the literature highlighted issues of inadequate representation among racialized individuals within both scholarly works and research participants. The literature highlighted epistemic racism in scholarly literature, impacting facets of the knowledge production process such as authorship, editorial roles, and publishing practices for diverse individuals. Several recommendations to advance a decolonial neuroscientific agenda were made. **Conclusion:** This project furthers the reflection on racism, whiteness, and epistemic oppression in the hopes to propel transformative change within neuroscience.

Poster Session 03

P3-A-332 - Wnt-calcium pathway instructs neurite pruning through CaMKII and PKC in C. elegans

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During development, neurons eliminate excessive neurites via a process called neurite pruning. Previously, we showed that Wnt instructs neurite pruning of a cholinergic motor neuron PDB in *Caenorhabditis elegans* (Lu and Mizumoto, 2019 PMID: 31804181). However, the mechanisms by which Wnt instructs neurite pruning remain unknown.

From a candidate screening, we found that two calcium-dependent protein kinases, calcium/calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC), are required for PDB neurite pruning. We observed that the double loss-of-function (lof) mutant of CaMKII and PKC exhibits a severe neurite pruning defect, similar to the Wnt (lof) mutant. Neither CaMKII nor PKC lof mutation enhances the pruning defect of the Wnt mutant, suggesting that CaMKII and PKC function in the same genetic pathway as Wnt. Consistently, the CaMKII gain-of-function mutation suppresses the pruning defect of the Wnt (*lof*) mutant. This result suggests that CaMKII functions downstream of Wnt to regulate neurite pruning.

We also found that inositol 1,4,5-trisphosphate receptor (IP3R), a calcium channel on the endoplasmic reticulum (ER), is also required for PDB neurite pruning. This raises the possibility that calcium influx from the ER is required for CaMKII and PKC activation. We are currently examining the local calcium dynamics at the pruning neurites using GCaMP7s, and induce calcium influx in Wnt mutants through channelrhodopsin.

P3-A-333 - Modeling neurodevelopmental defects of Tuberous Sclerosis in a novel 3D-micropattern system

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[1] Simon Fraser University

Introduction: Micropatterning of human pluripotent stem cells (hPSCs), by geometric restriction of the growth surface, can emulate the formation of self-organized embryonic tissues. One application is to model assembly of early neural tissues, providing a reproducible strategy to measure developmental impacts of disease-causing mutations. **Objective:** Our primary objectives are to 1) establish an accessible, high-throughput, cost-effective approach to derive micropatterned tissues using 3D bioprinting, and 2) quantify the impacts of *TSC2* deficiency, expected to prevent neural tube closure, on neural tissue assembly. **Method:** Using the CELLINK BIOX6 bioprinter, micropatterned colonies were formed by printing Cultrex into 600-800 Âµm diameter droplets. iPSCs seeded onto printed surfaces underwent a six-day standard neural induction protocol, followed by immunostaining for neural markers and measurement of tissue architectural features. **Results:** Micropatterned colonies consistently displayed PAX6/SOX2-positive structures containing a central lumen, indicative of developing forebrain tissue. Micropatterns formed by *TSC2*-deficient hPSCs exhibited abnormal central neural region formation, resembling neural tube defects observed in mouse models. **Conclusion:** This study introduces

a reproducible, scalable micropatterning approach of developing neural tissues, suitable for high-throughput assays and drug screening. Furthermore, quantification of tissues from hPSCs carrying disease-associated genetic variants can reveal early neural developmental defects like aberrant neural tube closure.

P3-A-334 - Examining the effects of a two-hit model of early life adversity on development: maternal separation stress and protein restriction delay development in male and female Wistar rat pups

Wendie Marks [1], **Khoi Tran** [1]

[1] University of Saskatchewan

Early life adversity is often characterized by exposure to both stress and malnutrition. Postnatal protein restriction in rodents is linked to stunted growth, altered metabolism, and delayed brain growth. Maternal separation stress in rat pups results in hyperresponsiveness to acute stressors in adult life. However, the effects of simultaneous exposure to early life stress and protein restriction are not well characterized. The objective of this research project was to explore the combined effects of maternal separation stress and protein restriction on development in male and female Wistar rat pups. Lactating Wistar rat dams were fed a protein restricted diet (8% relative to a typical 20%). Pups were subjected to maternal separation stress on postnatal days 2 â€“ 14. Measures of physiological development (i.e., righting reflex, negative geotaxis, eye and ear opening, and grip strength) were examined in pups. No significant effects of sex were observed, so male and female pup data were combined for analysis. Maternal separation stress significantly increased the time required for negative geotaxis, and age of ear opening. Protein restriction significantly reduced grip strength. Protein restriction significantly reduced body weight, however, maternal separation stress moderated this effect reducing the severity of weight loss in pups. These results demonstrate that maternal separation stress and protein restriction significantly delay developmental in Wistar pups, however, maternal separation stress may have a protective effect on weight in protein restricted pups.

P3-A-335 - Investigating the role of the deubiquitinase USP15 in cortical development

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The cerebral cortex helps with intricate brain processes like interpreting sensory input, language and awareness. Unsurprisingly, it has an intricate and highly regulated development that is sensitive to changes in protein expression levels. Deubiquitinases (DUBs) are important regulators of ubiquitinated proteins that have been shown to play important roles in the brain, but are not well studied in the context of brain development. Our objective is to explore whether the DUB USP15 plays a role in cortical development.
Using qrtPCR and immunohistochemistry, we assessed USP15 expression levels in the developing cerebral cortex of male and female mice from embryonic day 12 (E12) to postnatal day 21 (P21). We found that USP15 is expressed in the cortex throughout development, peaking at P3. USP15 also shows differential expression amongst different cell populations in the cortex, especially downregulated in newborn neurons. To address USP15 function, we performed in utero electroporation of embryos at E13, and five days later neurons with ectopic expression of USP15 showed a significant alteration in their distribution across the cortex compared to control neurons. This suggests that USP15 impacts how cortical neurons migrate during cortical development. Interestingly, changes to USP15 nucleocytoplasmic localization appears to alter neuronal migration in the developing cortex as well. In conclusion, our study reveals that USP15 may play an important role in cortical development, and that maintaining proper subcellular localization is critical to its function.

P3-A-336 - Kirrel3 modulates dendrite morphogenesis in the developing olfactory system

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The formation of dendritic arbors in the nervous system is dependent on the dynamic outgrowth and branching of dendritic processes early in development, which is followed by the pruning of exuberant branches. This process is especially important for the tuning of responses in sensory systems, such as the main olfactory system, which is responsible for processing odorant signals from the environment that are essential for rodent survival. In this system, olfactory sensory neurons project their axons to synaptic structures termed glomeruli in the olfactory bulb, where they form synapses with second order neurons, the mitral cells. Proper wiring of this olfactory circuit ensures that these signals are correctly represented in the olfactory bulb through the activation of glomerular maps, which are needed for processing odor information involved in the modulation of food foraging and predator avoidance, for example. The cell adhesion molecule Kirrel3 is expressed on olfactory sensory neuron axons and in mitral cells of the OB, suggesting they may modulate the development of both the pre- and postsynaptic sides of this circuit. We have previously shown that Kirrel3 is necessary for the accurate coalescence of olfactory sensory neuron axons in the olfactory bulb. Here, we investigate whether Kirrel3 contributes to the elaboration of mitral cell dendrite arbors during development. We demonstrate that Kirrel3 is

differentially expressed in populations of mitral cells and that it is essential for the maturation of their dendritic arborization.

P3-A-337 - ER stress underlies altered cell fate during brain development in a human model of the cortical malformation syndrome Tuberous Sclerosis

Shama Nazir [1], **Kaitlyn Locke** [1], **Lisa Lin** [1], **Lisa Julian** [1]

[1] Simon Fraser University

Introduction: Accumulation of unfolded proteins, leading to endoplasmic reticulum (ER) stress and the Unfolded Protein Response (UPR), is implicated in various neurological disorders, including the cortical malformation syndrome tuberous sclerosis (TS). Evidence suggests the UPR also impacts cell fate decisions in the brain.

Objectives: To determine if ER stress-UPR signaling during neural lineage induction produces aberrant neural stem cells (NSCs), neurons and astrocytes, and in skewed ratios.

Methods: Human pluripotent stem cell (hPSC) lines carrying inactivating *TSC2* mutations (which causes TS), and isogenic wild-type lines, are induced into the neural lineage and subsequently NSCs, neurons and astrocytes using standard protocols. High-content imaging and biochemical approaches are used to investigate alterations in the organelle, metabolic, cell fate, and ER stress-UPR pathways.

Results: *TSC2*-deficient hPSCs produce abnormal hypertrophic NSCs, neurons and astrocytes reflecting TS brain lesions. In *TSC2*-deficient cells, ER stress-UPR markers are acutely upregulated during neural induction and chronically at later stages. Chemical ER stress activation during neural induction strikingly mimics and, at higher doses, exacerbates long-term cell fate changes.

Conclusion: The neural lineage is susceptible to proteostasis-ER stress during neural induction. When experienced, this early stress leads to aberrant, dose-dependent, pro-neurogenic fate decisions that underly altered cortical development reflecting TS lesions.

P3-A-338 - Localization of melanopsin (a non-visual opsin) in the retina and brain of developing sablefish (Anoplopoma fimbria)

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[1] University of Victoria

Light regulates development, physiology, and daily and seasonal behavior in a great diversity of species. Light sensitivity is mediated by visual and non-visual opsins, which are GPCRs expressed in eyes and in non-ocular tissue including the brain. We are characterizing the expression of the non-visual opsins in the melanopsin (Opn4) subfamily in aquaculture-raised sablefish (*A. fimbria*). We quantified the transcript abundance of five melanopsins at 22 stages of development using qPCR. A fluorescent antibody that binds three of the five paralogs was used to characterize melanopsin proteins expression in 4 Âµm eye and brain sections at five stages of development. We also used immunogold labeling and EM to localize these proteins in 89 nm retinal and brain sections. Melanopsin expression began seven days after fertilization, and showed the highest expression at age 47 days. Sablefish develop slowly and a 47dpf larva is comparable with a 5dpf zebrafish. The fluorescent probe exposed at least one (perhaps all three) of the stained melanopsins in the developing retina (all cell types) and in neuro-progenitor cells of the forebrain and midbrain. Strong staining in radial glia end-feet suggests a light sensing role in early optic tectum (TeO) development. Immunogold labeling data showed these proteins in the outer segments of the cone photoreceptors. We also found evidence of melanopsin expression in mitochondria in the inner segments of cone photoreceptors.

P3-A-339 - The intestinal microbiome modulates microglia development

Jordan Hamden [1], **Claire Sie** [1], **Shreya Gandhi** [1], **Vivien Dang** [1], **Jatin Choudhary** [1], **Morgan Towriss** [1], **Carolina Tropini** [1], **Annie Ciernia** [1]

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Microglia are innate immune cells resident in the central nervous system that protect against damage and disease and modulate brain development. Microglial development and activity are regulated through local and peripheral signals, including metabolites released by the gut microbiome. Studies in mice lacking a microbiota (germ free, GF) and mouse models of neurodevelopmental disorders suggest microbiome modulation of microglial development may be an important factor in neurodevelopmental disorders like autism spectrum disorder. However, it has yet to be determined which microglial developmental pathways are altered through microbiome modulation. In this study, we examined the role of the gut microbiome on gene expression in microglia during early postnatal development. Studies were conducted in male and female conventional (specific pathogen-free) and GF C57Bl/6J mouse pups 7-, 14-, and 21-days post-birth. Gene expression dynamics were compared between microbiomes, sexes and developmental timepoints using RNA-seq on isolated brain microglia. Peripheral metabolites were

extracted from serum and measured by mass spectrometry. RNA sequencing and mass spectrometry analyses revealed widespread impacts of sex, age, and microbiome on differentially expressed genes and circulating metabolite levels. *These data demonstrate that the intestinal microbiome modulates microglial gene expression during early-life and provide possible mechanisms that may be opportunity for therapeutic targets.*

P3-A-340 - Impact of prenatal alcohol exposure on cognitive functions and inflammatory responses in aging male and female rats: A comprehensive longitudinal study

Sunny Qureshi [1], **Carolina Luft** [1], **Maddy Maheu** [1], **Jordan Albanese** [1], **Victoria Vella** [1], **Kingston Wong** [1], **Parker Holman** [1], **Tamara Bodnar** [2], **Charlis Raineki** [1], **Paula Duarte-Guterman** [1]

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Prenatal alcohol exposure (PAE) can have long-lasting detrimental effects in the developing brain. Among these effects are deficits in cognition and increased inflammation. However, little is known about how PAE-related cognitive deficits change as an individual ages. We filled this gap by using a PAE rat model and examining the relationship between inflammation and cognitive performance on learning and memory tasks in males and females as they age. Pregnant rats were randomly assigned to: ad libitum PAE â€“ liquid ethanol diet throughout gestation; or control â€“ pelleted diet. Their offspring were subjected to the Barnes Maze and Novel Object Recognition (NOR) tasks at 6 and 12 months. Blood was collected following behavioural testing for analysis of peripheral cytokine levels. Analysis of NOR indicated that by 6 months, PAE females displayed recognition memory deficits. By 12 months, PAE males also exhibited deficits in recognition memory. Interestingly, at 12 months, control females displayed similar deficits in recognition memory alongside PAE females. Cytokine data indicated that all PAE animals had lower levels of IL-4 regardless of age. Moreover, PAE females at 12 months showed significantly lower levels of IL-10 compared to controls. Currently, analysis of Barnes Maze is ongoing but preliminary findings at 6 months indicate deficits in PAE animals. These findings suggest PAE resulted in sex-dependent cognitive deficits with PAE females showing earlier decline. Serum cytokines do not seem to mediate this effect, however analysis of neuroinflammatory markers is ongoing.

P3-A-341 - The impact of socioeconomic status on white matter network organization and general cognitive ability in adolescents

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Socioeconomic status (SES) is associated with the development of general cognitive ability; however, the biological underpinnings of this relationship are still not understood. To test whether white matter network organization mediates the relationship between SES and general cognitive ability in adolescents, we constructed white matter connectomes and extracted multiple measures of SES for 814 cases from the Adolescent Brain Cognitive Development study. Graph-theory based metrics of whole brain white matter network organization were produced and general cognitive ability was estimated using NIH toolbox measures. No significant mediation effect of white matter network organization metrics on the relationship between SES and general cognitive ability was observed. Global efficiency and network clustering were both associated with general cognitive ability. Only males showed an association between network clustering and parental monitoring, school engagement, neighbourhood safety, and financial stability. These findings suggest that different aspects of SES have unique impacts on white matter network development and cognition that do not impact all children equally. To test the stability of these findings, further processing and analysis will be conducted on the rest of the approximately 11,000 participants available through the Adolescent Brain Cognitive Development study.

P3-A-342 - Investigating the role of the RNA-binding protein, CELF2, in the etiology of a rare neurodevelopmental disorder

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Regulation of neural progenitor cells (NPCs) self-renewal and differentiation is highly dependent on cell-fate determination gene expression, however the mechanisms that regulate this process are still largely unknown.

Our collaborative research team has identified a cohort of children with a rare neurodevelopmental disease marked by global developmental delay, cognitive impairments, and autism spectrum disorder. Our studies have revealed that self-renewal of radial glia progenitors (RGPs) is maintained by repression of mRNAs that promote neurogenesis by cytoplasmic CELF2. Shuttling of CELF2 into the nucleus releases these mRNAs for translation, allowing for the differentiation of RGPs. CELF2 variants have been shown to accumulate in the cytoplasm due to disrupted nucleocytoplasmic shuttling, which in turn, perturb the balance of self-renewal and differentiation of RGPs.

Here, we use patient-derived induced pluripotent stem (iPS) cells, neural precursor cells (NPCs), and gene-editing technology to i) assess the pathological impact of these variants on brain development, and ii) dissect the mechanisms regulating CELF2 translocation.

Overall, this study will provide key insights regarding the role of CELF2 in brain development, and ultimately, pave the way toward the development of precision medicine for this cohort of patients. This study is funded by the Azrieli Foundation and the Canadian Gene Cure Advanced Therapies for Rare Disease.

P3-A-343 - A bioinformatics approach using microelectrode arrays to characterize the relationship between electrical activity and neuronal growth

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Perturbations during neurodevelopment are linked to various neurological disorders, yet our understanding of neuronal activity during growth and synaptogenesis remains limited. To better characterize electrical activity during neuronal growth phases, we can leverage simple model systems with individually identified neurons like the *Lymnaea stagnalis* (pond snail). For example, LPeD1 and VD4 neurons form excitatory cholinergic synapses in the presence of a trophic factor-rich conditioned media (CM). Pairing these individual neurons with microelectrode arrays (MEAs) uniquely positions us to non-invasively investigate long-term activity of neurons during different stages of development.

Isolated LPeD1 and VD4 neurons, plated with conditioned media on MEAs, were monitored via simultaneous timelapse imaging, extracellular recordings, and live calcium labeling for 12 hours. Our findings revealed a transition from tonic to bursting activity during the branching phase before synaptogenesis, followed by intermittent long bursts and a return to tonic activity post-synapse formation. Our custom analysis code statistically quantified differences in interspike interval (ISI), spike amplitude, and burst qualities across growth stages. Interestingly, there was a decrease in total spikes and an increase in ISI during pre-synaptogenesis growth phases. This study shows, to our knowledge, the first long-term simultaneous characterization of neuronal activity and stages of growth, branching, and synaptogenesis, supported by Ca2+ visualization that correlates activity patterns with growth stages.

P3-A-344 - Defining the impact of interneuron-specific 16p11.2 microdeletions on mouse cortical development and behaviour

Anastasia Zhong-Vorkapic [1], **Jean-François Cloutier** [1]

[1] McGill University

In neuronal networks, a precise balance between excitation (E) and inhibition (I) is required for proper neuronal development and cognitive brain functions. An imbalance between excitatory and inhibitory synaptic transmission is proposed to contribute to altered brain function in some neurodevelopmental disorders, such as autism spectrum disorder (ASD). Such an imbalance may arise from changes in the relative number of neurons or synapses generated, impaired synaptic function/plasticity, or disrupted circuit connectivity. One of the most common genetic factors associated with ASD patients is a gene copy number variation resulting from a microdeletion in a segment of chromosome 16 (16p11.2). Preclinical mouse models bearing 16p11.2-like microdeletions show altered development and function of several populations of neurons, including excitatory and inhibitory cortical neurons. These developmental abnormalities are accompanied by ASD-related behavioural defects that include impaired novelty seeking behaviour, anxiety-like behaviour, hyperactivity, and motor deficits. However, since multiple populations of neurons are affected in these mice, the specific contribution of *16p11.2* genes to the development of cortical inhibitory interneurons and to the behavioural phenotypes observed in these mice remains to be determined. To address these questions, we have generated mice bearing an interneuron-specific deletion of *16p11.2* genes, examined the development of cortical interneurons in these mice, and assessed a battery of behaviours.

P3-A-345 - The chromatin remodeler ATRX regulates entorhinal cortex identity, laminar organization, and circuitry with the hippocampus

Alex Cordova [1], Valerie Cardin [1], Edward Sun [1], Kelly Xu [2], Érik Harvey-Girard [2], Keqin Yan [1], Magid Fallahi [1], Natasha Keshavjee [1], Jing Wang [1], Leonard Maler [2], Jean-Claude Béïque [2], David J. Picketts [1]

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Neurodevelopmental disorders are complex genetic diseases often characterized by altered synapse function and neuronal circuitry. Many causative genes encode chromatin regulators, including the ATRX gene as the cause of the ATR-X syndrome. However, linking altered neuronal circuitry to aberrant chromatin regulation remains poorly understood. Here we leverage a new mouse model by breeding $Atrx^{fl/fl}$ mice with an Emx1-Cre driver (AtrxEcKO) to ablate ATRX in excitatory neurons of the developing forebrain. Unlike other Atrx cKO models, proliferation deficits and early postnatal lethality were avoided, allowing the analysis of adult animals. Neuroanatomical analysis of AtrxEcKO mice showed an increased entorhinal cortex (EC), with a marked reduction in the hippocampus (HP) size. RNAseq analysis of dissected adult HP tissue revealed ~900 differentially expressed genes, including downregulation of

axonal guidance genes (e.g Ntng1 and Ntng2). Ntng1/2 axons traverse from neurons in the EC, where we observed striking alterations in neuronal lamination and loss of the cytoarchitectural border between the EC and the neocortex (NC). Additional immunohistochemistry and differential expression analyses support the idea that many EC neurons retain an NC fate. Consistent with this idea, impaired EC-HP connectivity was shown by AAV-mediated anterograde and retrograde neuronal tracing, which correlated with performance deficits in an EC-HP specific spatial learning task. **Conclusion**: Our findings suggest novel roles for Atrx in the formation of NC/EC border and in the formation of the EC-HP circuitry.

P3-A-346 - Characterization of oligodendrocyte-derived extracellular vesicles across cell differentiation

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Oligodendrocytes (OLs) are the myelinating glia of the central nervous system (CNS), and their dysfunction is a hallmark of several neurodegenerative disorders such as multiple sclerosis. Intercellular communication pathways between OLs and other cell types in the CNS is critical for the proper formation and maintenance of the myelin sheath. Extracellular vesicles (EVs) are a heterogeneous population of secreted membrane vesicles that differ in biogenesis, cargo, and biological functions they exhibit on target cells. OL-derived EVs have been shown to shuttle metabolic and stress proteins to neurons to confer protective effects and regulate myelination. However, the existence of different subpopulations of OL-derived EV over the stages of cell development have not been well characterized. This study assessed several antigens in isolated EVs collected across cell differentiation using the CG4-OL cell line following the minimal information for studies of extracellular vesicles (MISEV2018) guidelines. Relative to the endosomal marker TSG101, the constitutive secretion of EVs containing the tetraspanin markers CD44, CD63 and CD81 remain consistent across cell differentiation. On the other hand, the expression of CD9 increased suggesting a different population of exosomes and/or microvesicles are secreted from mature OLs. Continued work is focused on identifying cargo of these different populations. This will lead to a better understanding of OL-derived EVS, which is a key step to elucidate their role(s) in myelination in the context of health and disease.

P3-A-347 - Spatial transcriptomics reveals region-specific and cell-type-specific gene dysregulation in a Mecp2 mouse model of Rett syndrome

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Rett syndrome (RTT) is a rare neurometabolic disorder primarily attributed to mutations in the X-linked methyl-CpG-binding protein 2 (Mecp2). In female RTT patients, a period of seemingly normal development spans from 6 to 18 months, followed by a gradual regression of speech and motor skills, accompanied by stereotypic hand movements, movement disorders, and sleep disturbances. While heterozygous (Mecp2/+) female mice are more clinically relevant, hemizygous male mice (Mecp2/Y) are the preferred model due to their penetrant phenotype. Notably, in male mice, the phenotype becomes evident at 4-5 weeks of age, although molecular perturbations manifest earlier. Our previous results from RNAseq and immunofluorescence experiments unveiled a critical developmental 'switch' within the Mecp2 brain, occurring between postnatal day 14 and postnatal day 21, leading to the onset of RTT symptoms. To illuminate the precise timing and cellular underpinnings of this 'switch,' we harnessed MerFISH, a spatial transcriptomic technology with high spatial resolution and sensitivity. Employing this approach, we discerned distinct region-specific and cell-type-specific differentially expressed genes associated with cholesterol synthesis, signaling pathways, synaptic function, development, circadian rhythms, and various other vital processes. These dysregulated genes potentially hold developmental significance in the Mecp2 brain, which may underlie the fundamental pathophysiology of RTT.

P3-A-348 - Imaging topographic map reorganization in the growing Xenopus optic tectum

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The retinotectal projection in the *Xenopus laevis* optic tectum is topographically organized. Neurons in the retinotectal system undergo extensive activity-dependent plasticity during early development, which refines the retinotopic map to form a more precise representation of the visual world. We have previously observed marked changes in the three-dimensional layout of the retinotopic map within the tectal volume over a span of several days in development. This raised the question of whether such functional reorganization is accompanied by comparable migration and structural remodeling of tectal neurons as the brain grows. To examine this change in map topography in the context of individual tectal neuronal morphologies and localizations, we performed calcium imaging in the optic tectum of GCaMP6-expressing tadpoles in which individual or small numbers of tectal cells were sparsely labelled with Alexa594-dextran dye. We imaged animals at stage 45-46 and again at stage 48, recording the morphology of the dextran-labelled cells and quantifying the developmental changes in their position and spanning volume of their dendritic fields. By comparing these results to changes in the functional

retinotopic map occurring over the same developmental stages, we explore how morphological changes translate to functional changes in the retinotectal system.

P3-A-349 - Impact of early life insult on neurodevelopmental trajectory, from synaptic to brain-wide connectivity and behavior in zebrafish

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The establishment of a properly wired nervous system to support all of its functions is astonishingly complex. In addition to an extremely elaborate genetic program at play, the composite of every exposure to which the developing nervous system is subjected (the exposome) is highly influential, and occasionally detrimental. To investigate the impact of the exposome on brain circuit structural and functional development from synaptic to brain-wide scales, we adopted the larval zebrafish model. To address the impact of the exposome on brain circuit development, we are exploring the effects of, and recovery from, early insults in zebrafish embryo. We chose to use a brief pro-inflammatory insult (liposaccharide) on embryos of ~5 days post fertilization in various zebrafish lines that express fluorescent markers or activity sensors in specific brain cell populations or synaptic compartments. We monitor with fast multi-plane two-photon imaging at single cell resolution, the spontaneous and visually-evoked activity of nearly every 100,000 neurons of non-anesthetized, non-paralyzed, behaving transgenic larval zebrafish expressing GCaMP6s. We also combine measurements on synaptic remodeling. We then monitor over time the consequences and recovery of the early insult on neuronal activity, microglial activity, synaptic connections and sensory-motor processing and behavior. By performing fast brain-wide two-photon imaging, we are exploring putative changes across the brain in circuit connectivity, which we aim to correlate with local changes in synaptic remodeling. Our experiments should provide a broad and unbiased set of multi-scale data on neural circuit alterations caused by neurodevelopmental insult, which may help understanding the synaptic remodeling mechanisms underlying cognitive dysfunction

P3-A-350 - Electrophysiological interrogation of V2a descending neuron dynamics in zebrafish illuminates the mechanism of sequencing locomotor primitives

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Brain development during infancy is crucial in the formation of complex behaviours in animals. Throughout this period, the rapidly increasing number of neuronal connections facilitates progressively intricate brain functions. Recent research shows that the formation of new connections leads to the sequential creation of parallel circuits that enable the brain to acquire new functions without disrupting existing abilities. Yet, how the brain dynamically orchestrates these distinct circuits for flexible behaviour remains unknown. We investigate this issue by focusing on the hindbrain V2a descending neurons in larval zebrafish, using them as a model to understand circuit interaction dynamics. In this model, early-born neurons are associated with fast, whole-body movement, while late-born neurons are involved in slower, tail-restricted movements. To gain insight into the precise temporal dynamics of these two circuits, we conducted systematic characterizations of their spiking activity during the locomotive transition in escape and spontaneous swim episodes. Fluorescence-based targeted loose-patch recordings of V2a neurons were performed on immotile relaxed (canb1ts25) mutants at day 4 of development. Our results from 77 cells spanning across hindbrain segments 3-5 reveal an unexpected heterogeneity in spiking during swim episodes, including a subset that switches spiking patterns during the transition from fast to slow swimming. Investigating the mechanisms involved with such neural populations may further our understanding on the integration of behavioural neural circuits.

P3-B-351 - Stimulation of human derived microglia via HRH2 alters cell surface prion protein expression; a potential role for PrPC in neuroinflammation

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Although the physiological role of the cellular prion protein has been highly contested, recent evidence indicates this protein may be involved in neuroinflammation and immunity. Microglia are the resident innate immune cells of the CNS that enrich healthy brain functions and we have recently indicated that these cells express prion protein. Microglia can be stimulated by inflammatory mediators such as histamine, a neurotransmitter and neuromodulator involved in neuroinflammation. Here, we demonstrate that a human microglial cell line, HMC3, expresses mRNA and protein for three of the four known histamine receptors, and these cells can be stimulated by histamine to increase proinflammatory cytokine production and alter microglia morphological signaling molecules including Iba1 and CD45. We next demonstrate that these microglia express cellular prion protein and, following histamine-induced stimulation, these cells alter their expression of prion protein on the cell surface in a time- and concentration-dependent manner. These findings suggest that prion protein may indeed be involved in neuroinflammation and neuromodulation in the innate immune response. Lastly, we utilize histamine receptor agonists and antagonists to demonstrate that these histamine-induced changes in microglia cytokine release and prion protein expression are mediated by the HRH2 histamine receptor subtype.

Ultimately, our work is the first to demonstrate that human-derived microglia can be stimulated by a neurotransmitter to alter prion protein expression.

P3-B-352 - Excitatory glycine receptors in the adult hippocampus

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The GluN3A subunit of N-methyl-D-aspartate receptors (NMDARs) plays an established role in synapse development, but its contribution to neural circuits in the adult brain is less clear. Recent work has demonstrated that in select cell populations, GluN3A assembles with GluN1 to form GluN1/GluN3A receptors that are insensitive to glutamate and instead serve as functional excitatory glycine receptors (eGlyRs). Our understanding of these eGlyRs, and how they contribute to intrinsic excitability and synaptic communication within relevant networks of the developing and the mature brain, is only beginning to be uncovered. Here, we demonstrate that GluN3A subunits are present in the adult hippocampus, particularly in the ventral hippocampus (VH), where they localize to synaptic and extrasynaptic sites and assemble as eGlyRs on CA1 pyramidal cells. Mice lacking GluN3A had hyperpolarized CA1 resting membrane potentials and increased paired-pulse ratios, suggesting a role for GluN3A in CA1 pyramidal neuron excitability and Schaffer collateral release probability. GluN3A loss influenced the expression of classical NMDAR subunits, we demonstrate mis-localization of conventional GluN2A and GluN2B subunits. GluN3A loss also enhanced NMDAR-dependent calcium influx, presynaptic glutamate release, and increased the magnitude of NMDAR-dependent synaptic plasticity in the VH. GluN3A did not alter postsynaptic density or morphology. Together, these data reveal a novel role for GluN3A and eGlyRs in the control of hippocampal circuits in the mature brain.

P3-B-353 - The Ly6 protein, Witty, is a novel presynaptic regulator of postsynaptic Glutamate receptor subunit accumulation at the Drosophila NMJ

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During development, motor neuron synapses at neuromuscular junctions (NMJs) undergo tremendous growth and strengthening to meet the demands of growing muscles. The Drosophila NMJ has long served as a paradigm for discovering mechanisms that controlling these processes, due to *Drosophila*â€™s genetic tractability and ease of applying imaging and electrophysiological technologies, as well as the conservation of molecular mechanisms to mammals.

Growth and strengthening of the Drosophila NMJ requires retrograde Bone Morphogenetic Protein (BMP) signaling from the NMJ to motor neuron nuclei and subsequent gene regulation by BMP-activated Smad transcription factors. Taking advantage of this, we screened for BMP-regulated genes in motor neurons, and the *cis*-regulatory sites bound by Smads. We decided to focus on a genomic locus encoding an uncharacterized *ly6/uPAR* family protein, which we named *without maturity (witty*), that is highly upregulated by retrograde BMP signaling. Genes of the Ly6 family are expressed in *Drosophila* and mammalian nervous systems, and numerous members play critical modulatory roles in neuronal physiology relevant to human disorders.

We found that *witty* null mutants show a severe reduction of Glutamate Receptor IIA subunit (GluRIIA) accumulation at NMJ. Using an RNAi knockdown approach, we showed that presynaptic Witty regulates postsynaptic accumulation of GluRIIA, but not other GluRII subunits, at the NMJ. These results indicate a novel trans-synaptic role in post-synaptic receptor localization that may be conserved to mammalian synapses.

P3-B-354 - Chronic upregulation of endocannabinoid signalling in vivo normalizes impaired experience-dependent striatal synaptic plasticity and motor learning in YAC128 Huntington disease mice

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Huntington disease (HD) is a dominantly inherited disorder characterized by degeneration of the striatum, a key structure involved in motor function and learning. Prior to neurodegeneration, studies in animal models of HD have found synaptic and circuit dysfunction. Previously, we reported that endocannabinoid-mediated high frequency stimulation (HFS)-induced long-term depression (LTD) at corticostriatal synapses ex vivo is impaired in YAC128 HD mice, a deficit rescued by application of JZL184 to block degradation of endocannabinoid 2-arachidonyl glycerol (2-AG), thereby increasing CB1 activation. We have now investigated whether in vivo treatment with JZL184 could rescue impaired

synaptic plasticity and behavioural phenotypes. Two weeks of daily JZL184 treatment was sufficient to normalize motor coordination and learning deficits in 5-month-old YAC128 mice on the rotarod test. Interestingly, in brain slices collected from both JZL- and vehicle-treated WT mice, striatal HFS resulted in long-term potentiation (LTP) after behavioural testing in contrast to LTD shown in naÃ¨ve WT mice, suggesting the change in ex vivo plasticity results from experience-dependent changes in vivo. While no significant HFS-induced plasticity was observed in vehicle-treated YAC128 mice, JZL-treated YAC128 mice showed comparable LTP to WT mice, indicating that JZL184 treatment rescued impaired synaptic plasticity in these mice. Targeting impairments in endocannabinoid signaling may be an effective strategy for recovering neuroplasticity and treating the early motor symptoms of HD.

P3-B-355 - Exploration of the role of the TrkC-PTPσ complex in selective regulation of glutamatergic synaptic transmission in mouse CA1

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Proper organization of pre- and postsynaptic elements is crucial for brain function, with adverse organization linked to neurological disorders such as autism and epilepsy. Synaptic organizer proteins are primary regulators of synaptogenesis and synapse organization. TrkC is an established neurotrophin-3 receptor which also promotes excitatory synapse development through transsynaptic interaction with presynaptic PTPÏƒ. To further explore the synapse organizing properties of TrkC, mice harboring TrkC point mutations (TrkC knock-in) that selectively prevent PTPÏƒ binding were generated and assessed for changes in synapse properties. Analysis of fEPSPs in TrkC KI hippocampus slices revealed a significant increase in basal synaptic transmission within area CA1. Further investigation revealed that paired pulse facilitation (PPF), a measure of presynaptic function, was significantly increased at TrkC KI synapses, consistent with decreased presynaptic release probability. These data indicate central roles for TrKC-PTPÏƒ complexes in maintaining basal properties of synaptic transmission at CA1 synapses, independent of TrkCâ€™s role as a neurotrophic receptor. Further study is needed to determine the mechanistic nature of these deficits, which may reflect functional compensation for changes in glutamatergic transmission.

P3-B-356 - The fast and the furrious: Intermittent fasting gives a boost to hippocampal synaptic plasticity

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Intermittent Fasting (IF) is a form of dietary restriction that involves alternating periods of eating and caloric abstinence. It has therapeutic applications for chronic illnesses that include obesity and Type II diabetes, but also has benefits for the brain; IF induces morphological and physiological changes at the neuronal level that are correlated with improved cognitive functioning. Brain-derived neurotrophic factor has been shown to be upregulated in the hippocampus following IF where it could play a role in enhancing synaptic plasticity and neurogenesis. This research sought to identify the effects of IF in modulating long-term potentiation (LTP) in the Dentate Gyrus (DG) of the hippocampus. Male Sprague Dawley rats were randomly assigned to either: 1.) Fasting Condition (2-hour daily *ad libitum* feeding period; water only outside the feeding time), or 2.) Cage Control (*ad libitum* access to chow and water) for 3 to 4 weeks. When the rats reached young adulthood (PND 70-90), *in vitro* electrophysiology was conducted on transverse hippocampal slices (400 Âµm). After recording field excitatory postsynaptic potentials generated by stimulating the medial perforant pathway of the DG, a High-Frequency Stimulation Protocol (HFS; 100 Hz, 4 pulses) known to induce LTP was applied. Preliminary findings indicate that enhanced LTP accompanied a reduction in weight gain following the IF paradigm. These findings support IFâ€™s potential as a multifaceted intervention for metabolic and neurological wellness.

P3-B-357 - Age, region, and sex-dependent NMDA receptor mislocalization in a mouse model of Alzheimer's disease

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N-methyl-D-aspartate receptors (NMDARs) are essential for neuronal health as well as synaptic plasticity, circuit building, learning, and memory. Although these receptors are primarily found on the postsynaptic membrane of excitatory synapses, they can also be found extrasynaptically. Work over the last 10-15 years has suggested that activation of synaptic NMDARs (syn-NMDARs) primarily promotes cell survival and synaptic plasticity, while activation of extrasynaptic NMDARs (ex-NMDARs) promotes synapse weakening and activates cell death pathways. Unfortunately, most methods to assess NMDAR subcellular localization are time consuming, making it challenging to study putative age, sex, and region-dependent changes in NMDAR localization in disease states. Thus, our understanding of NMDAR mislocalization in diseases like Alzheimerâ€™s disease (AD) â€” the worldâ€™s most common neurodegenerative disease â€” remains limited. Here, we have addressed this challenge by developing an efficient super-resolution imaging and high-throughput analysis method that quantifies the nanoscale distances of thousands of

individual NMDAR subunit puncta to their nearest synaptic protein neighbor (e.g. PSD-95 or synaptophysin). We use this technique to profile NMDAR subcellular localization at different stages of disease progression in the 3xTg model of AD, revealing unique effects of age, region, and sex. Overall, our novel imaging and analysis method will serve as a valuable tool going forward to help identify the specific regions and circuits that are most susceptible to NMDAR mislocalization in disease.

P3-B-358 - Biophysically constraint generative algorithm synthesizing realistic astrocytes in sillico

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Astrocytes are prominent glial cells in the brain that contact multiple synapses and form lateral connections between them, where the activity at one synapse may modulate another through internal pathways of the astrocyte. To understand this connection, it is essential to characterize and reproduce astrocyte branching structures in 3D. However, such a framework does not exist currently. We extract essential morphological features from experimental 3D astrocyte tracings. Deploying techniques from statistics, machine learning, and graph theory, we develop a generative algorithm to grow artificial astrocyte branching structures inspired by biophysical constraints. The generated branching structures reproduce essential morphological features observed in experimental data. Therefore, our algorithms fill in the current gap of astrocyte anatomy characterization, and the inner workings of the generative process shed light on the potential biophysical forces that shape the astrocyte branching structure.

P3-B-359 - Drinking with Mary Jane: Prenatal ethanol and cannabis exposure causes sex-specific delays in motor development and impairs cannabinoid-dependent synaptic plasticity

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Combined prenatal cannabis (THC) and alcohol (EtOH) exposure is a growing public health concern due to the potential long-term effects on neurodevelopment. The hippocampus has a high density of CB_1 receptors (CB_1R) and is a shared target for THC/EtOH. It undergoes significant development during gestation, but also continues to mature into adolescence, and even adulthood, through the processes of

neurogenesis and synaptic plasticity. Disruptions to these processes during development can lead to learning and memory deficits. Pregnant dams were exposed to: 1) THC vapor (100 mg/ml); 2) EtOH (6 g/kg/day); 3) THC+EtOH. Controls received: 1) PEG400 vapor or 2) 0.9% saline. Offspring underwent early reflex tests (PND2-16) and behavioural tasks during adolescence (PND30-55). Preliminary results suggest there were no significant behavioural impairments, although some early motor behaviour deficits were apparent in male offspring exposed to prenatal THC. To examine CB_1R-dependent synaptic plasticity, transverse hippocampal slices (400Âµm) were made from adolescent male and female rats (PND55). The medial perforant path input to the dentate gyrus (DG) was stimulated and extracellular field excitatory postsynaptic potentials (fEPSPs) were recorded in the DG. After a stable baseline was obtained, long-term depression (LTD) was induced using low frequency stimulation (LFS; 10 Hz, 6000 pulses). fEPSPs were then recorded for an additional 60 min following the application of LFS. Initial findings show there is a trend toward reduced endocannabinoid-dependent LTD in adolescent males and females prenatally exposed to THC. These results show that combined THC/EtOH exposure can disrupt the development of CB_1R -mediated synaptic plasticity.

P3-B-360 - Investigating endocannabinoid-mediated neuroprotection in adolescent repeated mild traumatic brain injury

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Repeated mild traumatic brain injuries (RmTBIs) are particularly prevalent among adolescent populations, causing neuroinflammation, diffuse axonal injury and persistent cognitive and motor deficits. This current research will investigate the neuroprotective mechanisms of the endocannabinoid system (eCB) following injury as previous literature has pointed to a compensatory mechanism. The aim of this study is to establish eCB levels following a RmTBI paradigm in adolescence. This will be achieved by using a lateral impact model of RmTBIs in adolescent male Sprague-Dawley rats. Animals will either undergo RmTBI or remain injury free and will have a total of 5 hits administered 72 hours apart. Hippocampus, amygdala, frontal and motor cortex regions will be taken to assess eCB levels via mass spec immediately or one week after the last hit. Anxiety-like behaviour (light-dark box) and a working memory task (Novel Context mismatch) will be assessed a week after the last hit. Motor strength (Hanging Bar) will be assessed pre and post injury as well. We hypothesize that following injury, levels of endocannabinoids will be higher in injured rats than in injury free. Endocannabinoid levels between groups will be presented, with the aim of quantification of the eCB. This data will be used to identify the neuroprotective mechanism of the eCB which may allow for pharmacological intervention. Overall, these studies are anticipated to expand the current understanding of a compensatory mechanism the eCB has in neuroinflammation following RmTBIs.

P3-B-361 - Neuronal excitation and synaptic plasticity require TRPV4 activation in primary hippocampal cultured neurons

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Transient receptor potential vanilloid - 4 (TRPV4) is a bivalent cation channel. TRPV4 is polymodal, being sensitive to physiological temperatures (34-41oC), mechanical stretch, as well as endogenous ligands including arachidonic acid (AA) derivatives, 5â€™-6â€™ epoxyeicosatrienoic acid (EET) and anandamide. TRPV4 is highly expressed in the hippocampus of neuronal and glial cells. Subcellularly, it is localized to the plasma membrane, endoplasmic reticulum (ER)-mitochondrial contact sites. Previous studies have shown that TRPV4s regulates neuronal excitability at physiological temperatures and calcium release from ER stores. However, little is known of its role in long-term potentiation (LTP). Primary hippocampal neurons from rat embryos (E18-21) were generated. On DIV14, the role of TRPV4 in LTP was assessed by chemically inducing LTP (90mM KCl, 3x1s) in primary hippocampal neurons. Exposure to the TRPV4 antagonist RN9893 (3ÂµM) significantly reduced the number of GluA1-colocalised with mitotracker/100i⍰m dendrite following cLTP compared to control (56.6Â±18.9%), indicating a significant reduction in active synapses, and thus blockade of LTP (P<0.0001). RN9893 also blocked KCl-induced calcium transients, which were recorded using virally transduced SynGCaMP6f, (61.13Â±19.32% reduction compared to vehicle). These studies suggest that in primary hippocampal neuronal cultures, TRPV4 activation is necessary for neuronal excitability and LTP. These studies reveal a previously unknown role for TRPV4 in the regulation of synaptic plasticity.

P3-B-362 - Investigating the relationship between fMRI, eye-tracking, and self-reported pain measures in Fibromyalgia

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Fibromyalgia (FM) affects roughly one million people in Canada; despite its severe impact on the well-being and daily functioning of many people, it is poorly understood. Previous research in the field suggests that FM involves heightened pain sensitivity to pain and autonomic dysfunction. Differences in nociceptive processing in brainstem and spinal cord regions have also been identified in FM. The present study focuses on performing fMRI in the brainstem and spinal cord of FM and healthy participants while applying a noxious heat stimulus and eye-tracking. This is to identify the neural correlates of altered pain

processing in FM and the associated alterations in eye-tracking measures. We hypothesize altered nociceptive processing in fibromyalgia (FM) compared to healthy controls, affecting the brainstem and spinal cord's autonomic and descending pain regulation regions (parabrachial nuclei, nucleus tractus solitarius, locus coeruleus). Additionally, we predict less pupil diameter variation in FM due to tonically engaged sympathetic responses and altered neural signalling, reflected in coordinated BOLD responses and structural and physiological modelling models related to pain characteristics and eye-tracking measures. Our data consists of 25 healthy and 25 FM female participants. During fMRI data collection, a calibrated noxious heat stimulus was applied to the palm of the right hand. fMRI data were analyzed using SAPM, a connectivity analysis method providing a neural signaling model that explains observed BOLD signal characteristics and includes information about inhibitory and excitatory signaling. The results suggest differences in signaling linked to arousal and autonomic regulation, while variations in pupil sizes and gaze positions during heat stimulation correlate with pain ratings across trials.

P3-B-363 - Single-cell transcriptomics reveals pericyte-glia interactions during neurovascular dysfunction in glaucoma

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Purpose: Neurovascular dysfunction is a hallmark of glaucomatous neurodegeneration. This study investigates cellular and molecular mechanisms contributing to blood flow abnormalities in ocular hypertension (OHT)

Methods: Using magnetic microbeads, OHT was induced in mice, and single-cell RNA sequencing analyzed gene expression in retinal cells. Dimensionality reduction identified cellular clusters, and gene set variation analysis focused on calcium (Ca^{2+}) signaling in pericytes. Cell communication was assessed using CellChat, with findings validated by immunohistochemistry and electron microscopy.

Results: **:** scRNA-seq analysis revealed two distinct retinal pericyte populations: one characterized by high levels of *Cacna1* (L-type Ca^{2+} channel subunit) and another expressing *Kcnj8* (ATP-sensitive K^{+} channel subunit). OHT enhanced Ca^{2+} pathway activity, including *Cacna1* upregulation. Pericytes increased interactions with macroglia, which was validated by FIB-SEM. OHT also upregulated S100B levels in astrocytes and MÃ¼ller glia, with increased S100B protein confirmed by ELISA and immunolabeling.

Conclusions: The study highlights changes in Ca^{2+} pathways in OHT-affected pericytes, particularly in L-type Ca^{2+} channel activity, and points to stronger glia-pericyte connections mediated by S100B. It emphasizes the importance of Ca^{2+} balance and neurovascular unit communication in glaucomatous blood flow alterations.

P3-B-364 - Quantitative analysis of astrocyte properties in a Syrian hamster model of COVID-19

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BACKGROUND AND AIM: Neurological sequelae, like impaired cognition and fatigue, are commonly associated with COVID-19. Heightened peripheral inflammation associated with respiratory infection may impact blood-brain barrier integrity, ultimately resulting in neuroinflammation. Neuroinflammation is commonly associated with increased astrocyte proliferation and process extension. As such, we hypothesize that mild COVID-19 respiratory infection will promote similar changes in astrocytes. Here we created an unbiased quantitative imaging and analysis pipeline to investigate the impact of COVID-19 on astrocyte properties. METHODS AND RESULTS: We used an established Syrian Hamster model, which recapitulates several aspects of most human COVID-19 cases. Brains were collected at 1, 3, 5, 7, and 31 days following intranasal inoculation with SARS-CoV-2. The samples were subsequently sliced, immuno-labelled with marker antibodies, and imaged via confocal microscope. We digitally isolated brain regions involved in cognition and susceptibility to inflammation and subjected these images to a MATLAB-based analysis pipeline that we developed. Using our pipeline, we are now quantifying astrocyte density via the nuclear marker SOX9, and analyzing the distribution of GFAP which increases in neuroinflammation and may also provide insight into process extension. CONCLUSIONS: Our findings will shed light on the impact of COVID-19 respiratory disease on astrocytes, and advance understanding of COVID-19-associated neurological symptoms.

P3-B-365 - Dynamics of lipid droplets in microglia regulates neuroinflammatory and behavioural responses to LPS

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Lipid droplets (LD) are organelles that store neutral lipids such as triglycerides (TG). While LD are primarily known to generate fatty acids (FA) acting as energy substrates, they also play a role in inflammation by sequestering or releasing FA that are precursors for the synthesis of lipid signals of inflammation. Accumulation of LD has been observed in microglia and macrophages in response to pro-inflammatory stimuli. Recent studies suggest that adipose triglyceride lipase (ATGL), the enzyme catalyzing the first step of TG lipolysis in LD, is a key regulator of inflammatory responses in different cell types. Thus, we aimed to establish the role of LD accumulation in microglia on inflammatory and behavioral responses to acute pro-inflammatory insult. First, lipolysis inhibition in mouse primary microglia by ATGListatin led to LD accumulation and blunted the expression and secretion of pro-inflammatory cytokines induced by lipopolysaccharides (LPS). Targeted and untargeted lipidomic studies revealed that ATGL inhibition reduced the production of pro-inflammatory prostanoids induced by LPS and affected the ceramide profile. Finally, specific ATGL deletion in microglia reduced LPS-induced IL-6 expression and alleviated sickness behavior in male mice. Our findings demonstrate that ATGL inhibition reduces LPS-induced inflammation and suggest that inhibiting LD lipolysis may play a beneficial role in neuroinflammation. Ongoing studies are aimed at testing the role of LD formation in inflammatory responses by targeting the main esterification enzyme (DGAT1) in microglia.

P3-B-366 - Test-retest reliability of homeostatic plasticity induced and assessed in the primary motor cortex using thetaburst stimulation

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Homeostatic plasticity regultes synaptic plasticity to maintain an optimal level of neuronal activity by preventing excessive neuronal excitability or silencing. Abberant homeostatic plasticity is thought to play a role in chronic pain, hence a reliable approach to induce homeostatic plasticity is needed. Recently, it has been demonstrated that transcranial magnetic theta burst stimulation (TBS), can be used to induce

homeostatic plasticity in the human cortex, by implementing a priming-test TBS paradigm. However, the reliability of TBS induced homeostatic plasticity has yet to be investigated. We investigated the test-retest reliability of TBS induced homeostatic plasticity in the primary motor cortex (M1) in 11 healthy individuals, across days 0, 2, and 7. Using a cross-over design, homeostatic plasticity was investigated in response to both an excitatory and inhibitory priming-test paradigm. To assess homeostatic plasticity, 20 MEPs were recorded from the first dorsal interossei (FDI) muscle at baseline, between the priming and test blocks, and at 0-, 10-, and 20-minutes post-stimulation. Homeostatic plasticity was successfully induced for both the excitatory and inhibitory paradigm ($F = 3.72$, $p = .045$). Overall, cortical excitability was highly consistent across days (ICC = .89). Reliability for the inhibitory priming paradigm was slightly higher (ICC = .86) compared to the excitatory paradigm (ICC = .82). Our findings show that the priming-test TBS paradigm could serve as a dependable non-invasive tool to investigate homeostatic plasticity, paving a novel way for studying the neural underpinnings of chronic pain.

P3-B-367 - The collapse of degeneracy: How a loss of intrinsic biophysical diversity in deep Subiculum Pyramidal neurons paves the way for seizure activity

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A neurological insult has the potential to bring about an epilepsy phenotype, while at other times a healthy state in the brain is preserved. The concept of degeneracy, the ability of distinct entities to compensate for one another and adapt to unpredictable changes, provides an elegant solution to explain the homeostatic mechanisms that maintain healthy functioning of the brain when threatened by insults. However, a degenerate system requires heterogeneous components and may collapse when variability of these components is reduced. We hypothesized that a loss of neuronal heterogeneity, a precondition to degeneracy, underlies the failure of homeostatic mechanisms leading to an increased vulnerability to insults and emergence of seizure activity. Using the kainic acid model of epilepsy, we investigated the heterogeneity of intrinsic biophysical properties of deep subiculum neurons, an essential participant in seizure propagation. By comparing whole-cell patch-clamp recordings, we found a significant loss of heterogeneity in the spike threshold of subiculum neurons engaging in seizure-activity. This finding was not influenced by inter-animal variability or accompanied by a difference in the mean threshold value. The loss of heterogeneity in the threshold property suggests that deep subiculum neurons are more selectively tuned to respond to a tight range of inputs and lack flexibility to seizure perturbations. While the exact mechanisms underlying this loss have yet to be investigated, this finding underscores a potential collapse of degeneracy that accompanies epilepsy.

P3-B-368 - Deciphering the path to seizure activity: Unraveling the loss of intrinsic biophysical diversity in human cortical slice cultured neurons

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Epilepsy, a complex neurological disorder marked by synchronized electrical activity in the brain, has remained an intricate puzzle despite a decade of research. The challenge is heightened by the fact that approximately 30% of patients do not respond to anti-epileptic medications that target the E/I balance, emphasizing the necessity to explore epilepsy beyond the conventional excitatory/inhibitory (E/I) balance paradigm. In the context of this ongoing challenge, we hypothesize that the occurrence and spread of seizures may be accompanied by a loss of neuronal intrinsic biophysical diversity. To elucidate this, we suggest that placing cortical neurons in an environment characterized by highly synchronized input, resembling the conditions of epilepsy, can induce a homogenizing effect on intrinsic biophysical properties over time through mechanisms of intrinsic plasticity.
To test our hypothesis, the kainic acid (KA) model of temporal lobe epilepsy and cultured human cortical slice were used to investigate the diversity of intrinsic biophysical properties among cortical pyramidal neurons.
Using whole-cell patch -clamp, preliminary findings reveal a significant reduction in some intrinsic biophysical diversity such as resting membrane potential and inter spike interval among pyramidal neurons in both KA and slice culture conditions models.
Our research sheds light on how shared environments, whether induced through synchronized activity or within slice culture conditions, lead to a decrease in intrinsic biophysical diversity.

P3-B-369 - Huntingtin plays an essential role in the adult hippocampus

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Huntington's disease (HD) is a devastating neurodegenerative disorder caused by a CAG repeat expansion in the huntingtin (*HTT*) gene, resulting in the production of a mutant huntingtin protein (mHTT). The expression of non-pathogenic wild type HTT (wtHTT) is low in HD brains and is at risk of being reduced even further by non-selective HTT-lowering strategies for the treatment of HD. Thus, it is imperative that we better understand the putative consequences of wtHTT reduction in adulthood.

Here, we conditionally deleted (cKO) wtHTT in the hippocampus of adult *Htt*$^{fl/fl}$ mice by AAV-Cre injection. wtHTT cKO, confirmed by Western blot, increased GFAP staining intensity and impaired synaptic plasticity at Schaffer collateral synapses 1-2 months post-injection. We observed reduced intrinsic excitability but an increase in the amplitude of spontaneous excitatory postsynaptic currents recorded from CA1 pyramidal neurons in wtHTT cKO mice. Furthermore, striking morphological defects were noted in a subset of cKO brains, primarily driven by a thinning of the stratum oriens and pyramidale layers. Lastly, cKO mice exhibited clear deficits in the Morris water maze, indicating a spatial learning impairment.

In all, these observations contribute valuable insights to our evolving understanding of wtHTT's intricate role in cellular and synaptic function in the mature brain. Importantly, while the striatum is particularly sensitive to the toxic effects of mHTT presence, our results highlight the hippocampus as a potentially vulnerable region to the effects of non-selective HTT lowering.

P3-B-370 - The role of phosphatidylserine in synaptic development and plasticity at the Drosophila melanogaster neuromuscular junction

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Numerous studies have focused on how various proteins regulate synaptic development and plasticity. However, the roles of lipids, such as phosphatidylserine (PS), have been less studied. PS is a phospholipid synthesized by *Phosphatidylserine synthase* (*Pss*) and upon localization to the cell membrane is transported from the outer to inner leaflet by *dATP8B* (*ATP8B*), a phospholipid flippase. *Drosophila Pss* loss-of-function mutants have previously been shown to have reduced synaptogenesis/axonal growth. The effects of loss of *dATP8B* on synaptic growth have not been characterized. The primary objective of this study is to determine if PS is required for normal levels of synaptic growth and plasticity. We hypothesize that PS is required for proper synaptic growth as well as activity-dependent synaptic growth. We found that synaptic growth in *ATP8B* mutants were generally not significantly different in the number of boutons or active zones when compared to their controls. *Drosophila* larvae raised at high temperatures (30Â°C) have previously been shown to have increased locomotion, resulting in activity-

dependent synaptic growth. We found that this activity-dependent synaptic growth is largely absent in *ATP8B* mutants. Current experiments are examining whether presynaptic or glial *Pss* and *ATP8B* are required for activity-dependent growth. Future experiments will use Lactadherin-C2-mCherry, a fluorescent probe specific to PS, to determine if PS localization changes in response to increased synaptic activity and whether these changes are important for activity-dependent synaptic growth.

P3-B-371 - Expression and localization of 5-HT receptors across sexes in the dorsal horn of rats and humans

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The serotonergic system contains promising molecular targets for the development of new pain therapeutics. Preclinical rodent studies using agonists and antagonists against serotonin (5-HT) receptor subtypes have tied serotonergic signalling to spinal pain processing, yet the specific modulatory mechanisms within defined dorsal horn nociceptive circuits are largely unknown. The expression and localization of 5-HT receptors across laminar regions of the spinal cord have remained relatively unexplored in human samples, and minimal consideration has been given to potential sex differences in either rodents or humans. This study employed a multidimensional approach to map the distribution of 5-HT receptors in rat and human spinal pain circuits. Through immunohistochemistry, qRT-PCR, and single-cell/nuclei RNA sequencing, we investigated the spinal expression of all 5-HT receptor subtypes as well as localization of 5-HT2C in dorsal horn circuits, with analyses across spinal cord region, sex, and species. Our findings suggest that the 5-HT2C receptor, along with other receptor subtypes such as 5-HT4 and 5-HT7, are densely expressed across the dorsal horn of both rats and humans, with preferential localization to the nociceptive superficial laminae. We are investigating differential expression of 5-HT2C across sexes in both lumbar and thoracic spinal regions. The pronounced expression of specific subtypes of 5-HT receptors in both rodent and human spinal pain processing circuits suggest a critical role for descending serotonergic modulation in the perception and treatment of pain.

P3-B-372 - Miglustat selectively protects small diameter axons against Cuprizone-induced demyelination

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Multiple sclerosis (MS) is an autoimmune disease characterised by phases of axonal demyelination that eventually provokes axonal degeneration. Miglustat (Zavescaâ„¢; Actelion Pharmaceuticals) is a drug approved for the treatment of Gaucher and Niemann-Pick type C genetic diseases that is designed to inhibit the pathological accumulation of glycosphingolipids in cells. In the EAE mouse model for MS, Miglustat has recently been shown to inhibit astrocyte-mediated inflammation in the CNS. To test its impact on demyelination, we treated mice with Miglustat during a cuprizone (CPZ)-induced demyelination protocol. Brain sections were immunolabelled for myelin, astrocyte and microglia specific proteins to assess demyelination and potential protective effects of Miglustat in the corpus callosum. Spectral confocal reflectance (SCoRe) imaging was used to quantify global myelin integrity and myelin debris in the corpus callosum. Using transmission electron microscopy, we measured G-ratio, myelin thickness, major dense lines number and periodicity to characterise myelin ultrastructure. Our results indicate that Miglustat treatment did not improve global myelin integrity when given during demyelination, but provide evidence that it specifically protects smaller axons from demyelination, and may protect axonal integrity. If confirmed in humans, both impacts could be beneficial to MS patients. Further investigation will be required to determine if the partial protection conferred by Miglustat during demyelination may promote remyelination following the end of CPZ treatment in mice.

P3-B-373 - Structural homeostatic plasticity fine tunes trans-synaptic size relationships of spine synapses

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The size and shape of dendritic spines in the mammalian nervous system is thought to reflect the functional state of the synapse. Multiple elements of synapse structure appear to scale with one another; large active zones with multiple docked synaptic vesicles often appear on large spines, while smaller spines are associated with smaller active zones and fewer docked vesicles. Here we examine how the ultrastructural size relationships of spine synapses change when their function is altered by activity perturbations that engage presynaptic homeostatic plasticity (PHP). Using serial section transmission electron microscopy and quantitative 3D modeling we find that partial AMPAR antagonism drives the coordinated growth of active zones, docked vesicles, and dendritic spine heads in the adult mouse hippocampus. We define this homeostatic synapse growth as *structural homeostatic plasticity* (SHP). Notably, SHP preserves and strengthens the size relationships between trans-synaptic elements, suggesting the engagement of a coordinated growth program across the synaptic cleft. Mechanistically, we find that a point mutation in the secreted semaphorin, Sema3a, impairs SHP by blocking the

expansion of active zones and docked vesicle numbers, though the growth of spine heads remains intact. Consequently, we find that trans-synaptic size relationships are altered in Sema3a mutant mice. Taken together our results indicate that Sema3a is a homeostatic factor that coordinates the ultrastructural size relationships of spine synapses in the adult mammalian brain.

P3-B-374 - Investigating pathogenicity and rescue of the P369R Kv7.5 channel variant associated with intellectual disability and epileptic encephalopathy

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Epilepsy is a neurological disorder that is characterized by uncoordinated neural activity and recurrent seizures. Among children with epilepsy, 30-40% suffer from idiopathic epilepsy with a genetic cause. Genetic sequencing has enabled identification of several monogenic causes of idiopathic epilepsy. These include genes that encode ion channels, such as voltage-gated K^+ (Kv) ion channels, which control neuronal excitability by producing a sustained voltage-dependent current, known as the M-current. Previously, novel missense variants in Kv7.5 channels were identified in children affected by idiopathic epilepsy. One variant, P369R, produces a gain of function, which would be expected to increase the suppression of excitability, leading to pathophysiology. To further investigate the physiological consequences of the P369R variant, we measured gating properties at physiological temperature in the HEK cell heterologous expression system. In contrast to WT channels, P369R variant channels remained open even at strongly hyperpolarized voltages, suggesting a left-shifted voltage-dependence of activation. Compared with WT, P369R variant channels also conducted large persistent repolarizing current at rest and during periods of excitation. The Kv7 channel blocker, XE991, reduced aberrant P369R channel activity, blocking with significantly greater affinity than in WT channels, suggesting variant-specific rescue. Using CRISPR/Cas9 genome editing, we have introduced the P369R variant into human induced pluripotent stem cells (hiPSCs), which we differentiate down neuronal lineages to provide a translational model to investigate variant dysfunction and targeted rescue. This approach will provide opportunity for the development of personalised therapeutic strategies for patients with idiopathic epilepsy with a genetic basis.

P3-B-375 - Impact of SARS-CoV-2 Infection on Microglia in the Hippocampus

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Besko [1], **Mareya Valeva** [1], **Ifeoluwa (Hiphy) Awogbindin** [1], **Elisa Gonçalves De Andrade** [1], **Juan Sanchez-Arias** [1], **Katherine Picard** [3], **Leigh Wicki-Stordeur** [1], **Darwyn Kobasa** [2], **Leigh Anne Swayne** [1], **Marie-Ève Tremblay** [1]

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Infection with the novel coronavirus, SARS-CoV-2 (COVID-19), acutely, as well as long-COVID, are associated with a number of neurological symptoms. One cell type implicated in these symptoms are microglia, the brainâ€™s resident innate immune cells. Altered microglia reactivity is thought to contribute to altered functions of other brain cells, leading to aberrant brain function. In this work, we examined the impact of SARS-CoV-2 infection on microglia in dorsal hippocampus, a brain region important for some of the symptoms observed with COVID-19. In this work, adult male and female Syrian hamsters were infected with SARS-CoV-2 and examined 1-, 3-, 5-, 7- and 31-days post-infection (dpi), to investigate both the acute and post-acute phases following virus infection. Following extraction and fixation, brains were stained for antibodies against IBA1 (a marker of microglia and macrophages), and their density and distribution, and morphology, were examined. Our preliminary results show that IBA1 positive cell density in the dorsal hippocampus dentate gyrus was increased following virus infection, from 1-5 dpi, peaking at 3 dpi, before resolving at 7 dpi. At 31 dpi, there appears to be potential increased variability with microglial distribution. This may indicate that there is altered surveillance post-acute SARS-CoV-2 infection in some individuals. We hope that this work can contribute to the understanding of the manifestation of COVID-19 neurological symptoms. We will follow up with morphological and ultrastructure analysis of microglia and their interactions with other cells.

P3-B-376 - Functional synaptic modelling of deep brain stimulation

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Hypothesis:

Modelling the impact of deep brain stimulation through functional synaptic impact will allow for unified modelling as well as better integration of temporal dynamics in network stimulations.

Materials and Methods:

Detailed biophysical models of DBS were developed to study how physical characteristics of electrodes, stimulation parameters, and tissue substrates contribute to neural activities. However, these models are

extensively complex to study how DBS modulates dynamics of a large population of neurons. Building on recent computational models of DBS that capture various temporal dynamics of instantaneous firing rates of stimulated neurons in different sub-cortical regions (DBS targets), we propose a unified algorithmic framework that enables modeling the impact of DBS across different neural simulators consistently. Specifically, we model the impact of DBS using a module, referred to as parrot neuron, to generate DBS-induced spikes on both afferents and efferent of stimulated neurons in a neuronal network. We show that the use of parrot neuron preserves interactions of spike times of neurons and those generated by DBS, thereby enables accurate adjustments of synaptic dynamics altered by DBS.

Results:

In our simulation study, we demonstrate that the impact of DBS on the firing rate of neurons in a neural network can be consistently generated across three neuro-simulators, namely, NEST, BRIAN, and SpiNNaker.

Conclusion:

Deep brain stimulation (DBS) modulates synaptic plasticity and alters neuronal activities of stimulated neurons. Further, DBS can change neuronal dynamics at the network level in either/both short and long time scales. We anticipate that this work provides a standard modeling approach for studying how DBS alters dynamics of large neuronal populations.

P3-B-377 - Mapping the intricacies of layer 6b through connectivity, gene expression, and spatial transcriptomics

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Exploring the neocortex and the multimodal properties of its neuronal populations is vital to understanding brain composition and function. The composition and function of the deepest neocortical lamina, Layer 6b (L6b), remains largely enigmatic. To investigate this lamina, we performed a multimodal investigation to assess the heterogeneity within L6b of the mouse neocortex, including connectivity, gene expression, and single-cell spatial transcriptomics. First, we performed retrograde AAV injections into various subcortical targets to assess the long-range projections (LRP) of L6b neurons. We found that L6b neurons project to subcortical structures in a region-dependent manner and that the projections form largely parallel circuits. We then performed mFISH to assess the gene expression of these LRP L6b neurons and the expression of the dopamine 1 receptor (D1R) and other layer 6b marker genes, which we found to overlap with many of these LRP L6b cells. In addition to assessing the gene

expression of these LRP neurons, we mapped the spatial organization of four scRNA-seq-derived, transcriptomically distinct L6b subpopulations *in situ* and uncovered their spatial mosaicism-like organization. In collection, these returns illustrate that L6b is a heterogeneous layer, transcriptomically organized as a mosaic that can be associated with various subcortical targets. While complex, beginning to understand the properties of L6b is a vital step in understanding the function of the neocortex.

P3-B-378 - Chrna5 nicotinic receptors in the mouse interpeduncular nucleus: an optogenetic, electrophysiological, and pharmacological interrogation

Claire Richter Gorey [1], **Sanghavy Sivakumaran** [1], **Yupeng Liu** [1], **Evelyn Lambe** [1]

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The Î±5 subunit of nicotinic acetylcholine receptors (nAChRs) plays a key role in cholinergic signaling relevant to executive function, mood regulation, and addictive behaviour. The expression of the Î±5 gene, Chrna5, is strongest in the interpeduncular nucleus, but its local contributions to endogenous cholinergic signalling are not well understood. The interpeduncular nucleus contains GABAergic neurons that innervate key targets in the serotonergic raphe and the cholinergic lateral dorsal tegmental nucleus. A key excitatory input to the interpeduncular nucleus is from the medial habenula via combined cholinergic and glutamatergic co-transmission. Acetylcholine and exogenous nicotinic agonists can directly depolarize interpeduncular neurons, yet endogenous activation of nicotinic receptors has been suggested to retrogradely suppress incoming synaptic inputs from the medial habenula. Here, we probe deeper into the paradox of cholinergic excitation/inhibition with opto-physiological experiments to assess the impact of specific cholinergic manipulations (e.g. allosteric modulators and acetylcholinesterase inhibition). These manipulations in brain slices from compound wild-type and Chrna5 knockout mice are an important next step to clarify the impact of endogenous nicotinic signalling in the interpeduncular nucleus and its dependence on Chrna5.

P3-B-379 - Brain pericytes are highly sensitive to oxidative stress

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Brain dysfunction in disorders such as stroke, Parkinson's, and Alzheimer's disease is mediated by oxidative stress (OS) - a state caused by an excess of reactive oxygen or nitrogen species. These molecules impede brain function by oxidizing proteins, lipids, and DNA and can cause cell death. As such, it is vital to identify the key cellular and molecular targets modified by OS which drive brain dysfunction. We pursued this using two-photon microscopy to examine cell death during OS in rodent cortical tissue. Using propidium iodide (PI) uptake as a death assay, we found that pro-oxidants elicited rapid cell death. Interestingly, the rapidly dying cells were associated with the vasculature. Based on their morphology and association with the basal lamina, we examined whether the vulnerable cells were pericytes. Live imaging of pericytes labelled with the fluorescent marker NeuroTrace (NT), revealed that tissue oxidation produced simultaneous PI uptake and NT loss in a large fraction of pericytes within minutes. By contrast, there was significantly less PI uptake by non-pericyte cells. Our results demonstrate that pericytes are highly sensitive to pathological changes in redox state. Pericytes have important roles in regulating blood vessel stability, blood brain barrier integrity, and cerebral blood flow. As such, our data indicates that pericyte dysfunction could be a central contributor to brain pathologies associated with OS. Future studies examining the mechanisms of heightened sensitivity of these cells to cell death during OS will be critical to test this hypothesis.

P3-B-380 - Astrocytic Cav1.2: A potential novel target for restoring synaptic plasticity in the presence of neuroinflammation

Samantha Carew [1], **Craig Moore** [1], **Matthew Parsons** [1]

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Astrocytes are multifaceted glial cells that play intricate roles in both synaptic communication and the brainâ€™s immune response to neuroinflammation. Hippocampal long-term potentiation (LTP) is a form of synaptic plasticity that necessitates a precise balance of Ca^{2+} and is impaired under inflammatory conditions. $Ca_v1.2$ is a high voltage-gated Ca^{2+} channel with long lasting activity that is expressed throughout the hippocampus, characteristics that imply altered $Ca_v1.2$ expression could perturb the balance of Ca^{2+} required for LTP. Acute inflammation increases $Ca_v1.2$ expression in neurons and astrocytes in vitro, but it is unclear whether this occurs in the brain, or if $Ca_v1.2$ contributes to impaired LTP. Here, we stimulated hippocampal slices ex vivo for 3hr using lipopolysaccharide (LPS) as a model of acute inflammation. $Ca_v1.2$ expression from whole hippocampus was not altered significantly by LPS, but both the cell and dendritic layers of CA1 exhibited LPS-induced increases in $Ca_v1.2$ expression. Further super-resolution imaging revealed that $Ca_v1.2$ expression was preferentially increased on astrocytic, not neuronal, processes in the dendritic layer of CA1. As expected, LPS exposure induced a significant reduction in LTP of CA3-CA1 synapses. Importantly, LTP was fully restored with nifedipine, a $Ca_v1.2$ antagonist. Nifedipine is FDA approved with potential to treat cognitive symptoms in neurodegenerative disease. Here, we propose that impaired LTP under acute inflammation is driven by increased astrocytic $Ca_v1.2$, and demonstrate the capacity of nifedipine to restore LTP.

P3-B-381 - Lactate modulates brainstate in urethane anesthesia through signaling mechanisms

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Lactate, an abundant by-product of Astrocytic Glycolysis, is thought to be preferentially used by neurons to make energy via oxidative metabolism, and has signaling properties mediated especially by the hydroxycarboxylic acid receptor 1 (HCAR1). During sleep, when the neural energy needs are fluctuating with sleep states, it was found that extracellular lactate levels and not those of glucose or glutamate changed in time with sleep states - increasing during activated states like wakefulness and Rapid Eye Movement (REM) sleep, and decreasing during deactivated states like Non-REM (NREM) or Slow Wave Sleep. Using simultaneous Local Field Potential (LFP) and enzymatic biosensor recordings, we have demonstrated that this relationship between lactate and brainstate persists in urethane anesthesia. When brain lactate levels were increased via an Intravenous (IV) injection, it led to an increase in the slow wave sleep-like state. Additionally, we found similar effects with the physiological isomer L-Lactate (oxidative substrate + signaling) and the non-physiological D-Lactate (signaling only), leading us to conclude that this enhancement of the slow wave sleep-like state is mediated by lactate's signaling mechanisms, not its oxidative effect. Lactate, a sign of work having been done in the brain, through its signaling effect via the HCAR1 may be a key prompt to initiate waste clearance processes in the brain which are particularly active during slow wave sleep. Lactate could be a cost effective and simple way to augment slow wave sleep, a state which is attenuated in aging and age related diseases. Our research empirically illuminates the underpinnings of its effects.

P3-B-382 - Effects of the novel notch-sparing presenilin 1 mutation on cell death, synaptic activity, and behaviour

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Presenilin 1 (PS1) mutations are the primary cause of familial Alzheimerâ€™s disease (AD). They increase beta-amyloid levels and neurotoxicity while inhibiting Notch signalling. Recently, our lab identified a unique mutation, called PS1Î”S169 that, unlike other PS1 mutations, does not affect Notch signalling,

making it an ideal therapeutic target. This study examines the mechanisms underlying PS1Î”S169â€™s effect on AD pathogenesis particularly on neuronal cell death, synaptic activity, and behaviour. PS1 plasmids corresponding to the PS1 wild-type (control) and five AD-related PS1 mutations including the Notch-sparing PS1Î”S169 were transfected into a PS1-knockout neuro2A cell line called N2A-KO. The in vivo study included the experimental group corresponding to the PS1Î”S169 knockin mouse model compared to the wild-type control. Various molecular techniques were performed to determine how PS1 mutations affect cell viability and synaptic activity, including Western Blots, and cell death assays. Overall, our findings indicate that the pathogenic PS1 mutations significantly decreased cell viability in N2A-KO cells, and affected markers related to pyroptosis, and apoptosis. Similarly, synaptic markers and learning and memory-related behaviours were significantly altered in our PS1Î”S169 knockin model. These findings provide novel insights into the pathological effects of the PS1Î”S169 mutation on different cell death pathways, synaptic activity and learning and memory which offers a foundation to facilitate a novel therapeutic target.

P3-B-383 - Neuronal lipid droplets: An energy story where sex matters

Romane Manceau [1], **Danie Majeur** [2], **Colin Miller** [3], **Audrey Labarre** [4], **Anthony Bosson** [5], **Sebastien Audet** [4], **Khalil Bouyakdan** [1], **Demetra Rodaros** [1], **Martine Tetreault** [5], **Alex Parker** [6], **Baptiste Lacoste** [7], **Marie-Ève Tremblay** [8], **Ciaran Murphy-Royal** [4], **Stephanie Fulton** [4], **Elizabeth Rideout** [3], **Thierry Alquier** [4]

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Background: While lipid droplets (LD) are emerging as important triglyceride storing organelles in glial biology, the function of LD in neurons remains elusive. Lipid and fatty acid (FA) metabolism in hypothalamic neurons are essential for the regulation of energy homeostasis. Yet, the potential role of LD in neuronal functions and control of energy balance are unknown. Using electronic microscopy (EM) we observed LD in hypothalamic neurons of male and female mice in physiological states. To investigate the role of LD in these neurons, we inhibited or deleted ATGL, the enzyme that catalyzes the first step of LD lipolysis and release of FA, and studied its metabolic impact at the cellular and whole-body levels.

Results: At the cellular level in flies and murine hypothalamic neurons, lipidomic, metabolomic, electrophysiology and EM studies demonstrate that impaired LD lipolysis leads to: decreased cell size, membrane phospholipids remodeling, mitochondrial and cell metabolism alterations associated with reduced neuronal firing.

These cellular modifications induced by LD lipolysis inhibition affect whole body metabolism. Knockdown of neuronal ATGL in *C. elegans* and male, but not female, *D. melanogaster* inhibits peripheral fat breakdown, suggesting that neuronal ATGL promotes peripheral lipolysis in a sex specific manner. In male mice, ATGL-KO in hypothalamic arcuate neurons or specifically in AgRP neurons affects energy expenditure, feeding behavior and thermoregulatory responses to cold. Such changes were not observed in female mice, highlighting a sex-specific regulation of LD in hypothalamic neurons that influences the energy homeostasis.

Conclusion: Taken together, our findings reveal a previously unrecognized roles for LD in neuronal physiology and regulation of whole-body energy homeostasis by hypothalamic neurons.

P3-B-384 - Predictive biomarkers in bipolar disorder, suicidality, and treatment response: Novel findings on neuronal signaling pathway genes

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Bipolar Disorder (BD) often exhibits neurodevelopmental, structural, and functional neuronal abnormalities. Clinical genetics and bioinformatic research on BD revealed the importance of certain molecular pathways. Wnt signaling pathway, due to its crucial role in neurodevelopment and in regulating the function and structure of the adult nervous system, has been examined in several psychiatric studies. The objective is to better understand BD's underlying mechanisms, with a particular emphasis on the neuronal signaling pathway's involvement.

In this study, 250 bipolar patients and 250 matched healthy controls were recruited. The ARMS-PCR was used for genotyping 15 variants from 9 genes. Obtained results were confirmed by Sanger sequencing. The identified genetic variants were further investigated using bioinformatics tools to assess their potential functional implications in neuronal function.

The findings revealed the association between variants of *MARK2*, *BDNF*, *GSK3B*, *PPARD*, and *ADCY2* genes and certain aspects of BD. The study identified previously unreported *ADCY2* variant (rs2290910) as a potential biomarker for BD risk ($p = 0.001$), suicide tendency ($p = 0.004$), and response to lithium therapy ($p = 0.001$), particularly in the female population. In-silico analysis using tools such as Haploreg revealed potential regulatory elements and predicted their involvement in neuronal networks, further supporting the importance of these genetic biomarkers in neuroplasticity and BD pathogenesis. Replication in larger patient samples in Toronto (899) is in progress. The identification of genetic biomarkers can contribute to personalized treatment approaches and improved management of BD.

P3-B-385 - 'Occult' NaV-dependent spiking in retinal wide-field amacrine cells

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[1] University of Victoria

Objective: Retinal 'axon-less' wide-field amacrine cells (WACs), are endowed with processes that extend across large parts of the retina (~1 mm), and are thought to be involved in global computations. Surprisingly, previous electrophysiological recordings have revealed only a fraction of WACs support TTX-sensitive, voltage-gated sodium channel (NaV)-dependent action potentials at their somas. How non-spiking WACs process information over large distances remains unclear. Here, we used two-photon Ca2+ imaging techniques to examine the biophysical properties of WACs.
Methods: A Ca2+ indicator Oregon Green BAPTA was loaded into WACs through a patch electrode. Responses to small light stimuli were simultaneously recorded in the WAC soma and distal processes.
Results: Many WACs responded to light with graded signals and TTX did not affect their somatic light response. However, small spots of light (300 um diameter) presented near the soma evoked Ca2+ responses in WAC neurites up to 1mm away, indicating efficient signal propagation. Bath application of TTX abolished distal signals, indicating that they were mediated by NaV.
Conclusion: Our results demonstrate the presence of occult TTX-sensitive spike activity in the distal processes of WACs that are not visible at their somas. This isolation may enable individual WAC processes to function as independent units.

P3-B-386 - Oligodendroglial UNC5B is required for organization of paranodes and axonal domain segregation in the central nervous system

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In the mature CNS, netrin-1 is expressed by neurons and oligodendrocytes and is required to maintain the stability of axo-oligodendroglial paranodal junctions. The netrin receptor UNC5B is highly expressed by mature oligodendrocytes and enriched at paranodes; however, its function in mature myelin remains unknown. In this study, we aimed to characterize the contribution of oligodendroglial UNC5B to myelin structure and function. For this purpose, we used Olig2cre to selectively delete a floxed allele of *Unc5b* from the oligodendrocyte lineage. The ultrastructure of internodes and paranodes was examined in the optic nerve of wild-type (WT) and UNC5B conditional knockout (cKO) mice. Along the internodes of UNC5B cKO mice, the ultrastructure of compact myelin was preserved and the expression of compact myelin

proteins MBP and PLP remained unchanged. In contrast, the ultrastructure of paranodal junctions was disorganized, with glial loops everted away from the axon, and gaps between loop-loop interfaces. Confocal immunofluorescence analyses revealed disruption of the segregation of axonal domains around nodes of Ranvier. Field recordings of compound action potentials detected a delay in conduction velocity in the corpus callosum in the absence of oligodendroglial UNC5B. Our findings indicate that *Unc5b* expression by oligodendrocytes is required for the organization of paranodal junctions, the segregation of functional axonal domains associated with nodes of Ranvier, and the normal conduction of axon potentials along myelinated axons.

P3-B-387 - Repeated LPS drives priming and tolerance of microglial responses in the brain

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Neuroinflammation is involved in the pathogenesis of almost every central nervous system disorder. As the brainâ€™s innate immune cells, microglia fine tune their activity to a dynamic brain environment. Previous studies have shown that repeated bouts of peripheral inflammation can trigger long-term changes in microglial gene expression and function, a form of innate immune memory. In this study, we used multiple low-dose lipopolysaccharide (LPS) injections in adult mice to study the immediate cytokine, transcriptomic, and microglia morphological changes that contribute to the formation of immune memory in the frontal cortex, hippocampus, and striatum, as well as the long-term effects of these changes on behavior. We identified a shared pattern of priming and tolerance of gene expression across regions, and enrichment for IRF and NFkB family transcription factors, two key regulators of innate immune memory. We quantified shifts in microglia morphological populations and found that while the proportion of ramified and rod-like microglia mostly remained consistent within brain regions and sexes with LPS treatment, there was a dynamic shift from ameboid towards hypertrophic morphological states across immune memory states. Together, findings support the dynamic regulation of microglia during the formation of immune memories in the brain and support future work to exploit this model in brain disease contexts.

P3-B-388 - Neurophysiological investigation of nucleus accumbens shell medium spiny neurons in mice lacking Cntnap2.

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Oxytocin (OT) is a peptide hormone synthesized in the hypothalamus and released to major social brain regions including the nucleus accumbens (NAc). Mice lacking *Cntnap2*, an autism spectrum disorder linked gene, exhibit lower sociability and a dysfunctional OT system. Our recent study has shown that stimulation of endogenous OT release strongly activates the NAc in *Cntnap2* KO mice. Furthermore, OT receptor agonist infusion into the NAc shell (NAcSh) sufficiently rescues their lower sociability (Choe et al., 2022). Although the OT-mediated activation of NAcSh appears to be a critical process for the social rescue of *Cntnap2* KO mice, the underlying neurophysiological mechanisms remain unknown. To investigate this, we performed whole-cell patch-clamp recordings of NAcSh medium spiny neurons (MSNs) of *Cntnap2* KO and wild type (WT) mice with or without bath-applied OT receptor agonist (TGOT 1ÂµM, n=10-12 cells/group). We did not observe genotype differences in the resting membrane potential, input capacitance, or membrane resistance of MSNs. However, current-clamp recordings revealed lower excitability in KO MSNs compared to WT. Furthermore, voltage-clamp recordings revealed, on average, a lower frequency of spontaneous excitatory post-synaptic currents (sEPSCs) in KO MSNs than WT MSNs at baseline. Finally, bath-application of TGOT increased the average sEPSC frequency in KO, but not WT MSNs. These neurophysiological characteristics of NAcSh MSNs in *Cntnap2* KO mice may serve as potential mechanisms for their low sociability phenotype and the OT-mediated rescue.

P3-B-389 - Neuroinflammatory activation of microglia drives piezo1 expression in astrocytes

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Numerous brain disorders are associated with mechanical alterations in the tissue microenvironment. Recent evidence indicates that such mechanical changes are sufficient to initiate mechanosensory signaling pathways and impact neuropathology. However, the precise mechanisms which regulate mechanosensory signaling in brain pathology remain uncertain. In our study, we addressed this by examining how the expression of astrocyte Piezo1, a mechanosensitive ion channel, is modulated by inflammatory triggers. To do this, we used rodent primary astrocyte and microglia cultures as our cell models. Using qPCR and Western blotting we found that either LPS or oligomer AÎ²(oAÎ²) treatment of astrocyte cultures had minimal effects on Piezo1 expression. However, when LPS or oAÎ² were added to microglia cultures, the conditioned media from these microglia cultures significantly increased piezo1 expression in astrocytes. Additionally, we found that proinflammatory cytokines released by microglia

after these treatment, contained interleukin 1Î±, interleukin 1Î², and TNFÎ± that alone can significantly increase Piezo1 expression in astrocytes. Collectively, our results suggest that upregulated Piezo1 expression in astrocytes is triggered indirectly, through microglia-dependent activation and cytokine release. This microglia-astrocyte cross talk may play a critical role in facilitating astrocyte mechanosensation and modulating the homeostatic functions of astrocytes.

P3-B-390 - Widefield calcium imaging to investigate and optimize plasticity in prefrontal cortex

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Long term synaptic plasticity in the adult prefrontal cortex is challenging to examine using conventional whole-cell patch clamp electrophysiology. Protocols optimized in the hippocampus are acknowledged to result in more variable potentiation in brain slices from prefrontal cortex of adult mice. Yet, in the prefrontal cortex in vivo, dendritic "calcium spikes" arising from NMDA receptor plateau potentials are a robust and cognitively-relevant substrate for long term plasticity. Accordingly, we turned to calcium imaging ex vivo and discovered that wide-field imaging with genetically-encoded calcium indicators can detect plasticity-relevant events at the neural population level in prefrontal brain slices. We therefore developed an all-optical approach to study long-term synaptic plasticity using Thy1-GCaMP6f mice. Here, we measure neuronal population fluorescence in response to stimuli before, during, and after induction paradigms. These experiments employ low frequency test pulses and theta-burst stimulation (TBS) protocols. Calcium imaging allows the quantification of events during induction episodes which predict long-term potentiation (LTP). Initial results suggest that there is a strategic advantage in using calcium signals in this manner to refine preclinical stimulation paradigms. Ongoing work is evaluating pharmacological interventions and modified induction paradigms to improve the reliability of LTP in prefrontal brain slices from adult male and female mice.

P3-B-391 - Where not when: The spatial nature of reward anticipation in the rat medial prefrontal cortex

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It is well established that the rodent medial prefrontal cortex contains numerous cells exhibiting activity tied to reward. Particularly noteworthy are cells that show firing rate modulation on approach to a rewarding goal location. While this anticipatory activity is easily discernible, it remains unclear whether this firing rate modulation is determined by anticipated time or distance to arrival, as these two dimensions typically covary. In our experiment, rats ran towards a series of target locations around the circumference of a circular platform, reinforced by brain stimulation rewards. The number of trials was sufficient to encompass a wide range of running speeds, allowing effective disentanglement of the influence of time and distance on the anticipatory neural activity. Employing a linear model, we demonstrated that, on a trial-by-trial basis, distance provided a better fit to the modulatory activity than time. These results suggest that, at least within the context of goal-directed spatial navigation, the medial prefrontal cortex organizes its representations based on the anticipated location of the animal, rather than how much time it will take to get there.

P3-C-392 - SerpinE1 unique role in stroke risk and recovery

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Ischemic stroke can lead to a long-lasting disruption of blood flow in microvessels surrounding the infarct site, exacerbating injury beyond the initial insult. Homeostatic blood clotting and proteolytic (clot-busting) pathways are likely fundamental to regulating post-ischemic capillary blood flow, and thus functional recovery. We have recently discovered that the SERPINE1 gene, encoding for Plasminogen Activator-Inhibitor-1 (PAI-1), is highly expressed along blood vessels following photothrombotic stroke in the rodent somatosensory cortex. Therefore, we explored the role of SERPINE1/PAI-1 on cortical blood flow following experimentally induced ischemic stroke. Using *in vivo* 2-photon imaging of iv. injected dyes to label circulating plasma we longitudinally imaged superficial cortical blood flow in adjacent and distant areas to the infarct in both wildtype and cerebral endothelial SERPINE knockdown (KD) mice. We discovered reduced blood flow in the penumbra and distant regions following stroke in the subacute (3d) and chronic phase (21d). Surprisingly we found that the total number of stalls increased in SERPINE KD-mice compared to WT-mice, despite the presumed anti-coagulant role of PAI-1, which was mediated by a greater number of leukocyte stalls over red-blood cell stalls. These findings reveal that SERPINE1 KD leads to reduced blood flow and greater leukocyte recruitment, suggesting that merely increasing clot degradation following stroke is not sufficient to improve local blood flow and may in fact worsen injury through greater recruitment of leukocytes.

P3-C-393 - The role of the X-Linked Intellectual Disability gene, Zdhhc9, in neuronal connectivity

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Loss-of-function (LOF) variants in the human *ZDHHC9* gene are identified in 2% of patients diagnosed with X-Linked Intellectual Disability (XLID). Patients with *ZDHHC9* LOF mutations exhibit speech and developmental delays, intellectual disability, and an increased susceptibility to focal seizures. Magnetic resonance imaging studies have shown that these patients have structural abnormalities including a reduction in the volume of subcortical structures and a reduction in the volume of the corpus callosum (CC). Our lab has previously shown that ZDHHC9 promotes dendrite outgrowth and the formation of inhibitory synapses *in vitro*. Here we show that ablation of *Zdhhc9 in vivo* disrupts axon outgrowth, pathfinding and targeting. In *Zdhhc9* knock-out (KO) mice cortical neurons committed to a callosal-projection fate are able to extend their axons medially and cross the CC, however there is a decrease in branching in the contralateral cortex and a lack of projections to the more superficial layers of the cortex. Moreover, while axons do cross the CC in *Zdhhc9* KO mice, medial and lateral projections are not spatially segregated to the dorsal and ventral CC as they are in control mice. This suggests that ZDHHC9 is important for axon outgrowth, targeting and pathfinding. Notably, our RNAseq analysis from control and *Zdhhc9* KO CC demonstrates changes in the expression of several guidance molecules including *Epha8, Ephb2, Ephb1, Sema3a, Tenm3*, *Foxp1,* and *Flrt3,* offering mechanistic insight into the pathophysiology of patients with XLID and ZDHHC9 LOF mutations.

P3-C-394 - The role of the palmitoylating enzyme, ZDHHC9, in oligodendrocyte development and myelination

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Two percent of all patients with X-linked intellectual disability exhibit loss-of-function mutations in the palmitoylating enzyme, *ZDHHC9*. One of the main anatomical deficits observed in these patients is a decrease in corpus callosum volume and a disruption of white matter integrity. Here we demonstrate that ablation of *Zdhhc9* in mice substantially decreases the myelination of axons in the corpus callosum. Using RNA sequencing and fluorescent in situ hybridization we demonstrate that reduced myelination in *Zdhhc9* knockout mice is due to an impairment in the maturation of oligodendrocytes, specifically in the proportion of mature oligodendrocyte (MOL) subtypes. Specifically, we observed fewer MOL2/3 cells that have a transcriptional profile suggestive of an important role in mediating myelination compared to other MOL subtypes. Ultrastructural analysis of the remaining myelinated axons in the corpus callosum

revealed further disruptions in myelin integrity. This can be explained by proteomic analysis demonstrating a decrease in the expression of proteins involved in lipid metabolism, cholesterol synthesis and myelin compaction. Beyond a role for ZDHHC9 during development, we have also demonstrated that ZDHHC9 is required for remyelination following chemical demyelination by cuprizone. These results reveal a previously underappreciated and fundamental role for ZDHHC9 in regulating oligodendrocyte maturation and myelinogenesis and provide mechanistic insights into the deficits observed in white matter volume in patients with mutations in *ZDHHC9*.

P3-C-395 - Aberrant astroglial metabotropic glutamate receptor 5 signaling in Alzheimer's disease

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Alzheimer Disease (AD) is a neurodegenerative disorder that is characterized by progressive memory loss and cognitive decline. Though the etiology of AD remains predominantly unknown, one of the key hallmarks, amyloid-B (AB_{42}) oligomers, is linked to the neurotoxic effects associated with AD. AB_{42} oligomers targets several receptor systems in the brain, including metabotropic glutamate receptor 5 (mGluR5). Interestingly, mGluR5 can function as receptors that transmit the pathological signaling of AB_{42} to trigger neurotoxicity, in neurons. Interestingly, astrocytes also express significant levels of mGluR5 as the most abundant mGluR in this cell type. It remains unclear, however, whether the disruption of mGluR5 signaling in astrocytes contributes to AD pathology and whether it can be corrected by mGluR5-targeted ligands. By exposing cultured astrocytes to AB_{42} oligomers, we were able to demonstrate changes in glycogen synthase kinase 3-beta (GSK-3B), protein kinase b (Akt), and extracellular signal-regulated kinase (ERK) levels, that were reversed when the cultures were pretreated with a selective negative mGluR5 allosteric modulator. This study was able to display that AB_{42} oligomers disrupt canonical/non-canonical mGluR5 signaling pathways in astrocytes. These findings also provide evidence that astrocytes are involved in the pathological mechanisms of AD via mGluR5 signaling pathways. Further investigations on the plausible mechanisms relating disruption of AB_{42}-mGluR5 signaling in astrocytes to impaired neurovascular coupling will be conducted in AD mouse models.

P3-C-396 - Expression, Phosphorylation, and Intercellular Transfer of α-Synuclein in the Retina

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In diseases associated with prion-like spread of Î±-synuclein (Î±S) like Parkinsonâ€™s (PD), visual deficits may precede motor ones. Retinal thinning and phosphorylated Î±S (p-Î±S) deposits are seen in PD patients. To develop therapeutic targets, we assess (1) retinal Î±S expression in established mouse models of synucleinopathy, (2) the spread of Î±S between retinal neurons and (3) whether this pathology can serve as a biomarker. TgM83, Î±S -/-, Nrl -/-, and C57Bl6 mice are used in this study. Î±S pathology is accelerated by intracranial injection of MSA brain lysate (MSAbl) or pre-formed Î±S fibrils (PFFs). Î±S and p-Î±S are assessed with IHC. For donor-recipient experiments, retinal cells from perinatal TgM83 mice are cultured with a lenti-GFP reporter for 2 days, then transplanted into the subretinal space of adult mice, which are sacrificed 21 days post-transplant. The results show transgenic human Î±S (h-Î±S) is expressed in the retinas of perinatal and adult TgM83 mice and confirm p-Î±S in the adult retina. TgM83 photoreceptors (PRs) express h-Î±S in the adult retina that may transfer from transplanted to host PRs in vivo, which localizes to the host PR layer in transplanted mice. Effects of MSAbl, PFFs, and experimental therapies on Î±S and p-Î±S in the retina are being evaluated. Establishing a model for in vivo transfer of Î±S and p-Î±S seeding using retinal neurons supports distinction of mechanisms that enable intercellular spread of Î±S. Describing Î±S and p-Î±S in the retina for PD models and therapeutics may yield biomarkers for therapeutic efficacy and predict therapeutic validity.

P3-C-397 - A role for microglial ligands in the promotion of adult neural stem cell-mediated oligodendrogenesis and myelin repair

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Adult neural stem cells (NSCs) in the ventricular-subventricular zone (V-SVZ) produce myelinating oligodendrocytes, presenting a potential therapeutic avenue for myelin repair.

We used lineage tracing and single-cell (sc) transcriptomics to assess NSC-mediated oligodendrogenesis at homeostasis and during recovery from cuprizone/rapamycin-induced demyelination. During remyelination, NSCs are activated and generate transit amplifying progeny which make more oligodendrocytes. While these cells are transcriptionally similar before and after demyelination, the V-SVZ environment is significantly altered.

During recovery, microglia increase ~3-fold and change their ligand expression. Using scRNA-sequencing and proteomics we created predictive models of microglia ligand-NSC receptor communication. Two ligands, *Insulin-Like Growth Factor 1* (*Igf1*) and *Oncostatin M* (*Osm*), were increased in microglia with a distinct transcriptional state during remyelination. Using sc-spatial transcriptomics, these microglia were specifically enriched in the corpus callosum and V-SVZ dorsal wall during remyelination.

In vitro neural precursor cell (NPC) assays showed that IGF1 and OSM increased proliferating cells and oligodendrogenesis. Both ligands when infused into the lateral ventricle increased OPCs, NPCs and their proliferation in the corpus callosum. In the V-SVZ dorsal wall, IGF1 increased NPCs, and IGF1 and OSM increased OPC number and NPC proliferation.

These data suggest a positive role for predicted microglial ligands, IGF1 and OSM, in NSC-mediated oligodendrogenesis and remyelination.

P3-C-398 - Islets of Langerhans transplantation into the eye anterior chamber improves retinal ganglion cell function and visual behaviors in glaucoma

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Our recent work identified a role for insulin on retinal ganglion cell (RGC) dendrite regeneration. However, the delivery of insulin has several drawbacks including loss of biological activity and inconsistent levels in the retina. The transplantation of islets of Langerhans (IL) into the eye anterior chamber is a low-invasive strategy. Here, we tested the hypothesis that islet-based delivery of insulin is an effective strategy to improve RGC function. IL were isolated from donor mice pancreas and transplanted by trans-corneal injection into the anterior chamber. To measure IL-secreted insulin levels, aqueous humor was collected at 1, 2, 3 or 4 weeks after transplantation and analyzed by ELISA. Blood glucose was monitored weekly. To assess IL vascularization, fluorescent dextran was injected retro-orbitally and vessels were imaged with confocal microscopy. Following transplantation and induction of glaucoma, light-evoked single-RGC calcium (Ca^{2+}) dynamics were recorded using two-photon microscopy. Optomotor responses were assessed weekly post-transplantation and glaucoma induction. Transplanted IL rapidly attached to the iris and were stable for several weeks. Insulin levels increased in the posterior chamber at 2 and 4 weeks after transplantation relative to saline-injected controls.

Imaging of IL revealed dextran-positive vessels suggesting that IL were vascularized by the underlying iris. Blood glucose levels remained unaltered. IL transplantation restored light-evoked RGC Ca^{2+} dynamics and improved visual behaviors in glaucomatous mice relative to controls. Our data show that IL transplantation into the eye anterior chamber increases insulin production *in situ* without adverse effects, restores light-evoked RGC responses and improves visual behaviors during glaucoma.

P3-C-399 - Overexpression of FXR1 (FMR1 autosomal homolog 1) in the prefrontal cortex increases resiliency to stress

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Mechanisms responsible for maintaining allostasis under chronic stress are key regulators of mental stability. Dysregulated allostasis leads to the development of many mental disorders associated with depression/anxiety-related symptoms. Interestingly, the RNA-binding protein FXR1 (FMR1 autosomal homolog 1) has been associated with various disorders (insomnia, schizophrenia, bipolar disorder) and modulating synaptic homeostasis. However, direct effects of prolonged environmental stress on FXR1 remain unknown. Here we characterized the effects of chronic stress on FXR1 by subjecting C57BL/6J mice to chronic restraint stress (CRS: 1h, 2-times/day, for 5 weeks), followed by various behavioural and molecular/cellular measurements. CRS reduced FXR1 levels in the prefrontal cortex (PFC), without changing its X-linked homolog FMRP. Additionally, exposure to BDNF increased FXR1 levels, while Corticosterone decreased FXR1. Subsequently, FXR1 overexpression or knockout (via CRISPR) viruses were injected into the PFC bilaterally. Overexpression of FXR1 resulted in rescued apathy/anhedonia/anxiety-like behaviours after chronic stress exposure, while FXR1 knockout increased anxiety-like behaviours at baseline. Together, our results suggest chronic stress reduces FXR1 levels in the PFC, and overexpressing FXR1 can increase resiliency. This highlights an integrative mechanism involving FXR1 in dysregulated homeostatic stress responses leading to disease progression, and the rescue of FXR1 as a viable therapeutic avenue.

P3-C-400 - Metabolomic profiling unveils retinal energetic stress in early glaucoma

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Metabolic dysfunction plays a pivotal role in the pathophysiology of glaucoma, contributing to retinal ganglion cell (RGC) death and subsequent vision loss. This study aimed to investigate whether, prior to neurodegeneration, ocular hypertension (OHT), a major glaucoma risk factor, disrupts retinal metabolism, leading to bioenergetic deficits, with a focus on central carbon metabolism.
OHT was induced by intracameral injection of magnetic microbeads in mice. Retinas were collected two weeks post-injection, a timepoint with established OHT but undetectable RGC loss. Retinas from sham and OHT groups were randomized, blinded, and subjected to metabolomic testing. Liquid chromatography coupled with mass spectrometry (LC-MS) quantified concentration levels of glycolysis, tricarboxylic acid cycle (TAC), and pentose-phosphate pathway metabolites.
Metabolomic analysis unveiled a substantial decrease in glycerol-3-phosphate and lactate levels in glaucomatous retinas, indicating disturbed glycolysis and TAC. Moreover, OHT-induced damage significantly reduced energy carriers, including ATP, ADP, GTP, GDP, and NAPDH. Notably, OHT led to a negative shift in ATP/AMP and ADP/AMP ratios, suggesting diminished oxidative phosphorylation (OXPHOS) activity and limited energy availability. These findings align with our previous work demonstrating AMP-activated protein kinase (AMPK) hyperactivation and mitochondrial dysfunction in early glaucoma. Our study identifies potential targets within key metabolic pathways for retinal neuroprotection, presenting novel avenues for further research.

P3-C-401 - Endocannabinoids as a potential therapeutic in the SSP-Saporin "Trojan Horse" model of epileptogenesis

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The existing models of epileptogenesis, such as Status Epilepticus and Pilocarpine, suffer from issues of high mortality rate and/or not fully representing the process of epileptogenesis. [GT1] The neurotoxin SSP-Saporin (SAP) can be used to create a more valid model of epileptogenesis. Based on previous studies endocannabinoids that act as CB1 agonists or FAAH inhibitors may aid in slowing down epileptogenesis. We stereotaxically injected the neurotoxin SSP-SAP (0.04 ng/nL) within the hilar region of the dentate gyrus to selectively ablate inhibitory interneurons and implanted bipolar recording electrodes. In rats receiving an endocannabinoid treatment, they were administeredWIN55 212-2 (2 mg/mL, i.c.v.) or URB597 (8.3 mg/mL, i.c.v.) over a 2-week period. Continuous 24-hour video-EEG was recorded for a period of either 2 weeks following surgery, or one month, after which brains were extracted and imaged for sclerosis, and the spread of SAP. Injecting SAP into the hilar region resulted in reactive electrographic and behavioural seizures beginning on day 4 and concluding by day 7. Self-generated electrographic seizures start after approximately one month. Preliminary results with the WIN55 212-2 and URB597 do not reduce the number of reactive seizures. Based on the results, SAP can

be used as a model for epileptogenesis. However, CB1 agonists and FAAH inhibitors do not aid in reducing reactive seizures.

P3-C-402 - Characterizing the neuronal role of CK2 in Drosophila melanogaster

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De novo variants in either *CSNK2A1* or *CSNK2B* cause neurodevelopmental disorders with overlapping features and variable symptoms. These two genes encode Casein Kinase 2 (CK2) complex subunits. CK2 is enriched in the central nervous system and is believed to be constitutively active. There are currently no in vivo models to study CK2 complex in the adult central nervous system. Moreover, the variants, particularly missense, found in *CSNK2A*1 or *CSNK2B* have yet to be functionally assessed in vivo. There are two objectives of this study. First, we will assess the role of CK2 in neurons and glia in development and the adult organism. Secondly, we will generate CK2 disease-associated variants and assess their function in flies. CK2 (*CkIIÎ±* and *CkIIÎ²*) are knocked down in Drosophila neurons by RNAi using elav-GAL4, and nSyb-GAL4 drivers. Drosophila behavioural assessment was performed by negative geotaxis. Seizure induction was performed by bang-sensitivity assay. Variant transgenic flies were generated via site-direct mutagenesis, followed by Sanger verification. We found that neuronal knock-down of either *CkIIÎ±* or *CkIIÎ²* in neurons with nSyb-GAL4 resulted in lethality where some escapers had wing defects. No obvious phenotype was observed with elav-GAL4. We successfully generated 16 variants in *CSNK2A1* or *CSNK2B* to generate transgenic flies. We assessed CK2 variants in the fly by overexpression and variants show a spectrum of function in vivo. We have established that neuronal CK2 is critical for the development of Drosophila Melanogaster. Future studies will examine the adult-specific role of CK2 in neurons.

P3-C-403 - Mapping of the Akt and Beta-Arrestin interface for drug development

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Beta Arrestin mediated GPCR signaling is believed to mediate specific biological outcomes of receptor activation. However, this form of signaling has been mostly studied using functionally biased ligand having higher or lesser efficacy for beta-Arrestin recruitment. Verification of the Dopamine D2 receptor in vivo, results in a beta Arrestin 2 dependent inactivation of the protein kinase Akt resulting from the formation of a signaling complex comprised of Akt, beta Arrestin and the protein phosphatase 2A (PP2A). Interestingly this complex is disrupted in vivo following administration of lithium salts, thus suggesting a way by which lithium can interfere with D2 receptor signaling in the context of bipolar disorder therapy. Here we used a combination of In silico and in vitro approaches to characterize the interface between Akt and beta-Arrestin 2. This characterization allowed the identification of a putative lithium biding site and the generation of lithium resistant mutants. These finding provide a new molecular mechanism of lithium action and suggest possible approaches for the direct disruption of a beta Arrestin signaling complex for the development of bipolar disorder therapy.

P3-C-404 - Unraveling the pathogenesis of Spinocerebellar Ataxia Type 1 (SCA1): A comprehensive investigation using patient-derived cerebral, cerebellar, and choroid plexus organoids

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Spinocerebellar ataxias (SCAs) are a group of ~50 genetic disorders, leading to brain tissue degeneration in the cerebellum and often the cerebral cortex. Symptoms include loss of balance, coordination, speech and swallowing difficulties, muscle stiffness and spasms. Current treatments can alleviate symptoms but are not curative. We use induced pluripotent stem cells (iPSCs) to study cells and tissues of the human brain carrying patient-specific mutations of the SCA1 subtype, enabling the precise study of the causative biological factors in SCA1 patients in order to develop effective treatments.

Here, we generate cerebral, cerebellar and choroid plexus (ChP) organoids from SCA1 patient-derived iPSCs. Using microscopy and biochemical approaches to analyze these tissues, focusing on organelles, morphological and metabolic markers of degeneration to uncover SCA1 disease onset and progression. As cerebral spinal fluid (CSF) biomarker analysis is a priority amongst the SCA1 patient community, we generated ChP organoids from SCA1 iPSCs to perform CSF biomarker analysis. Using western blots and ELISA to assess the metabolic signatures and proteins indicative of the SCA1 disease state, we provide a link to translational clinical applications and an alternative to the current invasive methods of extracting human CSF.

Our long-term goals are to use this approach to elucidate commonalities and differences among SCA subtypes and to understand mechanisms of disease and identify a reliable biomarker that can point to better therapies for SCA patients.

P3-C-405 - Restoration of corticosterone-induced neurochemical and behavioral changes in post-partum depressed dams through a single peripheral injection of reelin

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Reelin, a synaptic plasticity protein, has demonstrated rapid antidepressant-like effects in adult corticosterone (CORT)-induced depressed rats, whether administered repeatedly or acutely. However, these effects remain unexplored in the context of post-partum depression (PPD). This study investigated the antidepressant-like effect of acute reelin in a CORT-induced model of post-partum depression. Long-Evans female dams received either daily subcutaneous CORT (40mg/kg) or saline injections (controls) from the post-partum day (PD) 2 to 22, and on PD22 were treated with a single intravenous reelin (3ug) or vehicle injection. Acute reelin treatment fully normalized to control levels the CORT-induced increase in FST immobility (111%, p=0.0007) and partially restored the decrease in oxytocin-positive cells in the paraventricular nucleus (33%, p=0.286), the number of reelin-positive cells in the dorsal (43%, p=0.134) and intermediate hippocampus (iHi) (52%, p=0.102) and the percentage of DCX-positive postmitotic neurons (55%, p=0.021) in the iHi. Neither behaviour nor neurochemical measures were influenced by estrogen levels. This preliminary data brings new insights into the putative antidepressant-like effect of peripherally administered reelin in an animal model of PPD. Future studies should be conducted to investigate these effects on a dose-response paradigm and to further elucidate the mechanisms underlying the antidepressant-like effects of reelin.

P3-C-406 - Exploring the pathogenesis of claudin-11-mediated hypomyelinating leukodystrophy through cellular models

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Leukodystrophies are a group of rare genetic disorders that affect the white matter of the central nervous system. Leukodystrophies have variable clinical presentations with over fifty genetic causes identified to date. Recently, *de novo* heterozygous stoploss mutations in *CLDN11* have been identified as the cause of an early-onset neurodegenerative disease termed hypomyelinating leukodystrophy 22 (HLD22). *CLDN11* encodes the myelin tight junction protein claudin-11. Additionally, frame shift mutations in *CLDN11* have also been linked to similar clinical presentations to that of HLD22. How these mutations in *CLDN11* cause disease is not well understood. To investigate the underlying pathogenic mechanisms, we have developed immortalized cell lines with stable expression of wild-type (WT) or mutant (MT) claudin-11. Western blot analysis shows increased molecular weight and reduced stability of MT claudin-11. Furthermore, immunofluorescence imaging showed markedly reduced plasma membrane abundance of MT claudin-11 relative to WT. Using bafilomycin A1 and lactacystin treatments, we demonstrate that the reduced expression of MT claudin-11 reflects increased clearance. Endoplasmic reticulum (ER) stress has been implicated in the pathogenesis of several myelinopathies, therefore, we subjected immortalized MT and WT claudin-11 cell lines to ER stressors to evaluate their sensitivity to ER stress induced death. Overall, our results to date point to reduced protein stability and increased ER stress as potential mechanisms underlying the pathogenesis of HLD22.

P3-C-407 - Glial Fibrillary Acidic protein levels in Cingulate cortex in major depressive disorder with and without psychosis: Evidence for astrocyte-linked pathology

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Introduction: Astrocytes comprise a phenotypically diverse glial population that perform vital CNS functions important for cellular, synaptic and metabolic homeostasis. Astrocytes have been implicated in the pathophysiology of major depressive disorder (MDD), with prior studies reporting reductions in astrocyte density across multiple brain regions in this disorder. However, questions remain regarding the function of astrocytes in MDD and the influence of potential confounding factors, such as suicide, presence of psychosis, and medication use. Glial fibrillary acidic protein (GFAP) is an intermediate filament that provides astrocyte structural support and is upregulated under pathological conditions.

The primary aim of this study was to compare GFAP protein and mRNA expression in postmortem brain tissue between MDD and control groups.

Methods: Frozen samples of cingulate cortex were obtained from the Stanley Medical Research Institute. The sample consisted of 36 subjects (control n=12, MDD without psychosis n=12 and MDD with psychosis n=12). Protein levels of GFAP were assessed by immunoblotting and mRNA expression quantified using qPCR.

Results: GFAP mRNA expression was significantly increased in MDD. Compared to controls, GFAP mRNA expression was significantly higher in MDD without psychosis. GFAP mRNA expression was significantly higher in MDD subjects who did not die by suicide, relative to controls. While GFAP mRNA expression correlated with GFAP protein levels, protein levels were not significantly different between groups.

Conclusions: Our results suggest that astrocyte pathology may contribute to MDD pathophysiology, with increased GFAP expression potentially indicative of astrocyte activation or dysfunction.

P3-C-409 - Postnatal choline supplementation alters long-term potentiation threshold in adult prenatal ethanol exposed offspring

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Fetal Alcohol Spectrum Disorder (FASD) is a condition that impacts 2-5% of the North American population and is characterized by a combination of long-lasting physical and cognitive deficits. Choline supplementation is increasingly being explored as a FASD treatment as studies have found improvements in memory to be associated with it. Previous work in our lab has also shown acute improvements in hippocampal synaptic plasticity following adolescent choline supplementation, but it is unclear whether these changes would persist into adulthood. To examine this issue, pregnant dams were exposed to an ethanol-containing liquid diet or a control diet throughout gestation. Offspring were then treated with choline or saline from postnatal day (PD) 10-30. Long-term potentiation (LTP) was examined in the dentate gyrus of adult offspring (PD 60-90) using saturating and non-saturating LTP induction protocols. The magnitude of LTP did not differ between control and prenatal ethanol exposed (PNEE) offspring, regardless of choline administration, when a saturating LTP induction paradigm was used. However, choline treatment was able to rescue reduced LTP when a non-saturating induction protocol was used in male offspring. Choline treatment improved LTP in female offspring, however, there was no deficit in the PNEE condition. These changes in LTP may involve alterations in the function of GluN2B. This study suggests that choline supplementation may have persistent benefits for hippocampal synaptic plasticity and that these could underly long-term cognitive benefits for treatment of FASD.

P3-C-410 - Conditioning electrical stimulation and exercise have synergistic effects on nerve regeneration and functional recovery

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Distal nerve transfers (DNTs) are a reconstructive technique for peripheral nerve injuries where the motor targets of an injured nerve are reinnervated by a redundant branch of a nearby functioning nerve. DNTs shorten the distance required for regeneration, as the transfer is typically performed immediately proximal to where the motor nerve enters into the muscle, thereby decreasing the time to reinnervation. Acceleration of nerve regeneration is essential to promote functional recovery following DNT.

Conditioning electrical stimulation (CES) delivered to the donor tibial nerve one week prior to DNT to the common peroneal (CP) nerve improves reinnervation and functional recovery of the tibialis anterior muscle. Postoperative exercise (EX) therapy improves plasticity and enhances regeneration following a nerve injury however, its effects have not been evaluated in DNTs. The aims of this study are to a) compare the effects of CES and EX on tibial to CP nerve transfer, and b) determine whether the combination of CES and EX would have a synergistic effect.

Methods: One week following a CP nerve injury (to induce footdrop), adult rats (n=10/cohort) were distributed into 4 groups: 1) DNT alone, 2) CES +DNT, 3) DNT + EX, 4) CES+DNT+EX. EX rats ran 1 hour/day for 6 days/week for 6 weeks. Gait kinetics and kinematics were assessed between 7-10 weeks.

Results: CES+EX animals had accelerated nerve regeneration, increased NMJ innervation, tibialis anterior muscle weight, compound muscle action potential, foot dorsiflexion, as well as superior kinetic and kinematic gait compared to CES, EX or DNT alone. Micro-CT analysis revealed the tibial bone mineral density recovered in the EX-animals, only.

Conclusions: CES+EX had synergistic effects on nerve regeneration and functional recovery. EX, but not CES, is responsible for bone remineralization.

P3-C-411 - Leveraging microelectrode arrays to characterize the long-term anti-epileptic effects of cannabidiol on primary rat hippocampal neurons

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Epilepsy affects over 130,000 Canadians, and with no cure, patients are left with few options and a compromised quality of life. Current medications fail approximately 30% of patients, while trialing drug combinations to find a safe and effective mixture is difficult and often distressing for the patient. This is especially concerning since epilepsy and certain antiepileptic medications (AEDs) have negative long-term effects on learning, memory, and cognition. Thus, it is imperative that we improve our understanding of new AEDs and characterize their interaction with classic AEDs to reduce seizures and preserve neuronal health.

One AED of interest is cannabidiol (CBD), which was recently approved as an adjuvant drug alongside first-line AEDs for seizure prevention. However, much is unknown about its potential as a fast-acting rescue drug and its long-term impact on seizure resiliency.

Leveraging microelectrode arrays to record electrical activity, we pre-treated primary rat hippocampal neurons with CBD before inducing seizures with a low-Mg2+ solution. We found that CBD pre-treatment made the neurons more resilient to subsequent seizures over several weeks in vitro. We also found that CBD provided an immediate reduction in activity during active epileptiform episodes. To our knowledge, this is the first long-term characterization of the effects of CBD on neuronal network activity, synchronization, and spike clustering in preventing and arresting seizure-like activity in dissociated hippocampal neurons, which provides a reliable platform for future AED comparisons.

P3-C-412 - Challenging the regenerative potential of neural stem cells in the Zebrafish Forebrain using a Repeated Injury Model

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Neural Stem Cells (NSCs) play a central role in neural repair after brain injury. While mammals struggle with glial scarring, zebrafish are an exceptional regenerative model displaying NSC regeneration in the adult forebrain post-injury. Remarkably, upon injury activated NSCs generate newborn neurons to replace lost lineages. A pro-regenerative environment, marked by an acute immune response and upregulation of specific genes further facilitates timely repair. Despite this neurorepair capacity, whether these NSCs can maintain a sustained neuronal output in response to successive instances of injury remains unknown.

This study investigates the regenerative potential of zebrafish forebrain NSCs when exposed to a prolonged injury state, and the factors contributing to its pro-regenerative environment. A repeated adult forebrain injury paradigm was established and validated using H&E staining. Fish received 1-4 injuries at weekly intervals. Across all injury groups, NSC differentiation into neurons was assessed using EdU/HuC/D co-labelling. Pilot data indicates decreased neuronal output with increased injuries. In contrast, an increase in macrophage recruitment to the injury site was observed in the *Tg(mpeg1:eGFP)* reporter line that marks for macrophages, revealing an enhanced immune response. PCR analysis is further being conducted to assess the expression pattern of key regenerative genes, including *gata3* and *cxcr5*. This study will unveil whether a regenerative limit exists for adult zebrafish NSCs and if this is coupled with changes in the pro-regenerative environment.

P3-C-413 - Unravelling the Immunological Nexus: Role of complement factor I and Cub and sushi domains 1 in Schizophrenia Pathogenesis

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Introduction: Schizophrenia (SZ) is a chronic psychiatric disorder affecting 1% of the population. Although the pathophysiology of SZ remains poorly understood, components of the complement system, including Cub and Sushi Domains 1 (CSMD1), have been identified as potential risk genes for SZ through genome-wide association studies. The complement system, known for enhancing innate immunity, has recently been recognized for its vital role in brain development and homeostasis, regulating synaptic pruning and plasticity. Brain complement activity is tightly modulated to prevent aberrant synapse degradation by complement regulators such as CSMD1 and its cofactor, complement factor I (FI). However, little is currently known of the mechanisms by which complement regulators such as CSMD1 and CFI may influence SZ risk and pathogenesis.

Methods: Postmortem brain tissue was obtained from the Stanley Medical Research Institute, including frozen prefrontal cortex from 104 subjects (control n=35, SZ n=35 and bipolar disorder n=34). mRNA expression of CSMD1 and FI was quantified using qPCR. Protein levels will be quantified by Western blotting. The relationship between FI and CSMD1 and markers of synaptic density will be explored.

Results: While CSMD1 mRNA expression was not significantly different between diagnostic groups, expression was correlated with levels of SNAP-25, a pre-synaptic marker. Additional data to be presented.

Conclusions: Findings will extend knowledge of the mechanisms by which the complement system contributes to SZ pathophysiology, potentially paving the way for complement-based therapies.

P3-C-414 - Preliminary description of neurological blood-based biomarkers in survivors of intimate partner violence

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About 200,000 Canadian women experience Intimate Partner Violence (IPV)-caused Brain Injury (BI) annually. IPV-BI can occur from Non-Fatal Strangulation (NFS), head impact or both. Diagnostic tools for IPV-BI are lacking; blood-based biomarkers have the potential to improve diagnosis.

Aims of this study are to describe (1) biomarkers in survivors of IPV with and without suspected BI compared to age-adjusted Canadian normative Reference Intervals (RI) and (2) the relationship between biomarkers and acute symptoms.

30 females who experienced IPV in the last 30 days (median=10 days, SD=7 days) were divided into groups based on whether or not they experienced NFS and/or head impact (suspected BI vs. without BI). Plasma was analysed for Neurofilament-Light (NfL) and Glial Fibrillary Acidic Protein (GFAP) and expressed as within or above the 95th percentile of the RI. Mann-Whitney tests compared biomarkers between those with suspected BI who reported experiencing headache or dizziness at the time of the incident vs. those who did not.

Of 23 participants with suspected BI, 17.4% and 8.7% exceeded the RI for NfL and GFAP, respectively. Of 7 participants without BI, 14.3% exceeded the RI for NfL while none exceeded the RI for GFAP. Biomarkers were not different in those reporting headache or dizziness (n=18) vs. those who did not (n=5) (NfL: p=0.17, r=0.30; GFAP: p=0.75, r=0.078).

Low proportions of participants with suspected BI had biomarkers outside of population norms, prompting further study of this population. Biomarkers were not associated with reports of headaches or dizziness.

P3-C-415 - Investigating the role of vascular cells on stem cell function during postnatal systemic inflammation

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INTRODUCTION: Acute systemic infection during childhood can lead to long-term neurological impairment. Brain endothelial cells (ECs) are directly affected by infection and in the adult neurogenic/oligodendrogenic niche, the subventricular zone (SVZ), provide cues restricting neural stem cell (NSC) proliferation and differentiation. The role of the NSC vascular niche during development and postnatal infection remains to be elucidated. We hypothesize that postnatally, ECs similarly restrict these stem cell phenotypes, and that systemic infection disrupts these processes. OBJECTIVE: To investigate the role of the vasculature on postnatal NSC proliferation and fate following systemic infection. METHODS: We modeled postnatal systemic infection in mice by administering the bacterial endotoxin lipopolysaccharide (LPS). Utilizing single-cell transcriptomics, we examined SVZ EC transcriptional changes. Using light sheet microscopy, we evaluated the SVZ vascular network. We also assessed in vitro EC conditioned media effects on cortical precursor fate. RESULTS: Our data indicates that postnatal systemic LPS alters EC gene expression and increases SVZ vascular density. EC conditioned media from the postnatal SVZ restricts cortical precursor gliogenesis and neurogenesis in vitro, while conditioned media from LPS-exposed ECs disrupts this fate restriction. CONCLUSION: Future work will examine LPS-induced changes to NSC fate in vivo. Together, these results indicate that postnatal systemic LPS alters the SVZ vasculature and suggests the importance of EC signalling on NSC fate.

P3-C-416 - Spatial transcriptomics of the human prefrontal cortex in major depressive disorder

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Major depressive disorder (MDD) is a debilitating, heterogeneous disease characterized by depressed mood, diminished interests and impaired cognitive function. Roughly 380 million people are currently suffering with MDD, worldwide. Women are 2â€“3 times more likely to develop MDD than men, while exhibiting greater functional impairment and symptom severity. Although some genetic associations have been detected between MDD and underlying risk factors, it remains a challenge to delineate causal disease mechanisms from these findings. Using snRNAseq, our lab recently reported that the greatest dysregulation attributable to MDD occurred in deep-layer excitatory neurons and immature oligodendrocyte precursor cells (OPCs): contributing roughly half of all changes in observed gene expression. Though, questions still remain to understand the complex interplay of different cell-types and how their localization may contribute to MDD in both males and females. Using 10X Genomicsâ€™s Visium spatial gene expression assay, we aim to assess spatial patterns of differential gene expression between individuals with a history of MDD and healthy controls in the BA9 region of the prefrontal cortex (n=10). We have integrated canonical immunofluorescent markers (NeuN, GFAP) into our Visium assays to provide a further cell-type annotation. This study aims to be the first, to our knowledge, to evaluate spatial patterns of differential gene expression in the context of MDD and the first to study the co-localization of different cell types in the brains of depressed individuals who died by suicide.

P3-C-417 - Vascular alterations in the hypothalamus in Alzheimer's Disease

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Alzheimerâ€™s Disease (AD) is a complex and multifactorial neurodegenerative disease. AD has been extensively associated with peripheral metabolic disturbances, but changes in the brainâ€™s main center for metabolic regulation, the hypothalamus, have been relatively unexplored. This region is

associated with the control of whole-body activity, adiposity, glucose homeostasis, energy expenditure, feeding behavior, and many more. Here, we investigate vasculature changes in the hypothalamus of postmortem human AD brains and early presymptomatic onset of such vasculature changes in the 3xTg mouse model of AD. Single cell RNA sequencing (scRNAseq) of hypothalami from pre-symptomatic 3xTg-AD mice (i.e., prior to memory impairments) identified evidence of early transcriptomic changes within the vascular endothelial cell lineage. Differentially expressed genes in 3xTg endothelial cells related to stress response, inflammation and vascular dysfunction. Preliminary analyses of human familial and sporadic AD brains are consistent with vascular inflammation within the human AD hypothalamus. Deeper histological analyses and bio-informatic mining of transcriptomic data are currently underway. These findings support the potential involvement of early hypothalamic deregulation in the metabolic alterations associated with AD.

P3-C-418 - CanProCo study: Prognostic protein biomarkers in sera and plasma from multiple sclerosis patients

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The heterogenic clinical presentation and unpredictable disease trajectory of patients with multiple sclerosis (MS) remains a prevalent clinical challenge. Prognostic biomarkers are much needed but are lacking. The Canadian Prospective Cohort to Understand Progression in MS (CanProCo) study involves collection of biological, radiological, and clinical data from 452 MS patients during early disease over five years. **Objective**: Investigate protein biomarkers in sera and plasma from MS patients. **Method:** Unbiased clinical clusters were determined from 452 CanProCo patients using PHATE embedding. 71 immune-related proteins were quantified by nELISA (Eve Technologies) in matched plasma and serum from 125 MS patients. Detailed clinical parameters at collection date and one-year follow-up were examined with supervised approaches to stratify patients into clinical clusters. Protein levels were then compared across clinical clusters. The delta EDSS was calculated following one-year follow-up to examine the prognostic value of each protein. **Results:** There was a disparity in the protein composition between serum and plasma in MS patients. Eight distinct clinical clusters were identified. Eotaxin (CCL11) levels in serum and plasma were elevated in a clinical cluster with mostly PPMS patients with high EDSS, and slightly lower in clusters that defined RRMS patients. CXCL9 in serum were correlated with MS disease worsening within one year, as defined by EDSS. Matched sera and plasma when analyzed together improved the prognostic power of this association. **Conclusion:** Collation of clinical and radiological data spanning five years will be eventually analysed to examine the predictive power of the soluble proteins over the course MS disease. These data present promising results of the use of novel protein biomarkers to predict MS disease progression.

P3-C-419 - Pimototracks: Automated homecage system for monitoring naturalistic behavior and assessing motor learning in mice

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Background: Automated homecages have revolutionized behavioral neuroscience, particularly in studying group-housed mice. These systems allow for the observation of naturalistic behaviors in a stress-reduced environment, enhancing the understanding of motor learning processes.
Methods: This study utilized group-housed mice, either wild-type or zQ175 Huntington Disease (HD) models, crossbred with thy1-jRGECO transgenic mice (expressing red calcium indicator in cortical projection neurons). Each mouse was tagged with an RFID chip for individual activity tracking and task performance assessment. The mice were housed in spacious conventional rat cages, equipped with an overhead camera and a grid of RFID readers for continuous tracking of movements. An adjoining chamber enabled mice to perform a skilled lever-pulling task to obtain water, their sole hydration source. Data was collected via a Raspberry Pi, with offline analysis using YOLO and DeepLabCut.
Results: The system efficiently identified a range of behaviors and social interactions. Mice rapidly learned the motor task, reaching a performance plateau within ten days. This platform pretrains mice for a similar head-fixed lever-pulling task, allowing for widefield cortical neuron activity analysis via their jRGECO expression.
Conclusion: PiMotoTracks is a robust tool for studying spontaneous behaviors and task-dependent motor learning. It holds significant potential for understanding and characterizing disorders with motor phenotypes such as the HD mouse models, contributing valuable insights into neuroscientific research.

P3-C-420 - Autism Spectrum Disorder (ASD) variant prospecting in Drosophila with clinical predictive value

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Gene variant discovery is becoming routine, but remains frustratingly difficult to interpret the disease relevance of most identified rare variants. Experimental assays capable of interpreting variant function with clinical predictive value are helping to fill this interpretation gap. We take advantage of *Drosophila*

*melanogaster*â€™s molecular genetic tractability to perform inexpensive, reproducible, in vivo testing for hundreds of variants. We exploit genetic interaction analysis to selectively test variant function in disease-relevant pathways. This allows us to overcome challenges with assay reproducibility, scalability to hundreds of variants, and relevance to the pertinent disease mechanism. We have demonstrated the successful deployment of the ClinGen Sequence Variant Interpretation (SVI) Working Group guidelines for clinical interpretation of variant function. We will present our progress in establishing assays in *Drosophila* that test functional effects of rare coding variants in ASD related genes including MECP2, BRAF, STXBP1 and Î²-catenin. This includes: (i) An informatics pipeline for prioritizing genes amenable to *Drosophila* testing and identifying reference variants for calibrating assays. (ii) Generating the molecular genetic reagents for testing variants. (iii) Developing assays to test disease-relevant mechanisms in alignment with SVI guidelines. (iv) Designing semi automated quantification programs that quantitate phenotypic differences across variants allowing us to calibrate our results. As this project progresses, more ASD gene rare variants will be tested for clinical significance.

P3-C-421 - The neuromuscular junction in health and disease: functional impact of NMJ morphological alterations, from normal aging to ALS and nerve injury

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Voluntary movements rely on the integrity of the neuromuscular junction (NMJ), an essential synapse controlling muscle contractions. Several conditions impair NMJ functionality, causing motor dysfunction. For instance, NMJ denervation is a hallmark of amyotrophic lateral sclerosis (ALS), age-related NMJ dysfunctions are observed in aging, while nerve injury leads to NMJ denervation and motor deficits. We posit that properties of NMJ innervation governs the functional muscle properties in a motor unit-dependent manner during ALS, aging, and nerve injury. We studied NMJ innervation and functionality of the SOL (slow-twitch) and the EDL (fast-twitch) in a mouse model of ALS (SOD1G37R), in old WT mice, and in WT mice following nerve crush. Neuromuscular function was assessed using a force transducer and immunohistochemistry was performed. The EDL presented profound NMJ alterations in ALS and progressive functional deficits, which reflected disease stages. In the SOL, however, NMJ alterations occurred late in the disease. In addition, the SOL reinnervated faster than the EDL after nerve injury. In ALS, NMJs an muscles showed unique alterations that reflects pathogenesis and cannot be explained by nerve injury alone. Finally, aged mice presented mild neuromuscular alterations compared to ALS and nerve injury. Altogether, our findings reveal a strong link between structure and function in all conditions and highlight the dynamic state of the NMJ in health and disease. These results demonstrate the determinant impacts of NMJ structure on its function during ALS, aging, and after nerve injury.

P3-C-422 - Modelling spinal cord neuro-inflammation in polio-like paralysis, using human immuno-competent neural avatars

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Recurring outbreaks of Acute Flaccid Myelitis (AFM) following Poliovirus (PV) eradication in the western world have raised significant public health concerns. AFM cases coincide with outbreaks of non-polio enteroviruses, specifically EV-D68 and EV-A71, suggesting a viral link. Despite phenotypic similarities to poliomyelitis, viral antigens are rarely detected during AFM diagnosis, and the mechanism causing spinal cord injury remains unclear. Elevated systemic inflammatory cytokine levels in AFM patients indicate an immune response. Inflammatory responses, initiated for viral clearance, may lead to additional neurotoxicity without direct infection. Our hypothesis posits direct neural cell infection and inflammatory damage involving microglia and astrocytes. Using 2D and 3D human pluripotent stem cell (hPSC) derived models of the brain and spinal cord, we investigate cellular and host responses to EV-D68 and EV-A71 infection, aiming to delineate AFM injury mechanisms. Immunofluorescence, RT-qPCR, and cytokine release assays track viral and inflammation kinetics, while single-cell RNA-sequencing delves into transcriptomic changes. We demonstrate that EV-D68 infects hPSC-derived neurons and microglia, leading to cell death with prolonged infection. Additionally, preliminary results suggest neuronal infection to be productive and increased expression of essential cytokines and chemokines in microglial infection. These findings provide insights into potential AFM injury mechanisms, emphasizing the importance of understanding enterovirus infections for public health.

P3-C-423 - Aberrant hippocampal neurogenesis in a novel mouse model with ALS-like pathology

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Decreased adult hippocampal neurogenesis is reported in rodent models of Amyotrophic Lateral Sclerosis (ALS). While about10% of ALS has been linked to genetic mutations known as familial ALS, the majority of the cases have no underlying genetic cause. Our group has recently developed a pan-neuronal

Thioredoxin-1 knockout mouse model (Trx-nKO) using a Syn1-dependent Cre-recombinase system. Thioredoxin-1 is a major regulator of cellular redox status that is responsible for reduction of oxidized proteins during oxidative stress. We demonstrate that Trx-nKO mice display an ALS-like pathology, including robust motor deficits that is associated with loss of spinal cord motor neurons, axonal degeneration, loss of hindlimb muscle mass and premature death at 8-10 weeks of age. We have also identified accumulation of cytoplasmic TDP-43 and depletion of parvalbumin-positive inhibitory neurons in Trx-nKO mice that are similarly reported in ALS pathology. Given the positive role of Trx1 in cell proliferation, we aimed to examine whether hippocampal proliferation and neurogenesis is affected in Trx-nKO mice. Surprisingly, our analyses in adult 8-week-old mice revealed enhanced cell proliferation and neurogenesis evidenced by increased number of doublecortin (DCX+)-positive cells in these mice. The DCX+ cells had irregular shapes and also expressed the neuronal marker, NF200. Aberrant increased neurogenesis has been recently shown in ALS patients. Our *in vitro* analysis on neural stem cells isolated from dentate gyrus of Trx-nKO animals show that pharmacologic restoration of Trx can normalize neurogenesis and neuronal morphology. Our findings suggest a potential link between Trx dysregulation with ALS-like pathology that needs further elucidations.

P3-C-424 - Latrogenic gambling disorder: the effect of light cycle and ropinirole on risky behaviour in male rats

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Parkinsonâ€™s Disease (PD) is one of the most common neurodegenerative diseases, affecting 1-2% of individuals over the age of 65. D2/3 receptor agonists such as ropinirole are effective against PD motor symptoms, yet a significant minority of patients develop impulse control disorders (ICDs), such as gambling disorder (GD). We have previously shown chronic ropinirole administration on performance during the rat gambling task (rGT) significantly increased risky decision-making with win-paired cues. Interestingly, effects were prominent at the beginning of the dark cycle, suggesting an impact of the circadian rhythm on the dopaminergic system and reward-seeking behaviours. We sought to further characterise the effect of light cycle on ropinirole-induced risky decision-making. Male rats (n=64) were treated with 5 mg/kg of ropinirole (n=32) or saline (n=32). Half of the rats in each drug condition were kept on a standard 12-hour reverse light cycle (lights off 8 AM), and the remaining half were on a standard light cycle (lights on 11:30 AM). Rats performed rGT at 9-11 AM following minipump implantation. We show that ropinirole-treated rats towards the beginning of their dark cycle display a bias towards risky options and an increase in impulsive decision-making in comparison to ropinirole-treated rats at the end of their dark cycle on the cued rGT. Brains were wet-dissected following the final rGT session and flash-frozen for metabolomic analysis to assess differences in dopaminergic and melatonergic signalling. These results may provide evidence to previous findings showing dopamine-agonist receptor therapy-induced ICDs are most pronounced towards the evenings. Understanding how

the circadian rhythm and dopamine agonist therapy affect dopaminergic signalling and behaviour will inform treatment guidelines and personalised therapies for PD patients.

P3-C-425 - Laminin beta 2, a matrix protein as a potential biomarker in ALS

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease resulting in the death of motor neurons. Through the loss of these motor neurons, there is an early loss of neuromuscular junctions (NMJs), which progressively lead to complete muscular paralysis, resulting in death. The absence of a biomarker has considerably hampered therapeutic research. JNM elements include the presynaptic element, the postsynaptic element, the glial cell and the synaptic cleft. At the NMJ, the synaptic cleft is occupied by the basal lamina, composed of matrix proteins. Our laboratory identified a number of matrix proteins as potential biomarkers in ALS. To this end, this study aims at confirming that laminin beta 2 (LB2) and its modulation at NMJ reflect the stages of the disease, whereby its presence would be reduced as disease progresses. This basal lamina protein is uniquely present at the NMJ and is known for its architectural role in anchoring pre- and postsynaptic elements and the maintenance the NMJ organization. Absence of LB2 leads to synaptic deficits. Using immunohistochemistry, we found that LB2 is reduced at the symptomatic stages of the disease, but not related to the state of innervation. Furthermore, its presence was no longer restricted to the endplate area of the NMJ. in a mouse model of ALS. Our results show that the presence of LB2 reflects the state of the disease and supports its possibility as a biomarker linked to NMJ in ALS.

P3-C-426 - Fluoxetine and its major metabolite, norfluoxetine, both exacerbate Alzheimer-related amyloid phenotypes in vitro (HEK 293 cells) and in vivo (C. elegans)

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A diagnosis of depression has been associated with risk for developing Alzheimer disease (AD) in later life. There is evidence that some of this risk may be more associated with the type of antidepressant drug prescribed rather than the depression itself, while other studies implicate the 5-HT transporter (5-HTT). Selective serotonin reuptake inhibitors (SSRIs), such as Fluoxetine, block the 5-HTT and are associated with the greatest risk. The pathology of AD is thought to relate, in part, to the accumulation of the Î²-amyloid (AÎ²) peptide within the brains of patients with AD. We took advantage of a C. elegans model for ectopic AÎ²-aggregation to examine whether Fluoxetine or its major metabolite, Norfluoxetine, could exacerbate phenotypes associated with AÎ² accumulation in vivo. The drugs both increased paralysis. Human HEK293 cell cultures transfected with the human APP gene (encodes the precursor molecule that yields the AÎ² peptide) were treated with either Fluoxetine, the serotonin-norepinephrine reuptake inhibitor Venlafaxine, or the tricyclic antidepressant Imipramine. There was less AÎ² secreted into the culture medium, and more accumulating in the corresponding cell lysate, in Fluoxetine-treated HEK293 cells. This phenotype was not observed with either of the other two drugs. This is the first evidence that the 5-HTT may play a role in intracellular AÎ² accumulation. This has major clinical relevance as Fluoxetine has been increasingly prescribed for off-label purposes, particularly to younger individuals, which might be exposing many individuals to an iatrogenic risk of developing AD.

P3-C-427 - Defective oligodendrocyte maturation and myelination in EPRS1-related leukodystrophy

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EPRS1-related leukodystrophy (EPRS1-HLD) is a rare white matter disease associated with prominent hypomyelination and ensuing neurodegeneration. It arises from biallelic variants in the ubiquitously expressed glutamyl-prolyl-tRNA synthetase, *EPRS1*, an indispensable enzyme responsible for the charging of proline and glutamate tRNA species. Pathogenic variants in several other aminoacyl tRNA synthetases have been associated with hypomyelinating disorders, supporting a link between proper protein production and white matter diseases. Disease-causing variants in *EPRS1* result in impaired tRNA synthetase function, however, little is understood regarding the pathophysiology of EPRS1-HLD and the specific cell types most affected by this disease remains unclear. To address this, we generated induced pluripotent stem cells (iPSCs) from three patients with EPRS1-HLD and differentiated them into oligodendrocytes (OLs), the cells tasked with myelinating the central nervous system. EPRS1-HLD patient OLs exhibited defective maturation, as evidenced by a lower proportion of O4+ cells and reduced morphological complexity. Myelination capacity was severely impacted in patient OLs, as indicated by a reduced number of myelin sheaths formed per cell and decreased sheath length. This data provides insight into specific vulnerabilities of OLs to perturbations in protein synthesis and sheds light on the pathogenesis of EPRS1-HLD.

P3-C-428 - Long-term Effect of low-field magnetic stimulation on neurotoxin induced motor dysfunction and neuronal loss in an experimental mouse model of Parkinson's disease

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Parkinsonâ€™s disease (PD) is the second most common neurodegenerative diseases worldwide. Repetitive transcranial magnetic stimulation (rTMS) technology has been widely used for the treatment of neurological disorders including PD. We recently demonstrated that low-field magnetic stimulation (LFMS) restores motor function in an acute model of PD. The present study focused on the long-term effect of LFMS on MPTP induced motor dysfunction and neuronal loss in a chronic mouse model. Mice were injected intraperitoneally with MPTP (35 mg/kg X 10 injections), once in 3.5 days along with probenecid (250mg/kg) for 35 days. 24 h after first MPTP injection, mice were treated with LFMS (20 min), once daily until the end of the experiment. Mice induced with MPTP showed decreased movement and stride length compared to the normal mice, whereas LFMS treatment significantly restored motor function from day 7 as evidenced through beam walk, rota rod and stride length tests. Dopamine level was measured in striatal region, which showed that LFMS treatment significantly reversed the dopamine depletion in MPTP intoxicated mice brain. In addition, LFMS treatment improved neuronal recovery as evidenced by increased NeuN and tyrosine hydroxylase (TH) levels. GFAP, an astroglial marker was significantly decreased in LFMS treatment. The data obtained from the current study revealed that LFMS treatment significantly improved motor function and restores neurons in chronic MPTP mouse model. Further studies will generate a benchmark in restoring the PD neurodegenerative processes.

P3-C-430 - Origin of spinal cord fibroblasts after injury

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Injury to the spinal cord triggers an inflammatory cascade leading to the activation and migration of fibroblasts into the lesion. Fibroblasts produce a dense extra-cellular matrix, which inhibits axon regeneration after spinal cord injuries (SCI). Understanding the cell populations responsible for fibrotic scarring after SCI may help to target these cells pharmacologically, to reduce fibrotic scarring. Currently, there is debate whether, and to what extent, pericytes, perivascular fibroblasts or meningeal cells are the primary source of fibroblasts in spinal cord lesions of mice. To address this question, we have employed single cell sequencing of the mesenchymal cell progeny fate-mapped using a Hic1-CreERT2 mouse line which revealed distinct fibroblast populations before and after injury. This line was also used for histological fate mapping with a TdTomato reporter. In addition, we used fate mapping of the perivascular fibroblasts (and oligodendrocyte precursor cells, OPCs) in a PDGFRa-CreERT2 mouse in combination with a GFP reporter line. A thoracic contusion model SCI was administered to mice and fate-mapped cells were analyzed before injury and at 7- and 21-days post injury. To characterize fate mapped cells and distinguish fibroblasts from OPCâ€™s, we used antibodies against NG2, PDGFRb, and extracellular matrix proteins. Our results demonstrate that perivascular fibroblasts are major contributors to the fibrotic scar and may offer a therapeutic target to reduce fibrotic scarring following SCI.

P3-C-431 - Effects of low and high dose psilocybin administration on hippocampal cell proliferation

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Background: Psychedelics are novel, promising candidate molecules for clinical use in the treatment of a variety of psychiatric disorders. Mechanistic studies indicate that psilocin, the active component of psilocybin, acts as an agonist for serotonin receptors, particularly the 5-HT2AR receptors in the peripheral and central nervous systems. 5-HT2AR is primarily responsible for psychedelic effects. Studies indicate that a single administration of psilocybin can induce rapid changes to synaptic plasticity and neurogenesis via activation of pro-plasticity intracellular signalling cascades and consequent changes in gene expression.

Methods: 60 mice were randomly assigned into one of 5 varying dose treatment paradigms (12/group), then injected with BRDU 24 prior to perfusions and brain collection. Standard immunohistochemistry methods were used to quantify cell proliferation in the prefrontal cortex, amygdala, and hippocampus.

Results: Between-subjects ANOVA analyses were conducted independently for each of the five paradigms, with a focus on distinct brain regions. Our preliminary analyses indicate that significant

changes in cell proliferation were observed in both male and female mice administered the high-dose of psilocybin.

Conclusion: A single high dose of psilocybin facilitated rapid alterations in cell proliferation in brain regions associated to mood, learning, and memory. These mechanistic findings underscore the nuanced impact of dosage and sex-differences of psilocybin as a potentiator for neurogenesis, contributing valuable insights for further exploration and understanding in the novel treatment to psychiatric disorders.

P3-C-432 - Differential dopamine and somatostatin receptor expression in Alzheimer's disease mouse model

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The Dopaminergic (DA) system has been extensively studied as a key neurotransmitter involved with cognition and emotion. It has been documented that the overall DA declines with age. Around 35â€“40% of individuals diagnosed with Alzheimer's Disease (AD) exhibit extrapyramidal signs in addition to symptoms of cognitive decline, indicating disruption of the DA system with the pathophysiology of AD. The spatial proximity of Amyloid-Î² and Somatostatin (SST) exists in the human brain, particularly in areas relevant to AD, implicating that SST biology overlaps with AD pathology. In addition to the inverse correlation of SST with age in the frontal cortex, naturally decreased SST levels are further accentuated in AD with approximately 50% loss of SST-positive neurons. Despite the direct involvement of DA and SST systems in AD, their region-specific distribution remains debated. In the present study, we employed immunohistochemistry and western blot analysis to characterize the expression patterns of DA receptors (D1R and D2R) and SST receptors (SSTR2 and SSTR4) in the hippocampus and cortex of APP/PS1 transgenic mice. Our findings reveal region-specific distribution of DRs and SSTRs expression within the hippocampus and cortex of AD mice compared to age matched control. Our results indicate upregulation in the expression of D1R and downregulation of D2R and SSTR4 in both cortex and hippocampus. Moreover, SSTR2 expression was downregulated in the cortex and increased in the hippocampus of AD mice. This study will unravel insights into DA and SST system distribution in AD mice and shed light on molecular mechanisms underlying their dysregulation in AD pathology.

P3-C-433 - Comprehensive functionalization of DYRK1A mutations implicated in Autism spectrum disorders

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Autism Spectrum Disorder (ASD) is the most common genetic neurodevelopmental disorder, yet there are currently no effective treatments. This failure is in part due to complex genetics with hundreds of implicated genes, compounded by a poor understanding of how ASD-associated mutations alter physiology. One such gene, Dual specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A) is highly implicated in ASD and we present work detailing the functional impact of 58 disease-associated and population control missense mutations. We show by N-terminal GFP-tagging that DYRK1A is a highly cleaved protein and that single residue changes direct dramatic changes in how full length DYRK1A is processed. Furthermore we show by phospho-flow cytometry that variant subgroups exist, exhibiting variable decreases in pp53 and pS6, which are downstream catalytic targets of DYRK1A. These changes are associated with loss of tyrosine phosphorylation on DYRK1A. Finally, by aggregating multi-function impact scores we show that disease-associated missense variants of DYRK1A show significantly greater damaging phenotypes compared to population control variants. Our work counters prevailing models that missense mutations cause complete loss of protein function impacting physiology due to halpoinsufficiency. We show that protein variant dysfunction is stratified and can be due to a variety of causes such as substrate phosphorylation, protein stability, native DYRK1A phosphorylation and proteolytic cleavage status.

P3-C-434 - Elevation of SIRT3 as a disease-modifying strategy in preclinical models of Parkinson's disease

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Mitochondrial dysfunction is central to the pathology of PD. SIRT3 is the main deacetylase in the mitochondria involved in the regulation of metabolism, oxidative stress, mitochondrial dynamics, proteolysis, and inflammation. We previously showed that intranigral infusion of the gene therapy AAV.SIRT3 has neurorestorative effects in preclinical rat models of PD. In humans, SIRT3 levels decline with age, which is the primary risk factor for PD. We found that in the SNc of PD subjects, SIRT3 levels were reduced compared to age-matched controls (56.83Â±15.51 %), suggesting a link between loss of SIRT3 and PD pathology. Thus, upregulating SIRT3 may be a disease-modifying therapy for PD. As surgery is invasive, we assessed noninvasive methods of upregulating SIRT3 in preclinical models of PD. We assessed whether MR-g-FUS-mediated delivery of AAV.SIRT3 increases SIRT3 expression in the brain following IV administration. SIRT3 was elevated in the striatum, hippocampus, and SNc. In a parallel study, we determined whether hexafluoro honokiol (HFH), a synthetic derivative of honokiol from

Magnolia officinalis increases SIRT3 in the brain. In the mutant AAV.Î±-syn rat, intranasally administered HFH (0.83 mg/kg) upregulated endogenous SIRT3 expression in the striatum and olfactory tubercle and reversed motor dysfunction compared to control (18.5Â±9.2 vs. 32.4Â±14.1 % asymmetry, -36.3Â±22.1 vs. -66.7Â±21.4 s latency to fall). These studies provide evidence that noninvasive methods can upregulate SIRT3 in the brain and show that noninvasive elevation of SIRT3 has disease-modifying effects.

P3-C-435 - Lipidome of compact Myelin from aging murine cortex

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Neurodegeneration of cortical white matter tracts is associated with age-related cognitive decline. The trajectory of white matter degeneration follows a similar pattern in human and animal models, including the loss of myelin integrity and myelinated axonal segments. To better understand the biochemical determinants of age-related white matter degeneration, we profiled the lipid composition of cortical myelin isolated from young adult, middle-aged and old-aged murine brains. A refined 2-step gradient protocol was used to enrich compact myelin, and global lipidomics was performed by LC-MS/MS. Multivariate analyses showed that myelin from young mice was biochemically more distinct when compared to the aging cohorts, while little sex differences were observed. The relative abundance of 991 unique lipid species were found to be upregulated or downregulated across age groups. Early aging was characterized by changes in glycerophospholipids and sphingolipids, including sulfatides. Several of these changes persisted into old age, alongside increased accumulation of diacylglycerols and triacylglycerols. These data suggest that changes in the myelin lipid composition, which may impact membrane organization and fluidity, are already apparent by middle age when myelination peaks. The findings provide greater insight into age-related biochemical alterations in the myelin sheath and may aid with strategies to mitigate white matter degeneration over the lifespan.

P3-C-436 - Involvement of supraspinal neuroimmune mechanisms in a rat model of morphine withdrawal-induced hyperalgesia: Role of Toll-like receptor 4 in the periaqueductal gray

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Individuals with prolonged opioid use who seek treatment face a challenging barrier upon detoxification, namely opioid-withdrawal syndrome (OWS). OWS also contributes to opioid relapse, making its mitigation a critical step toward discontinuing opioid use. A crucial but under-appreciated aspect of OWS is opioid withdrawal-induced hyperalgesia (OWIH), which manifests as increased sensitivity to pain. Glial cells in the central nervous system play important roles in pain management and OWS. Of particular interest is the activation of glial cells by opioids via Toll-like 4 receptors (TLR4), a pattern recognition molecule of the innate immune system. Accordingly, we explored the role of TLR4 as a potential avenue for intervention in the reversal of OWIH. In this study, rats were given intermittent morphine (15mg/kg) for three weeks, during which time the pain threshold was measured via Von Frey tests. Systemic blockade of the TLR4-MyD88 pathway before morphine injections, using Tat-MyD88 peptide, successfully prevented the development of OWIH and when given after induction also reversed hyperalgesia. Moreover, the acute effects of systemic Tat-MyD88 on OWIH were accompanied by a less reactive microglial morphology. Importantly, local inhibition of TLR4-MyD88 in the periaqueductal gray (PAG) restored hypersensitivity to pain by accelerating recovery to pre-morphine treatment levels in OWIH rats. Together, these results identify TLR4-MyD88 as a novel therapeutic target for treating OWIH, which acts through microglia in the PAG, a supraspinal region of the central nervous system.

P3-C-437 - Sex-specific modulation of the gut microbiota-immune-brain axis in prenatally stressed mice fed a Mediterranean-based diet

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Prenatal stress may predispose to mental illness, possibly by disrupting the establishment of the gut microbiota and inflammatory signaling pathways along the gut-brain axis. As diet is a determining factor in gut microbiota development, early-life dietary interventions could modulate the effects of prenatal stress on the gut microbiota-immune-brain axis, with beneficial impact on mental health. This study examined if a Mediterranean (Med)-based diet limited changes in the gut microbiota-immune-brain axis and in anxiety- and depressive-like behaviors in prenatally stressed female and male offspring. Pregnant C57BL/6N female mice fed a Control or a Med-based diet experienced a restraint stressor in the second trimester. Offspring behaviors were assessed in adulthood using the elevated plus maze, open field, and tail suspension tests, after which intestinal and brain sections were collected for the analysis of microbiota, inflammatory, and tight junction markers. The Med-based diet improved fear and passive coping behaviors in females only. Likewise, the diet limited pro-inflammatory shifts in the microbiota and colonic elevations in pro-inflammatory cytokines promoted by the stressor mostly in females. Lastly, the tight junctions occludin and claudin-5 were increased in the hippocampus of mice fed the Med-based diet, again exclusively in females. These findings suggest that early-life diets could limit changes to

the gut microbiota-immune-brain axis consecutive to prenatal adversities, potentially improving mental health, and that these effects may be specific to females.

P3-D-438 - Subtype-specific modulation of cortical interneuron populations by noradrenaline

Emmeraude Tanguay [1], **Paul De Koninck** [1], **Vincent Breton-Provencher** [1]

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Noradrenaline (NA) plays a significant role in complex behaviors, however, the mechanisms by which it affects the distinct cellular components of cortical circuits remain poorly understood. Here, we aimed to map the expression and the function of each subtype of NA receptors - Î±-1(a,b,d), Î±-2(a,b,c), and Î²-(1,2,3) - onto the distinct subclasses of cortical interneurons. To achieve this, we analyzed the expression of NA receptors using a cell-type atlas of the mouse and human motor cortex. Our findings revealed varied expression of NA receptor transcripts in the cortex, with GABAergic interneurons expressing high levels of Î±-1a and b and pyramidal neurons expressing Î²-1 transcripts. To investigate the spatial distribution of these expression patterns in the cortex, we used fluorescence *in situ* hybridization to label the RNA of NA receptors alongside the main subclasses of interneurons. Our preliminary results showed a difference in the expression of Î±-1 receptor transcripts across cortical layers for Vip+ and Sst+ cells. Conversely, for Ndnf+ and Pval+ interneurons, we found a difference in the expression of Î±-1 receptor transcripts across cortical regions. We are currently investigating how this differential pattern of expression affects interneurons' spontaneous activity and their responses following reward and punishment using two-photon imaging, pharmacological, and gene-editing approaches. Thus far, our results suggest that NA modulates cortical circuits by differentially targeting subtypes of GABAergic cortical interneurons.

P3-D-439 - Disinhibition compromises spatiotemporal processing of touch by disrupting the receptive fields of spinal dorsal horn neurons

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The spinal dorsal horn (SDH) plays a crucial role in processing touch and pain signals. Different inhibitory circuit motifs in the SDH, including feedforward and lateral inhibition, influence the temporal and spatial integration of incoming signals. Spatial processing of somatosensory input depends on neuronal receptive

fields (RF). Specifically, spinal neurons have a center-surround RF structure formed via excitatory connections with primary afferents and inhibitory connections with other spinal interneurons. RFs have been shown to expand when synaptic inhibition is reduced, which may underlie the clinical observations that broad or dynamic stimuli produce more allodynia than punctate or static touch in patients with neuropathic pain. Despite recent findings, the impact of RF expansion on SDH neurons or circuit function remains unclear. To begin disentangling this synaptic connectivity in the spinal cord, we built a data-driven model of the SDH circuit to which we incorporated the synaptic connectivity underlying RFs to efficiently examine the spatial processing of different types of tactile stimuli (e.g. diffuse/punctate). Using experimental electrophysiology data recorded from spinal interneurons, we fit the network model to both normal and pathological RF sizes and firing rates. Neuropathic conditions, simulated by reductions in synaptic inhibition (i.e. disinhibition), disrupted feed-forward inhibition in ways that increased temporal summation of spikes and lateral inhibition in ways that reshaped RF organization and increased spatial summation of tactile input. Finally, our model made testable predictions regarding multiscale targets for combating the effects of pathological disinhibition on spatiotemporal processing in the SDH.

P3-D-440 - Abnormal Noradrenergic activity patterns in the primary motor cortex of 16p11.2 deletion mouse model of autism

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Children with autism spectrum disorders (ASDs) frequently experience delays in motor-related development. We have previously shown that in the mouse model with 16p11.2 deletion, a common copy number variation associated with ASDs, mutant mice also display delayed motor learning. Interestingly, they show abnormally high neuron activity in the primary motor cortex (M1), and activating locus coeruleus noradrenergic (LC-NA) neurons rescues the circuit deficits and delayed motor learning. Here, by using in vivo two-photon microscopy to record LC-NA axonal activity in M1 during motor learning, we found that persistent calcium events (>5 sec) in WT mice are highly correlated with the running epochs (RE) during the initial learning phase but this correlation is significantly reduced in mutant mice. In contrast, mutant mice exhibit an increase in the frequency of transient calcium events (~0.4 sec) during non-REs, suggesting non-behavior related activation of LC-NA. Lastly, through the disruption of behavior-specific NA activity by locally infusing noradrenaline reuptake inhibitor or using a dedicatedly designed closed-loop optogenetic manipulation, we can mimic the unspecific LC-NA activities observed in the 16p11.2 mice, and induce delayed motor learning in WT mice. Our findings unveil abnormal noradrenergic activity dynamics during motor learning in 16p11.2 deletion mice, shedding light on neural circuit dysfunctions linked to motor-related deficits in ASDs.

P3-D-441 - Mapping the connectivity between premotor areas and the motor cortex in the human brain

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Goal-directed movements are associated with unique neurophysiological alterations in motor-related brain regions. This study systematically examined the interhemispheric connectivity between the premotor (dorsal, PMd; ventral, PMv) and the primary motor (M1) cortices using dual-site transcranial magnetic stimulation. We had 30 right-handed young adults (15F; 26.5Â±4.3yrs) participate in three experimental conditions to examine interhemispheric inhibition (IHI) between: (1) PMd to M1, (2) PMv to M1 and (3) M1 to M1. PMd and PMv were located relative to M1 (PMd: 2cm anterior, 1cm medial; PMv: 3cm anterior, 2.5cm lateral), using each participantâ€™s structural MRI. A 3x3 grid (9 locations with 1cm spacing) was generated for each cortical region (i.e., PMd, PMv, M1). To investigate IHI between these cortical regions, conditioning stimuli were applied to each of the 9 sub-locations placed over the right PMd, PMv, and M1. The test stimulus over left M1 then occurred after a 10ms interval. Additional trials with only the test stimuli over the left M1 were collected in a pseudorandom manner. IHI ratio was calculated by comparing the mean peak-to-peak motor evoked potential amplitudes of the conditioned to unconditioned trials at each sub-location. The most significant inhibition was found at the center for M1, 1cm anterior and 1cm medial to M1 for PMd, and 2cm anterior and 1.5cm lateral to M1 for PMv. These results demonstrate the need to comprehensively understand the unique interactions between premotor and M1 areas influencing goal-directed movement.

P3-D-442 - Optic flow neurons in the pretectum have different direction tuning at very fast speeds

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The pretectum contains neurons responsive to global visual motion. These signals are sent to the cerebellum, forming a subcortical pathway for processing optic flow. Global motion neurons exhibit selectivity to both direction and speed, but this is usually assessed by first determining direction preference at intermediate velocity (16-32 deg/sec), and then assessing speed tuning at the preferred

direction. A consequence of this approach is that it is unknown if direction preference changes with speed. We measured direction preferences in 114 cells from 44 zebra finches *Taeniopygia guttata* across a range of spatial and temporal frequencies. The cells showed highest overall activity at intermediate speeds (32 deg/s) with lower overall activity as speed increased or decreased. 15% of the cells were omnidirectionally excited across most speeds. The remaining 85% of the cells had direction tuning that changed with speed. For at least at one tested speed, some cells were directionally-selective, some were bi-directional-selective, and some were omnidirectionally excited. One third of the cells were either directionally- or bidirectionally-selective at intermediate speeds and became omnidirectionally excited at fast speed (1024 deg/sec). Collectively, these results indicate that pretectal global visual motion neurons are most responsive at the stimulus speeds typical for locomotion, but that a large fraction of the cells also respond omnidirectionally, especially to fast speeds that could signify impending collisions.

P3-D-443 - Inhibiting oligodendrocyte-specific remyelination in aged spinal cord injured mice results in locomotor and cognitive impairments

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Spinal cord injury (SCI) is a debilitating affliction that, reflecting the growth of the worldâ€™s aging population, is occurring more commonly in aged individuals. SCI typically causes focal demyelination of spared axons near the site of injury. Surprisingly, no differences in locomotor recovery were seen post SCI when remyelination was inhibited in young adult mice (Duncan et al. 2018, Nat. Comm. PMID: 30076300). To assess the importance of remyelination for locomotor recovery and cognitive function in aged, injured mice, we compared young (3-5 month) versus aged (15-18 month) transgenic mice of both sexes. By cross breeding mice with an inducible knockout of *Myrf,* a key transcription factor for myelination expressed in oligodendrocyte progenitor cells (OPCs), with a PDGFRÎ±CreERT2 driver line we could inhibit OPC maturation into oligodendrocytes (OLs). This ultimately prevents new OL-based remyelination. Young and aged animals underwent a moderate/severe thoracic (T9/10) level contusion, or a sham injury (laminectomy), and were observed for three months. To examine the influence of age and remyelination inhibition on locomotion we completed a variety of behavioral tests including an open field locomotion test (Basso mouse score), horizontal ladder test, and more. We assess cognition through the Y-maze and the object relocation task. Overall, this project looks to address fundamental questions about the significance of remyelination after aged injury, which may give greater insight to personalized therapies post SCI.

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P3-D-444 - Pretectal optic flow processing in zebra finches during flight

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Many animals including birds and humans depend on global visual motion, commonly termed optic flow, to guide movement through natural environments.
In all tetrapods, the pretectal nucleus lentiformis mesencephali (LM) contains retina-recipient neurons that respond to optic flow stimuli, and demonstrate tuning with respect to direction, speed, and in the spatiotemporal domain. LM is homologous to the mammalian pretectal nucleus of the optic tract. Given the physiology and connectivity of LM and its role in image stabilizing optokinetic responses, it is expected to also play a role in the analysis of optic flow during locomotion.
However, LM function has never been investigated during natural locomotion. Our foundational hypothesis is that LM neurons are modulated by optic flow during flight.
We developed in vivo electrophysiology to record spiking activity of LM neurons in freely flying zebra finches. Analyses focus on the responses of neurons to flight-induced optic flow, head turn-induced optic flow, and exogenous optic flow cues presented along the lateral walls of the flight chamber.
We found that most neurons preferred forward motion and were suppressed by flight-induced backward motion. However, some neurons could be excited by exogenous forward motion during flight, suggesting a portion of LM neurons faithfully report sensory driven optic flow during flight. We also discovered neurons that display weak directional tuning but are highly modulated during flight. These findings suggest that LM contains neuronal sub-types that may not be fully captured by anesthetized recordings. LM likely participates in visuomotor transformations during locomotion, extending its role beyond optokinetic responses.

P3-D-445 - Cortical control of muscle coordination in the larynx, tongue and wings of the Egyptian fruit bat

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Most behaviours involve the coordination of muscles across the body. Among neural structures involved in motor control, the neocortex plays a major role in coding movements involving multiple muscles. How

adaptive behaviours, including complex muscle activation sequences, emerge from the fractured topography of the motor cortex is unknown. We explored the topography and temporal patterns of muscle representations in 4 Egyptian fruit bats (*Rousettus aegyptiacus*), which, like other members of Chiroptera, have evolved several unique behaviours, including self-propelled flight, lingual clicks used for echolocation, and complex vocal social communication. We studied cortical control of muscles involved in flight (including occipito-pollicalis, which is unique to bats), echolocation (several tongue muscles and mouth and jaw muscles), and complex social communication (cricothyroid muscle of the larynx). We delivered long train (500 ms) intracortical microstimulation (ICMS) to the sensorimotor cortex while recording electromyographic signals (EMG) from up to 16 muscles. Preliminary analysis reveals broadly overlapping representations of single muscles from across the body. Many muscle representations spanned motor and somatosensory cortex. As we have reported in rats, some muscles exhibited topographical differences in ICMS-evoked EMG waveforms. For example, the medial cricothyroid representation centred in motor cortex exhibited activity lasting hundreds of ms, while EMG activity evoked from lateral sites centred in somatosensory cortex comprised 1-2 spikes as brief as 20 ms.

P3-D-446 - Structural and genetic constraints on zebrafish brain networks

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Network analysis has considerably advanced our understanding of nervous systems at large scales, where critical cellular information is missing. Leveraging advances in optical imaging and genetically encoded sensors, we performed whole-brain imaging at cellular resolution in zebrafish larvae to investigate mesoscopic functional connectivity (FC). We recorded neuronal activity from roughly 50000 cells in head-restrained transgenic larvae, expressing GCaMP6s in every neuron, while simultaneously monitoring tail movements, then computed mesoscopic FC from regional calcium signals, revealing similar functional networks across larvae. Remarkably, FC fingerprinting allowed subject identification over a 24hr period. To probe the structure-function relationship of zebrafish brain networks, we used over 4000 neuron reconstructions to compute structural connectivity (SC). We found a strong correlation between FC and SC, with several structural network properties predicting over half the variance of FC. Next, we identified structural network communities, which constrained the shape of regional coactivation patterns observed in multiple animals. We then identified stimulus- and motor-correlated cells to generate anatomical maps of functionally specialized populations, which segregated along a rostrocaudal axis. This â€œsensorimotor axisâ€ could be recovered with a diffusion gradient derived independently from both spontaneous FC and SC. Finally, we used spatially resolved gene markers to identify a subset of genes whose co-expression significantly predicted regional FC. Our results highlight several key principles by which functional connectivity emerges from structural and genetic constraints, setting the stage for future investigations into the developmental trajectories of neural networks and their deviations in disease.

P3-D-447 - The visual world of free-flying pigeons: Insights from head-mounted cameras

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Vision is crucial for diurnal animals to control flight. Studies in birds, insects, mammals, and reptiles corroborate that visual information is essential for flying animals to negotiate tight and obstructed spaces, regulate speed, manage contact with surfaces, capture prey, navigate long distances, and maintain position. Yet our understanding of how animals discern critical visual cues from a myriad of available options and encode that information to control behavior remains limited. A significant obstacle in advancing our understanding of this process is the scarcity of empirical data describing the visual stimuli that flying animals encounter in their natural habitats. To address this knowledge gap, we developed a head-mounted camera system to record the visual environment experienced by free-flying homing pigeons.

P3-D-448 - The molecular and cellular logic of taste in the yellow fever mosquito

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The yellow fever mosquito, Aedes aegypti, is a vector for deadly viruses like Dengue and yellow fever. These mosquitoes use taste neurons distributed across their body to inform blood-feeding, egg-laying and other critically important behaviours. Hundreds of taste receptor genes from large multi- gene families are expressed in taste organs, however, it is unclear how these genes are expressed within and organized across sensory neurons to encode complex chemical stimuli and drive behaviour. Using the Q-binary expression system, we developed a technique to profile gene expression in genetically identified populations of sensory neurons. We generated a transgenic mosquito strain to express a nuclear-targeted green fluorescent protein (GFP) in cells of interest. Intact nuclei are then isolated from whole tissues using Dounce homogenization followed by fluorescence-activated cell sorting (FACS). We have used this protocol to generate a single-nucleus RNAseq dataset from a small population of neurons that are activated by both fresh and salt water and mediate egg-laying behaviour. This dataset has revealed the co-expression of multiple taste receptor genes within these neurons and will identify candidate salt receptor genes that can be further characterized using genetic techniques. Characterizing the logic of

chemoreceptor expression will help to elucidate the mechanisms by which a limited receptor repertoire encodes the inordinate diversity of the chemical world, helping us to understand the evolution of taste processing and informing future mosquito control strategies.

P3-D-449 - An injectable anisotropic hydrogel to direct aligned axon growth after spinal cord injury

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After a spinal cord injury (SCI), the damaged tissue fails to regenerate, leading to cavity formation at the lesion site. Previous studies indicate that longitudinally-aligned microchannel scaffolds can guide aligned axon growth across SCI lesions. Currently, these scaffolds must be implanted into the lesion site, posing risk of further tissue damage. We are investigating the use of an anisotropic hydrogel that can be injected minimally-invasively to direct aligned axon regrowth. It utilizes a surrounding hydrogel to deliver microrods (developed in the De Laporte lab) that serve as physical guidance cues for axons. These microrods contain magneto-responsive nanoparticles, enabling rostro-caudal alignment using a low magnetic field. When the hydrogel-microrod solution is injected into the lesion, a magnet can be placed outside the body to align the rods in under a minute, while the surrounding hydrogel crosslinks to fix the rods in their aligned orientation. This 3D platform has previously promoted aligned growth of chick dorsal root ganglia axons *in vitro;* in our lab it promotes neurite outgrowth of rat postnatal cortical explants. We hypothesize that it can induce aligned axon outgrowth *in vivo* after SCI. We are currently evaluating various hydrogel formulations for optimal gelation and microrod alignment parameters in rat SCI models. Once reliable rod alignment is achieved *in vivo*, we will evaluate the ability of the platform to induce aligned axon regrowth by combining it with treatments targeting scarring as well as the intrinsic growth response of injured CNS neurons.

P3-E-450 - Effects of neonatal endotoxin exposure on glucocorticoid regulation in the adult mouse brain

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Bacterial infections in early life have enduring consequences on brain development and function. Bacterial infections activate the hypothalamic-pituitary-adrenal (HPA) axis and increase glucocorticoids (GCs), such as corticosterone, in the blood. Exposure to lipopolysaccharide (LPS), an endotoxin found in gram negative bacteria, at postnatal day (PND) 5 also induces GC production in the mouse brain. It is unclear whether neonatal endotoxin exposure ("1st hit") alters GC regulation in the brain after adult endotoxin exposure ("2nd hit"). Here, male and female C57BL/6J mice were injected intraperitoneally with saline (vehicle control) or 50ug/kg LPS at PND 4 and 6 ("1st hit"). Mice were injected again with either saline or 50ug/kg LPS in adulthood (â€œ2nd hitâ€) (2x2 design). The brain and blood were collected 4 hr later (n=10/sex/group). GC levels were measured in the blood and microdissected brain regions, including the prefrontal cortex, hippocampus, hypothalamus, and amygdala via highly specific and sensitive liquid chromatography tandem mass spectrometry. Preliminary results show that neonatal LPS treatment alters GC responses to adult LPS treatment. This project will clarify how neonatal endotoxin exposure produces long-term alterations in brain GC regulation in adulthood, potentially underlying behavioural impairments seen upon subsequent immune challenges later in life.

P3-E-451 - Dissecting the cross-talk between H1R and CB1R in hypothalamic neurons in the Olanzapine-induced metabolic syndrome

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Background

Metabolic syndrome (MS) is the main burden of Olanzapine (OL) administration. We identified the hypothalamic histamine receptor 1 (H1R) as responsible for OL-induced weight gain. Moreover, we proved that the blockade of Cannabinoid receptor 1 (CB1R) restores the MS phenotype. Here we explore the cross-talk between H1R and CB1R.

Methods

C57BL/6J female mice are treated with OL for 28 days. The H1R and CB1R knockouts (KOs) are induced with a double viral CRISPR approach using AAV-MeCP2Cas9 administered through hypothalamic stereotaxic injection. The hypothalamic transcriptome is analyzed by performing differential gene expression analysis, pathway analysis, and gene co-expression network analysis.

Results

The H1R-KO in hypothalamic neurons disrupts the physiological response to a fat diet, upregulating the NPY expression. The OL treatment of the H1R-KO mice boosts this effect, further increasing the NPY transcriptional level, but it also amplifies the CB1R expression.

The KO of the CB1R restores the normal response to the calory intake, decreasing the NPY expression. A reshaping of the hypothalamic connectome is responsible for this rescue. The CB1R-KO dissociates the expression of the H1R and the NPY pathway.

Conclusion

Our findings highlight the complexity of the GPCRsâ€™ interplay. The CB1R-KO mediated phenotypical and transcriptional rescue is driven by intricated changes in the hypothalamic connectome and not by a single transcriptional factor of protein-protein interaction. Our results represent a proof of concept of the importance of deeper explorations in the study of GPCRs.

P3-E-452 - Triglyceride- and lipid droplet-associated genes act in neuroendocrine cells to regulate sex differences in body fat

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In most animals, males and females show differences in the amount of stored body fat, and in the rate of fat breakdown when nutrients are withdrawn. Our lab recently identified a key role for triglyceride lipase brummer (bmm), the Drosophila homolog of mammalian adipose triglyceride lipase (Atgl), in regulating these differences in fat metabolism. Specifically, we found that pan-neuronal loss of bmm/Atgl in Drosophila abolished the sex difference in fat breakdown by blocking fat breakdown during fasting. This project aims to identify the neuroanatomical focus of bmm/Atglâ€™s effect on fat breakdown, and to determine whether additional genes that regulate triglyceride and lipid droplets are similarly required in neurons to regulate fat metabolism. We used RNAi to perform bmm/Atgl knockdown in specific subgroups of neurons, and monitored the effect on fat breakdown. We found one group of neuroendocrine cells called the adipokinetic hormone-producing cells (APCs) that reproduced the sex-specific phenotype caused by pan-neuronal bmm/Atgl loss. RNAi knockdown on one other gene, triglyceride lipase midway (mdy), in the APCs additionally mimicked the pan-neuronal bmm/Atgl phenotype. These data suggest that the APCs require correct triglyceride breakdown and lipid droplet regulation to support their function, and disruptions of these pathways negatively affects the health of these cells and whole-body triglyceride storage. Ongoing studies aim to examine the functionality of these neurons, investigate potential behavioural consequences, and assess additional neuron populations.

P3-E-453 - Acute exercise buffers stress-induced metaplasticity in CRH-PVN neurons and mitigates stress-induced defensiveness

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Stress imprints biochemical, molecular, and synaptic changes in the brain to promote adaptation. However, these changes can become maladaptive and foster neuropsychiatric diseases. Surprisingly, there is limited understanding on how these imprints can be reversed. In humans, exercise is used to cope with stress despite inducing physiological stress itself. Here we examined the effects of exercise on stress-induced short-term potentiation (STP) of glutamate synapses on corticotropin release hormone cells in the paraventricular nucleus of the hypothalamus (CRH^{PVN}). Exercise (treadmill) for one hour after foot shock (FS) increased CRH^{PVN} activity and circulating corticosterone (CORT). Next, we obtained electrophysiological recordings from CRH^{PVN} neurons in hypothalamic slices and evaluated the effects of exercise after FS on STP. Following FS, high frequency stimulation of glutamate synapses elicited STP. Exercise after FS blunted STP. Exercise after FS increased brain-derived neurotrophic factor (BDNF) in the PVN. And incubation of brain slices from FS mice with a TrkB agonist and CORT blunted STP. At a behavioral level, mice subjected to FS showed lower exploration of the light compartment in a Dark/Light box. Exercise after FS reversed this phenotype. Our findings demonstrate that exercise increases BDNF in PVN and decreases STP induced by stress This is accompanied by a decrease in stress-induced anxiety.

P3-E-454 - Elucidating state-dependent activity dynamics of hypothalamic stress output neurons using a spiking network model and dynamic clamp

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Corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus of the hypothalamus (PVN) are critical in driving the neuroendocrine stress response. We recently showed that CRH_{PVN} neurons fire in two distinct modes: rhythmic brief bursts (RB) and single spikes (SS) of variable interval. Somewhat counterintuitively, RB constrained the overall firing rate at low, stable levels due to a long (~2 s) interburst silence. In contrast, SS showed a wide range of firing rates. These data pointed to the importance of the bistable firing pattern in gain control of CRH_{PVN} neuron activity. Here, we used

tight combinations of experimental and computational approaches to show that a recurrent inhibitory network generates RB, controls gain, and constrains the overall firing rate. We adopted a recently developed, conductance-based adaptive exponential integrate-and-fire model (CAdEx), which overcomes the limitations of current-based models. The CAdEx spiking network model showed that release from recurrent inhibition upon stress stimuli permits continuous SS that increases the overall firing rate, revealing a novel mechanism for bistable control of stress output neurons. To validate the spiking network model, we used ex vivo dynamic-clamp electrophysiology. CRH_{PVN} neurons in these experiments exhibited in vivo-like bursting behaviour that constrained the overall gain. Ultimately, we identified a novel network mechanism that underlies bistable firing activity dynamics at the effector neurons of hormonal stress response.

P3-E-455 - Differential distribution of Calbindin- and Calretinin-expressing neurons in mouse versus rat subfornical organ

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The SFO is characterized by extensive vascularization with fenestrated capillaries, and the presence of neuronal cell bodies surrounded by large extracellular spaces. This lack of a blood-brain-barrier allows solutes, hormones and signaling molecules to rapidly move across capillary walls, make contact with, and influence SFO neurons. SFO also receive synaptic input from, and projects synaptic output to autonomic control centres such as the paraventricular nucleus of the hypothalamus (PVN) and nucleus of the solitary tract (NTS). Thus, the sensory SFO represent windows through which levels of circulating satiety signals may be communicated from the periphery to autonomic control centres. Indeed, SFO is regarded as a "thirst centre" and primary site of action for ANGII in stimulating thirst and drinking. Historically, rat has been the predominant model for study of the role of SFO in water and salt balance, however with increasing use of transgenic mice, numerous studies have used mice in study of SFO neurophysiology. We present evidence of differing patterns of immuohistochemical staining of Calbindin and Calretinin calcium binding proteins in mouse vs rat SFO. Specifically, in rat SFO Calbindin-expressing neurons are typically localized to the inner core, whereas Calretinin-expressing neurons are restricted to the outer shell. In contrast, Calbindin- and Calretinin-expressing neurons are evenly distributed throughout the mouse SFO.

P3-E-456 - Anxiolytic effect of beta-klotho deletion in the ventral subiculum

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Beta-klotho (KLB) is a co-receptor required for the signaling of two metabolic hormones, fibroblast growth factor (FGF) 19 and FGF21, that mount a stress response via the paraventricular hypothalamus (PVH). However, as PVH *Klb* expression is low, upstream *Klb* activation must drive the stress axis. To identify candidate *Klb* regions, we performed *in situ* hybridization and found that the ventral subiculum (SUBv), which bidirectionally regulates the stress axis, is a *Klb*-rich region where *Klb* expression is higher in females. However, it is not known if SUBv *Klb* expression regulates responses to stress elicited by anxiety, thus we deleted *Klb* by delivering an adeno-associated virus encoding Cre recombinase-EGFP (or EGFP in control littermates) into the SUBv of male and female *Klb-flox* mice. We then tracked their movement in the anxiogenic, open center or elevated arms of an open field or elevated plus maze, respectively. Mice with SUBv *Klb* deletion tended to increase their entry and time exploring an elevated open arm, but this anxiolytic effect was not seen in the open field. Interestingly, when a food reward was placed in the open field, both sexes spent more time in the center after SUBv *Klb* deletion. However, their expression of anxiety-like behavior differed, as female mice make less entries but spent more time per entry into the open space. These findings support an anxiogenic role of SUBv KLB signaling and point to an anxiety-related pathway with metabolic implications, though high *Klb* expression, like in females, may not convey functional differences.

P3-E-457 - Exploring the impact of H1 receptor antagonist promethazine on high-fat diet fed rats: unveiling behavioural and neuroendocrine responses

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Histamine H1 receptors (H1-Rs) play a crucial role in body composition regulation, found in both peripheral tissues and hypothalamic regions. Our study investigated H1-Rs' mechanisms in obesity, utilizing high-fat diet and repeated Promethazine injections (2.5 mg/kg) in male Albino Wistar rats from Dow University of Health Sciences, Pakistan. Rats were divided into Controls (Standard lab chow; 10% fat) and Test (High-fat diet; 35% fat) groups, treated for four weeks following guidelines of the Institutional Bioethical Committee, University of Karachi, Pakistan. Subsequently, Promethazine (2.5mg/kg) was injected daily for 14 days. Weekly physiological and behavioural changes were monitored, analysed using SPSS version 14.0 with significance at $P<0.05$.

Results showed high-fat diet fed rats treated with Promethazine exhibited a substantial 35% weight increase compared to controls (P<0.01). Notably, significant increases in food intake (F=50.5, P<0.01) and body weights (F=35.3, P<0.01) were observed weekly. Impaired cognitive functions and anxiogenic-like effects were evident. Metabolic analyses revealed hyperglycemia (F=10.8, P<0.01) and insulin resistance (F=9.0, P<0.01), alongside elevated brain serotonin and plasma leptin, ghrelin, and corticosterone levels.

In conclusion, our findings underscore the exacerbation of hyperphagia and feeding behaviour in high-fat diet fed rats following 14-day Promethazine administrations (2.5 mg/kg). This emphasizes H1-Rs' pivotal role in appetite regulation, nutrient sensing and potential neuroendocrine modulation of metabolic disorders.

P3-F-458 - Contributions of the infralimbic (IL) cortex and ventral CA1-IL circuit in cued approach-avoidance conflict decision-making

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Approach-avoidance conflict (AAC) occurs when a stimulus imbued with both positive and negative valences is presented, resulting in opposing motivations to approach and avoid. Previous research has implicated the ventral CA1 (vCA1) in potentiating approach under AAC and the infralimbic (IL) cortex in the regulation of adaptive responding to punishment and reward cues. However, the specific contributions of the IL and vCA1-IL projections in arbitrating cued motivational conflict remain underexplored. To this end, male and female Long-Evans rats were conditioned to associate three distinct cues with either an appetitive, aversive, or neutral outcome. Animals then underwent a conflict test, in which AAC was elicited through the superimposition of the appetitive and aversive cues, as well as additional assessments of preferences for the appetitive and aversive cues. During these tests, optogenetic and chemogenetic (DREADDs-mediated) inhibition was induced in the IL and vCA1-IL, respectively. Inhibition of the IL and vCA1-IL significantly increased time spent in the aversive cued arm compared to controls, whereas inhibition of the vCA1-IL circuit also increased time spent in the conflict arm. These findings suggest that the IL and vCA1-IL circuit necessitate the effective avoidance of negatively valenced cues, and vCA1-IL projections facilitate avoidance under conflict. Taken together, we propose the vCA1 is a general regulator of behaviours under AAC, whereas the vCA1-IL circuit mediates conflict avoidance via preferential processing of negatively valenced cues.

P3-F-459 - Interoceptive and preliminary proteomic effects of nicotine vs cigarette smoke extract in male and female rats

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Introduction: Although nicotine (NIC) is generally the primary alkaloid investigated for tobacco use disorder, the other ~8000 constituents in cigarette smoke are thought to interact with NIC to affect its etiology. The primary study used a Pavlovian drug discrimination task; we hypothesized that rats could discriminate between NIC and cigarette smoke extract (CSE) of the same NIC concentration based on the presence of constituent chemicals. We have also begun investigation of brain proteomic changes resulting from long-term exposure to CSE, NIC, or vehicle (VEH). **Methods:** Behaviour is assessed using three types of occasion setting training: NIC discriminating from VEH, CSE discriminating from VEH, and CSE discriminating from NIC. Separate rats were injected daily for 28d with CSE, NIC, or VEH, and brains were excised for proteomic processing. **Results:** Subjects readily discriminate between NIC and VEH and between CSE and VEH; however, they are unable to discriminate between CSE and NIC after 72 sessions with 8 trials of each. Preliminary results suggest differential proteomic changes evoked by CSE vs NIC. **Conclusions**: Our results confirm that CSE is a successful occasion setter and adds to previous NIC literature. Interestingly, we demonstrate that CSE and NIC do not create distinct interoceptive environments under current training conditions and differences evoked by each substance may be occurring at the cellular level instead. **Significance:** This has important implications for ongoing discussions regarding nicotine as a proxy for tobacco in animal models.

P3-F-460 - Individual and sex differences in the effects of contingent versus non-contingent foot shock on oral morphine seeking in rats

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Purpose: Altering opioid circuitry through uncontrollable stress could expose an overlap in analgesic and reinforcement systems. The present study investigates the effects of contingent and non-contingent punishment on oral morphine (OM) intake. **Methods**: Male and female rats were randomly assigned to one of five groups: Morphine Control (MC); Punishment (P); Yoked (Y); Shock Control (SC); and Chamber Control (CC). Following OM acquisition, groups transitioned to the punishment phase. MC rats continued

self-administration with no foot shocks (FS). P rats received FS at a 15% probability, contingent on active lever pressing for OM. Y rats were matched with P to receive time-matched non-contingent FS during SA sessions. SC rats received matched FS, but never had access to OM; CC rats never experienced FS or had access to OM. **Preliminary results**: Male and female morphine controls are consuming similar amounts of drug in mg/kg. Contingent FS reduces drug intake in both, but the there appear to be strong individual differences between animals more resistant versus more sensitive to FS when it is linked with morphine seeking. Non-contingent FS decreases consumption in males below baseline morphine control and this decrease is not observed in females. Notably, both sexes show equal pain sensitivity in the tail-flick test. **Implications**: These results have an impact on theoretical notions of the role of compulsivity in substance use disorders as well as provide insight into individual differences between those sensitive and resistant to punished drug-seeking behaviour.

P3-F-461 - Multiple peptidergic signaling pathways underlying sensitization and dishabituation in C. elegans

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Nonassociative learning, the simplest form of learning, is thought to help animals selectively allocate attention or neural resources to promote survival. Sensitization and dishabituation are two forms of nonassociative plasticity: both sensitization and dishabituation facilitate the likelihood and/or magnitude of responses. Previous Work from *Aplysia* has shown that although sensitization and dishabituation both require the neuromodulator serotonin, they can be differentiated by the developmental stages they emerge, the electrophysiological profiles, and the downstream second messengers involved. Our previous work using *Caenorhabditis elegans* found a FLP-20/FRPR-3 neuropeptidergic pathway specifically mediating sensitization but not dishabituation of a nociceptive response.

In the present work, we demonstrated that sensitization and dishabituation are mediated by multiple neuropeptidergic signaling pathways. Using our previously established paradigms, we found that sensitization of response duration and response speed, two components of the nociceptive response, are regulated by different signaling molecules downstream of FLP-20/FRPR-3. We also found that sensitization and dishabituation of response speed are mediated by the same neuropeptides, however, they appeared to be recruited by different upstream signaling in these two forms of learning. We are currently performing a genetic screen to identify other molecules involved in these signaling pathways.

Taken together, these data show that sensitization and dishabituation can be mediated multiple distinct and shared, underlying molecular mechanisms, and that sensitization may not be a global, organism-wide, phenomenon, rather, sensitization of different aspects of behavior can be differentially regulated by multiple neuromodulatory pathways.

P3-F-462 - Cognitive mapping in rats: the role of the orbitofrontal cortex (OFC) in creating mental representations of a two dimensional auditory space

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This experiment seeks to elucidate the neural mechanisms underlying abstract cognitive mapping in rats, by examining the role of the orbitofrontal cortex (OFC) in the use of a two-dimensional auditory space. To test ratsâ€™ ability to make inferences based on cognitive maps, 21 rats were trained on a Pavlovian task in which two sets of auditory cues were configured to represent a 2D-space. The stimulus set consisted of 12 distinct tone frequencies and 12 distinct click frequencies which were combined for 144 compound auditory stimuli. We sought to determine whether learning about a reward paired with a single compound on the space would accelerate across multiple compound-reward learning episodes, and whether this accelerated learning would require the OFC. During training sessions, rats were exposed to â€œrandom-walkâ€ paths through the abstract space, such that they would listen to a progression of cues with a particular transition structure. This progression was designed to mimic physical movement through space, so each compound cue would only precede an adjacent compound cue on the 2D-space. Rats were trained on 6 separate reward locations, with each location presented for 12 days. Learning was assessed through reward anticipation. OFC was inactivated using DREADDs in 14 of the rats during learning of the 5th and 6th reward location counterbalanced with injection of saline or the DREADD agonist. Contrary to our expectations, OFC inactivation facilitated reward learning across the latter learning episodes. This observation aligns with the theory that the OFC may be required for determining latent states of abstract cognitive maps. Inactivation of OFC may reduce interference of prior learning events, which may act as competing latent states. By disrupting the competing states through OFC inactivation, learning on the task would thereby be improved.

P3-F-463 - Neurogenesis mediates contextual memory reorganization

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Memory storage is a dynamic and distributed process and adult hippocampal neurogenesis has been demonstrated to affect learning and memory in numerous ways. Previous work has shown that increasing neurogenesis prior to contextual conditioning renders memory recall more resilient to hippocampal disruption. These findings implicate neurogenesis as a potential mediator of memory reorganization, reshaping the functional connectivity supporting contextual memories to be less reliant upon the hippocampus. To further explore these ideas, we used a functional connectivity approach to assess the influence of neurogenesis on memory network reorganization. We used FosTRAP2 mice and c-fos immunohistochemistry, to capture brain-wide neuronal activity patterns at both recent (1 d) and remote (28 d) contextual memory retrieval timepoints. We used voluntary wheel running for 4 weeks prior to contextual fear conditioning, to increases neurogenesis. Compared to the sedentary group, running caused a decrease in the correlated activity between the dentate gyrus (DG) and CA1 at the recent memory recall timepoint. Conversely, the mean correlated activity between the DG and the cortical regions of these networks increased with running. This effect was greatest at the remote memory recall timepoint. Together, these results suggest that neurogenesis may promote the recruitment of cortical regions into memory networks.

P3-F-464 - Fear conditioning induces sex-specific changes in the blood-brain barrier and is modulated by a immune challenge

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Experiences are linked to emotions impacting memory consolidation and associated brain neuronal circuits. Posttraumatic stress disorder is an example of strong negative emotions affecting memory processes by flashbacks of past traumas. Stress-related memory deficits are also observed in major depressive disorder. We recently highlighted that stress induces blood-brain barrier (BBB) alterations in a sex and brain region specific manner in mice and human depression. However, little is known about the relationship between emotional valence, memory encoding and BBB function.
In this study, we studied the effects of negative emotional valence through an aversive memory experience: fear conditioning. We assessed the impact of this paradigm on BBB properties in brain regions related to memory and emotions processes. Male and female mice went through acquisition (auditory cue + footshocks) on day 1, context, cue and recall tests in the following 3 days. We observed sex differences in behavioral and biological variables: females froze more and had higher circulating corticosterone levels compared to males. Comparison of ventral hippocampus and prefrontal cortex BBB transcriptomes revealed striking increased genes expressions and variations between sexes. Fibroblast

growth factor 2, which is known to regulate BBB tight junctionsâ€™ expression, was upregulated in ventral hippocampus, amygdala and prefrontal cortex, for both males and females in a drastic way for mice receiving footshocks. We also evaluated how an acute immune challenge, via peripheral injection of lipopolysaccharide, affects BBB transcriptome and behavioral memory outcome. In summary, mice that experienced acute stressful situation showed BBB changes, different between male and female animals. This gives us additional information on the role of the BBB integrity in memory formation following traumatic events.

P3-F-465 - Yohimbine has cognitive enhancing, rewarding, and reinforcing properties in male and female Sprague-Dawley rats

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The indole alkaloid yohimbine (YOH) is anxiogenic and activates stress-sensitive neural circuits to precipitate drug craving and seeking. However, because there is also evidence of YOH-induced reward, this study employed male and female Sprague-Dawley rats to explore whether YOH shares three properties of pharmacological reinforcers. First, it was determined if intravenous infusions of YOH (0.25 mg/kg/inf) could maintain lever pressing, and whether intake would be adjusted based on dose per infusion (0.125 or 0.5 mg/kg/inf). Second, to assess YOHâ€™s effect on memory consolidation, rats received 0, 0.3, 1.25 or 3 mg/kg (IP) post-training and were tested for object recognition memory 72 hr later. Finally, place conditioning assessed whether doses of YOH that elevate serum corticosterone (1.25 or 3 mg/kg) could elicit conditioned approach. Here we report for the first time that both sexes acquired intravenous YOH self-administration, intake was modulated by dose per infusion, and that lever pressing persisted in the absence of YOH, or in the absence of the YOH-paired cue. Furthermore, it was found in both sexes that post-training injections of 1.25 mg/kg YOH enhanced consolidation of object memory, and that 1.25 and 3 mg/kg elicited conditioned approach. These novel findings in rats revealed a profile of YOH's cognitive and behavioral effects that is consistent with that of psychostimulant reinforcing drugs. As such, these results suggest the need for further investigation into YOH's potential for abuse and its utilization in clinical and pre-clinical research to study relapse.

P3-F-466 - Differential encoding of valence and reinforcement signals by dopamine release across multiple pathways of the dopaminergic system

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The dopaminergic system facilitates associative learning and motivated behaviors by signaling reinforcement valence and reward expectation within the brain. Previous studies measuring somatic and axonal activity suggest heterogeneous representation of reinforcement signaling by dopaminergic neurons, but it remains unclear how dopamine signals for valence and expectation interact in various brain regions. Moreover, the extent to which fluctuations in dopamine levels track reinforcement signals is poorly understood. Here, we used an improved fluorescent dopamine sensor to record dopamine signals associated with stimulus valence and reward predictions in various locations of the mesolimbic pathway (subregions of the nucleus accumbens, olfactory tubercle and amygdala), the nigrostriatal pathway (dorsal and tail striatum), and mesocortical pathway (medial prefrontal cortex). Our findings show dopamine release in response to rewarding stimuli across all locations. However, the encoding of reward expectation by dopamine release varied: reward expectation was the strongest in regions of the mesolimbic pathway, while it was the weakest in the mesocortical pathway. Additionally, when measuring dopamine release following an aversive stimulus, we observed that it peaked in regions where the encoding of reward expectation by dopamine was the lowest. Together, our results provide evidence of contrasting reinforcement signals across the dopaminergic pathways through which dopamine release supports learning.

P3-F-467 - The role of corticotropin-releasing hormone in the medial prefrontal cortex in stress-induced working memory deficits in mice

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The corticotropin-releasing hormone (CRH) is widely known as a hormone that mediates stress responses via the hypothalamus-pituitary-adrenal (HPA) axis. Besides its neuroendocrine release, CRH is also expressed and acts as a neuromodulator across various brain areas, including the medial prefrontal cortex (mPFC). Using the trial-unique non-match-to-location (TUNL) task that measures special working memory in mice, we showed that 4-hour (h), but not 1-h restraint stress impaired working memory. In agreement with the stress duration-dependent effects, 4-h, but not 1-h, restraint stress increased CRHR1 mRNA expression as examined using in situ hybridization. Further, both pharmacological blockade of CRHR1 and chemogenetic inhibition of CRH neurons in the mPFC â^'after 4-h restraint and before the TUNL taskâ^' prevented the working memory impairment. These results indicate that stress impairs working memory through mPFC CRH-CRHR1 signalling during the TUNL task. Using fibre photometry imaging, we monitored mPFC-CRH neuron activity (GCaMP6s was expressed in CRH neurons) and CRH release (a newly developed CRH sensor $GRAB_{CRF}$ was expressed in the mPFC) during the TUNL task. Our preliminary data showed that mPFC CRH neurons selectively increase their activity shortly after incorrect choices. I am currently examining CRH dynamics in the mPFC during TUNL. This study will advance our understanding of how the

CRH system mediates working memory deficits associated with stress and, potentially, cognitive impairment in stress-related psychiatric disorders such as post-traumatic stress disorder.

P3-F-468 - Inhibiting ventral hippocampal projections to the prefrontal cortex increases conflict preference and alters decision dynamics in an operant approach-avoidance conflict task

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A hallmark of psychopathology is disordered approach-avoidance decision-making processes. This includes resolving competing approach-avoidance tendencies, which arise when stimuli signal rewarding and aversive outcomes simultaneously. Converging human and rodent research has implicated the anterior/ventral hippocampus (vHPC) in reconciling such approach-avoidance conflict (AAC). However, the precise roles of the extensive vHPC projection pathways in mediating AAC are less clear. This study therefore investigated the role of vHPC projections to the infralimbic (IL) and medial orbitofrontal (MO) cortices in AAC resolution using an operant conflict task. Male Long-Evans rats learned to choose between a small reward (sucrose pellet) versus a larger reward (two sucrose pellets) paired with varying foot-shock intensities (conflict option). Inhibition of vHPC-IL or -MO projections occurred through a dual-viral, projection-specific chemogenetic approach (via modified human muscarinic receptor, hM4Di). Results suggest that vHPC-IL inhibition significantly increases conflict preference while vHPC-MO inhibition induces a trend of increasing conflict preference. Supplemental ethological anxiety assays were not impacted by inactivating either pathway. Analysis of response latency via drift-diffusion modeling revealed changes in decision dynamics (e.g. evidence criterion) due to vHPC efferent inhibition and varying shock intensity. These results illustrate differential regulation of AAC through distinct vHPC projections and provide future avenues for translational research.

P3-F-469 - Differential reward processing in circuits linking the amygdala and nucleus accumbens

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The neural circuits that guide motivated behavior converge in the nucleus accumbens, where motivations are translated into actions. The nucleus accumbens is a heterogeneous brain region made up of multiple anatomically and functionally distinct subregions, which receive and process inputs from many parts of the brain. Connections from the basolateral amygdala to the nucleus accumbens are abundant across all subregions of the nucleus accumbens, and relay information about motivational environmental stimuli. How these circuits are organized and how information about reward is relayed through these circuits to drive motivated behaviour is still not well understood. Here we use a combination of anatomical tools, slice electrophysiology, head-fixed Pavlovian reward conditioning and *in vivo* fiber photometry to examine how reward-predicting cues are encoded by circuits linking the basolateral amygdala to the nucleus accumbens. We demonstrate that distinct populations of amygdala neurons target different subregions of the nucleus accumbens, and that they are differently engaged in a reward learning task. Our results suggest that cues that drive motivated behaviour are differentially routed to distinct locations within the nucleus accumbens by inputs from the basolateral amygdala.

P3-F-470 - Spatial characterization of midbrain dopamine neurons using multiplexed error-robust fluorescent in-situ hybridization (MERFISH)

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The midbrain dopamine (DA) system contains a relatively small number of neurons yet has broad functions in the striatum and cortex. Despite their small number, DA neurons contain considerable heterogeneity in their transcriptomic identity, projection targets and function. How this heterogeneity is distributed spatially is not well understood. In this project we utilized Multiplexed Error-Robust Fluorescent In-Situ Hybridization (MERFISH) to create an atlas of mouse midbrain DA neurons. Mouse midbrain sections were imaged across 7 coronal planes and 4,563 DA neurons were identified. We integrated the data with single-nucleus RNA sequencing (sn-Seq) data of DAT enriched nuclei and found 2,297 cells that integrated with high confidence. Of the 22 distinct DA clusters found in the sn-Seq dataset we were able to resolve 17 with high DAergic characteristic. These clusters were broken up into three major families: a Sox6 positive family (6 clusters), a Calb1 family (8 clusters) and a Gad2 family (3 clusters). Spatial mapping of each cluster showed distinct distributions for each family, with Sox6 clusters mapping mostly to the substantia nigra pars compacta (SNc) and retrorubral field, Calb1 clusters mapping to the ventral tegmental area (VTA) and SNc and the Gad2 clusters mapping to the VTA and caudolinear nucleus. This is the most granular map of molecularly-defined mouse DA neurons atlas to date and future experiments will be needed to resolve their functional heterogeneity.

P3-F-471 - Investigating the interaction between cell adhesion molecule 2 (CADM2) genetic variation and cannabinoids using behavioural and neurochemical assessments

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CADM2, located on chromosome 3, is a synaptic adhesion molecule associated with cannabis use and cognition. It is primarily expressed in the brain, especially in addiction-related regions. Our lab has previously used a Cadm2 knockout (KO) mouse line to investigate the association between CADM2 and voluntary cannabinoid intake, THC response and cognitive phenotypes. Prepulse Inhibition (PPI) of startle is one form of sensorimotor gating that has been widely studied in mice and THC has been shown to impair sensorimotor gaining in rodents. Here we investigated the effects of CADM2 genotype and THC exposure on PPI. PPI trials were run on WT, HET and KO mice both before and after THC treatment. Our findings suggested that baseline PPI was attenuated in the KO mice. Additionally, it was also observed that the KO were hyperactive compared to the WT and HET, even without the startle stimulus. Furthermore, after 2mg/kg dose of THC, the differences previously seen between the KO and HET mice disappeared, showing that this dose may attenuate PPI in HET, but not in KO and WT mice. We are conducting Matrix-assisted laser desorption/ionization (MALDI) on THC-treated brains in WT, HET, and KO mice to evaluate differences in glutamate, GABA, dopamine, serotonin, and choline levels in the PFC and NAc. Since the same mice were used for PPI and MALDI, we will also be able to correlate neurotransmitter levels to their PPI measures. These studies aim to clarify THCâ€™s effect on behavioural phenotypes and neurotransmitter levels in relation to variation in Cadm2 expression.

P3-F-472 - Unveiling the role of hippocampal CA1 VIP interneurons in contextual fear memory encoding

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Contextual fear memory (CFC) serves as a widely utilized framework for examining the neurobiological underpinnings of fear acquisition and extinction within the rodent hippocampus. While previous investigations predominantly concentrated on hippocampal pyramidal neurons (PNs), limited attention has been given to exploring the potential involvement of interneurons

(INs) in CFC. Within the CA1 region of the hippocampus, vasoactive intestinal polypeptide-expressing (VIP) INs establish intricate connectivity patterns, exerting regulatory control over PNs and other INs, and have been implicated in memory formation. This study employed a combination of in vivo calcium imaging and optogenetic interventions in freely behaving mice to elucidate the contributions of VIP-INs to CFC. Furthermore, we investigated whether VIP INs modulate the activity of their downstream targets by conducting calcium imaging of PNs, as well as two major types of INs in CA1 â€“ somatostatin (SST) and parvalbumin (PV) expressing INs during CFC tasks in mice. Analysis of calcium activity recordings of VIP-INs during CFC conditioning revealed a consistent increase in activity following the administration of aversive stimuli. Intriguingly, both PNs and PV-INs exhibited robust increases in calcium transients in response to shock, while SST-INs' activity remained largely unaltered. Additionally, we assessed the impact of optogenetic manipulation of VIP activity on fear memory encoding. Taken together, our findings suggest that CA1 VIP-INs are responsive to aversive stimuli and may play a role in supporting the encoding of fear memories.

P3-F-473 - Sign-tracking is associated with self-reported impulsivity but not with risky decision making in human participants

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Attribution of incentive salience to reward cues, termed sign-tracking (ST), has been linked to addiction vulnerability in animal models with emerging data in humans suggesting similar associations. Addictive disorders have been linked to both impulsivity and risky decision making. While studies in both rodents and humans have reported associations of ST with impulsivity, associations with risky choice have been inconsistent in rodents and scarcely examined in humans. We examined associations of ST propensity with self-reported impulsivity and risky choice across three studies comprising 246 human participants. ST was assessed by quantifying gaze fixation on reward-predictive cues during Pavlovian conditioning, risky choice was assessed using a behavioral economic task, and impulsivity was measured using the UPPS-P Impulsive Behavior Scale. Given previously reported sex differences on all three measures, we considered sex as a moderator of these associations. Males and females did not differ in ST propensity. ST was associated with higher scores on negative and positive urgency UPPS-P scales. Males scored higher on positive urgency and sensation seeking. ST did not significantly predict risky choice on its own or in interaction with sex. Relative to females, risky choice in males was more determined by the expected values of the prospects. Our findings support the previously reported associations between ST and impulsive action. However, ST appears to have little overlap with risky choice suggesting that the two may represent unique risk factors for addictive disorders.

P3-F-474 - Exercise and its mimetic metformin restore spatial memory in diet-induced obesity

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Excessive consumption of high-fat and high-sugar (HFHS) foods can not only cause obesity but also lead to cognitive decline. Pattern separation â€“ the process of keeping similar stimuli as distinct memory representations â€“ is specifically vulnerable to HFHS foods even prior to weight gain. This study assessed the impact of aerobic and resistance exercise, along with metformin, an anti-diabetic drug & exercise mimetic, on reversing HFHS diet-induced impairments in pattern separation in mice.

Male and female C57BL/6 mice were divided into HFHS and control diet groups and further subdivided into one of six treatment groups for 28 days: 1) running wheel access (3hrs/day), 2) ladder access with weighted resistance (15min/day), 3) metformin injections (i.p.), 4) no running wheel access, 5) ladder access without weighted resistance (15min/day), and 6) saline injections (i.p.). Memory was evaluated through a spontaneous location recognition (SLR) task. SLR tests spatial memory by assessing the extent to which mice can discriminate locations of objects manipulated in the similarity of distance between each other - dissimilar (d-SLR), similar (s-SLR), and extra similar (xs-SLR) object locations. Neurogenesis markers were analyzed, and body weight, abdominal fat, and liver weight were measured.

We found HFHS-induced impairment in pattern separation, particularly when demands on pattern separation were high (s-SLR). Both exercise and metformin rescued pattern separation. Only aerobic exercise enhanced spatial memory at xs-SLR. HFHS diet did not distinctly affect neurogenesis, but exercise increased neuroproliferation and immature neuron numbers in the dorsal dentate gyrus.

Overall, aerobic and resistance exercise as well as metformin preserve pattern separation and mitigate metabolic risk factors associated with diet-induced obesity.

P3-F-475 - Investigating hippocampal-prefrontal interactions that represent and modulate contextual navigation

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The Hippocampus (HPC) and the medial Prefrontal Cortex (mPFC) are key regions involved in planning and execution of tasks involving spatial memory. Activities of cells in CA1 region of the HPC are believed to create a representation of oneâ€™s surroundings, referred to as cognitive map. Anterior Cingulate Cortex (ACC) region of mPFC is believed to encode context, and may generalize information across contexts. Our aim is to delve into neural representations in the HPC and mPFC as the contexts, rules, and spatial locations dynamically change. We are interested in the space of neural representation of the task, rule, and context. We designed and prototyped a rodent maze apparatus. It is a 3x3 grid of interconnected octagonal compartments with walls that can go up and down to have dynamically changing configurations. Our task consists of two distinct sounds cues and two different visual cues projected on the walls at the choice points. All requisite information about the task is provided at the task's outset. At the onset of each trial, one sound cue plays and this determines which visual cue is associated with the correct path, which the rat follows to reach the reward. We will implant trained rats with dual Silicon probe assemblies targeting CA1/ACC and wirelessly record neural activities as they perform the task. We predict that ACC neurons will maintain a consistent rule representation enabling information to be generalized across contexts, and HPC place cells will encode a graph-like representation that enables rule generalization.

P3-F-476 - Mediterranean-based dietary patterns modulate non-social cognitive impairments induced by chronic social defeat in male mice

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Dietary patterns based on the Mediterranean (Med) diet have been shown to improve cognition, potentially through their bioactive-containing compounds exerting anti-inflammatory and neurotrophic actions. This study examined a Med-based diet as a potential preventative strategy to attenuate cognitive impairments in a mouse model of chronic social stress. Male C57BL/6N mice were fed a Control diet (slightly modified standard purified diet) or a calorie-matched Med-based diet (Control diet enriched with olive and fish oils, fruits, vegetables, legumes, and walnuts) for 1 week, after which they were assigned to a No Stressor or a Chronic Social Defeat Stressor (CSDS) condition for 10 consecutive days. Twenty-four hours after the last No Stressor or CSDS session, social behaviors and recognition memory were assessed in the social interaction, three-chamber, and novel object recognition tests. The increases in social avoidance as well as the reductions in sociability and in preference for social novelty elicited by the CSDS were not prevented by the Med-based dietary intervention. In contrast, the Med-based diet increased long-term recognition memory for objects in stressed mice only. These findings suggest that the cognitive improvements promoted by the Med-based diet in the context of chronic social stress may be specific to non-social aspects, at least when long-term memory is evaluated.

P3-F-477 - Neuromelanin alterations associated with problematic social media use

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Background: Neuromelanin-sensitive MRI (NM-MRI) has demonstrated its potential as a proxy measure of dopamine functioning in the substantia nigra (SN). Elevated neuromelanin has been associated with more severe substance use and sensitivity to social reward. Structural alterations in the reward system have been found with problematic social media use, however dopamine functioning has yet to be explored. The current study aims to determine whether higher problematic social media use is linked to altered neuromelanin signal intensity in the substantia nigra.

Methods: Twenty-six young adults (18-35 years of age) completed the Bergen Social Media Addiction Scale to measure problematic social media use and underwent a NM-MRI scan. The contrast-to-noise ratio was calculated for each participant and at each voxel within the substantia nigra. The average change in NM-MRI signal intensity was correlated with problematic social media use scores, with age included as a covariate.

Results: Higher levels of problematic social media use were not significantly associated with average neuromelanin signal intensity across the SN ($p = 0.30$). 113 voxels with higher NM-signal intensity were significantly correlated to increased problematic social media use.

Conclusions: These findings suggest a nuanced relationship between problematic social media use and neuromelanin in the substantia nigra. Further exploration is warranted to uncover the mechanisms underlying problematic social media use for a more comprehensive understanding.

P3-F-478 - Hippocampal assembly dynamics in REM sleep reveal by large-scale calcium imaging in freely behaving mice

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Sleep plays a critical role in memory consolidation and is broken down into REM (rapid eye movement) and slow-wave (SWS) phases. In SWS, neurons in the hippocampus representing awake experiences are reactivated as compressed sequences, and disruptioning these reactivations impairs memory recall. Disrupting the dominant theta rhythms in REM sleep impairs contextual memory recall, suggesting that coordinated network activity is essential for consolidation. However, it is unclear how awake representations may be reactivated in REM sleep. Here we combine electrophysiology with large-scale CA1 calcium imaging in freely behaving mice (n=5, 500-1000 neurons per mouse) to determine how experience shapes network reactivations in REM sleep. Mice were recorded across novel, familiar, and anxiogenic tracks and during two hours of sleep before and after the task. We identified patterns of co-active CA1 neurons on the track using PCA-ICA and applied these patterns to pre- or post-task REM data to compute an assembly expression score. We find task assemblies were active during pre- and post-task REM sleep, well exceeding the chance level. Roughly 25% of the awake assemblies displayed coherent population-level spatial tuning and these spatial assemblies were present even before novel track exposure. The nature of the experience however, did not appear to alter the number of reactivated assemblies, nor their reactivation rate or strength suggesting that REM assembly reactivations may represent pre-wired hippocampal ensembles as has been shown in SWS.

P3-F-479 - AMPAR activation and exchange in memory destabilization and update

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Consolidated memories can be changed through reactivation-induced memory destabilization and subsequent reconsolidation and previous research suggests that the transient exchange of Î±-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) in the lateral amygdala is necessary for destabilizing auditory fear memories in rats; however, the importance of activation and exchange of AMPARs for destabilization of other forms of long-term memory has not been elucidated. Our findings indicate that AMPAR activation and exchange from calcium-impermeable AMPARs (CI-AMPARs) to calcium-permeable AMPARs (CP-AMPARs) via endocytosis is necessary in perirhinal cortex (PRh) for destabilizing object memories in rats. Using the spontaneous object recognition paradigm, we administered bilateral PRh infusions of an AMPAR antagonist, CNQX (0.7Âµg/Âµl), immediately before memory reactivation (RA; 24h after initial object exposure); the RA phase was followed by PRh infusions of the reconsolidation blocker, anisomycin. We then ran this same experiment using the AMPAR exchange blocker, $GluA2_{3y}$ (100Âµg/Âµl). Results from these experiments indicate that the pre-RA blockade of AMPARs and AMPAR exchange prevents object memories from destabilizing, thereby rescuing them from the memory deficits associated with the post-RA infusion of anisomycin. We are currently conducting follow-up experiments that directly evaluates the effects of pre-RA infusions of the CNQX and $GluA2_{3Y}$ in a context-dependent memory updating task. As well, we are running western blot experiments to visualize and quantify the ratio of CI:CP-AMPARs immediately after a RA session. This

research will further establish the loosely understood role of AMPARs in memory destabilization and will expand our current understanding of the neurobiological bases that underlie the dynamic nature of long-term memory storage.

P3-G-480 - Automated analysis of mouse behavior without human labeling or keypoint tracking: leveraging end-to-end self-supervised machine learning

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Machine learning promises to revolutionize behavior testing by enabling automatic segmentation of behavioral motifs (i.e. grooming, rearing, freezing). Current techniques require fine-tuning of keypoint trackers or direct labeling of behaviors, both of which add labor costs and introduce inter-experimental variability. We present a method to classify animal behaviors without human labeling, end-to-end. First, we leveraged self-supervised contrastive-learning to train a convolutional image encoder, modified from Selfee (Jia et al. 2022). We trained this to embed frames from >16 hours (30fps) of a recorded mouse experiment. Since frame embeddings lack the temporal information needed to represent behaviors, the second step was to train a recurrent neural network-variational autoencoder (RNN-VAE) to encode sequences of frame embeddings onto a latent space of motion embeddings. Unsupervised techniques were then used to cluster the motion embeddings into discrete behavioral motifs. We took inspiration from VAME (Luxem et al. 2022), which embedded sequences of keypoint positions using an RNN-VAE; we found that using sequences of frame embeddings instead yielded better segmentation of behaviors. When analyzing videos of individual mice acclimating to a new environment, we successfully identified various behaviors i.e rearing, sniffing, and different grooming variations, as well as how their frequencies change with acclimation. In short, this framework requires no human labeling (keypoints or behaviors), produces effective results, and is adaptable to various experimental contexts

P3-G-481 - Non-viral transfection techniques for targeting radial astrocytes in vivo

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Non-viral transfection is used extensively *in vitro,* whereas in vivo viral methods are more common. As viral techniques are limited for insert size, large cell type-specific promoters are more amenable to non-viral methods. Also, viral methods may induce inflammatory or immune responses in the transfected animal. We thus investigated modern, high-efficiency non-viral transfection techniques for use *in vivo*.

We compared three non-viral transfection techniques: electroporation, lipofection, and magnetofection. To target gene expression to radial astrocytes in the Xenopus *laevis* optic tectum, we injected circular plasmid DNA, complexed to cationic lipids, cationic lipids and ferrous nanoparticles or simply mixed with external recording solution reagents into the brain ventricle of GCaMP6s-expressing tadpoles. For each technique, a genetically encoded fluorescent protein was transfected to measure transfection efficiency. The number of transfected cells, the distributions, and types of transfected cells were quantified to assess the efficacy of the techniques. We also assessed the health of the animals and of transfected cells and nearby tissue to identify any undesirable side-effects.

Whereas electroporation transfects many cells with a high specificity for radial astrocytes, the current also leads to extensive neuronal cell death. Lipofection was less efficient, but did not cause neuronal death, instead slightly elevating intracellular calcium levels, perhaps resulting from an inflammation reaction. In contrast, we found that was ineffective at sublethal dosses in our model.

P3-G-482 - An activity-dependent enzyme for rapid and stable tagging of neural ensembles in vivo

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Recent technological advancements have revolutionized our ability to study the brain at the cellular and molecular levels. However, the ability to rapidly and stably tag functionally co-activated neurons remains a challenge. Here, we engineered a Calcium-dependent split-TurboID (CaST) enzyme for rapid and non-invasive neural activity mapping based on a biotin ligase split-TurboID. We fused each half of the split-TurboID to Calmodulin or MKII. Upon neuronal activation and calcium influx, split-TurboID will reconstitute and label proximal proteins in the presence of biotin. Biotinylated proteins can be visualized using streptavidin-AF647 (SA-647; which binds biotin) or pulled down using streptavidin beads. We characterized the specificity and temporal properties of CaST in vitro. We then performed an in vivo demonstration of CaST by expressing it in the medial prefrontal cortex (mPFC) of mice to tag psilocybin-activated neurons. We observed robust SA-647 labeling only when both biotin and psilocybin were injected, as opposed to controls without psilocybin. Quantitative analysis demonstrated an increasing ratio of SA-647/GFP fluorescence in mice treated with psilocybin (2.1Â±0.10) compared to controls (1.4Â±0.06; *n*=8 slices from 3 mice, each group). Additionally, psilocybin-treated mice had a higher

fraction of neurons with a SA-647 intensity over the threshold than control (0.72Â±0.086 vs. 0.12Â±0.026). These findings describe CaST as a novel technology for rapid and non-invasive detection of acutely activated neurons both in vitro and in vivo across different models and species.

P3-G-483 - Experience specific tuning of postnatally born hippocampal neurons

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Neurogenesis in the dentate gyrus continuously generates new granule cells (GCs) that gradually integrate into the hippocampal network and are required for functions such as learning, memory, and pattern separation. In mice, GC generation peaks at birth and declines with age. Currently, we do not fully understand how GCs that are born right after birth develop functionally. While it's known that experiences influence adult-born neurons' development, it's unclear if this applies to developmentally born neurons, even though they make up a large portion of the population.

It is proposed that these developmentally born GCs , like adult-born GCs, may undergo a period of high experience-dependent plasticity during their development that enables them to become attuned to features present in their environment, enabling enhanced learning of situations containing those features later in adulthood. We aim to test this proposal by exposing adolescent mice to textured visual stimuli and assessing their ability to discriminate these textures from other stimuli in adulthood. Our method includes a custom-built exposure setup and a touchscreen testing chamber. By using genetically modified mice, we will be able to silence newborn neurons at distinct stages of their development to examine its impact on learning. Furthermore, considering the role of the hippocampus in the development of Alzheimerâ€™s disease and depression, our work will establish a framework for how early-life experiences shape circuits in mental health disorders.

P3-G-484 - Plasma proteomic profiles of youth athletes at pre- and post- concussion

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Background: Sport-related concussions (SRCs) are a growing concern, with over 300,000 cases reported annually in Canada. The complexity of SRC symptoms reduces the reliability of current diagnostic methods that rely on subjective clinical measures. Recent research has shown the value of blood biomarkers in SRC diagnosis, offering an objective and minimally-invasive method to assess changes in the brain. While group-level differences have been found in some blood biomarkers following SRC, large overlaps of data have marginal clinical significance at the individual level. This study aims to use a novel proteomic approach to discover protein biomarkers with enhanced sensitivity and specificity for SRC diagnosis in youth athletes

Methods: Plasma was collected from a sub-cohort of participants enrolled in SHRed Concussions, a national prospective cohort study using validated concussion surveillance across high risk youth SRC sports. Paired pre and post-SRC samples will be analyzed by Alamarâ€™s NULISA platform, a proximity-extension platform capable of detecting over 120 central nervous system biomarkers with attomolar sensitivity.

Results:2144 specimens have been collected (45% female), with 144 paired pre and post specimen. We hypothesize youth athletesâ€™ proteomic profiles will help identify novel biomarkers that will have increased diagnostic utility for youth SRC.

Conclusion:Uncovering biomarkers that differ at baseline and post-SRC may serve as an objective measure for SRC diagnosis in athletes. These findings will aid SRC diagnosis enabling more precise and timely interventions.

P3-G-485 - More is better: a simple antibody-based strategy that enables recovery of all major mouse brain cell types from multiplexed single cell RNAseq samples

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Single cell RNA sequencing (scRNA-seq) is a powerful technique for studying cellular diversity within the complexity of the brain. Nonetheless, this technique can be daunting due to its high cost and complexity of execution. To reduce the costs of RNA sequencing, here we sought to establish an effective multiplexing strategy for the brain that could allow multiple experimental groups to be pooled into a single sample for scRNAseq, thus reducing sequencing costs. We first describe an optimized cold temperature single-cell dissociation protocol that permits isolation of a high yield and viability of brain cells from the adult mouse with minimal ex vivo activation gene signature. Using flow cytometry, we then screened a panel of antibodies to identify a single antibody that is capable of tagging the vast majority of isolated mouse brain cells. Finally, utilizing this primary antibody against a â€œuniversalâ€ neural target together with sample-

specific oligonucleotide-labeled secondary antibodies and the BD Rhapsody single cell system, we pooled 4 adult mouse brain samples into a single multiplexed run for scRNAseq. Bioinformatic analyses enabled efficient demultiplexing of the pooled brain samples, with high tagging efficiency and precise annotation and clustering of brain cell populations, including astrocytes, oligodendrocytes, microglia, etc. The integration of this cell dissociation protocol with the efficiency and flexibility of the two-step multiplexing strategy simplifies experimental design with elimination of sequencing batch effects and reduced sequencing costs.

P3-G-486 - Multiplex, single-cell CRISPRa screening for cell type specific regulatory elements

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CRISPR-based gene activation (CRISPRa) is a promising therapeutic approach for gene therapy, upregulating gene expression by targeting promoters or enhancers in a tissue/cell-type specific manner. Here, we describe an experimental framework that combines highly multiplexed perturbations with single-cell RNA sequencing (sc-RNA-seq) to identify cell-type-specific, CRISPRa-responsive *cis*-regulatory elements (CREs) and the gene(s) they regulate. Random combinations of many gRNAs are introduced to each of many cells, which are then profiled and partitioned into test and control groups to test for effect(s) of CRISPRa perturbations of both enhancers and promoters on the expression of neighbouring genes. We first applied this method to a library of gRNAs targeting candidate CREs in both K562 cells and iPSC-derived neurons, and identified gRNAs capable of specifically upregulating intended target genes. Next, we massively scaled our screening efforts to 3,183 Massively Parallel Reporter Assay (MPRA)-prioritized candidate cis-regulatory elements (2,422 enhancers, 761 promoters) surrounding 337 high-confidence haploinsufficient ASD and neurodevelopmental disorder risk genes in iPSC-derived neurons (n=200,517 cells with 10 gRNAs/cell). We identified hundreds of gRNAs/CREs capable of yielding target gene-specific upregulation. This work establishes a scalable single cell framework to screen promoters and enhancers, identifies functional gRNAs targeting ASD & NDD risk gene CREs, and contributes to our understanding of the circuitry of neuronal gene regulation.

P3-G-487 - Clinical Interpretation of VoUS in epigenetic syndromes using Drosophila

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Clinical and population exome sequencing are routine, driving the need for well-calibrated functional assays of variant function. These assays play crucial roles, from establishing causal genes in novel syndromes to screening rare population variants for potential risk factors in common diseases. We've developed a cost-effective, scalable screening platform using *Drosophila* for variant functional assessment with clinical predictive value. The RBBP4 gene codes for a histone-binding protein of the PRC2, NuRD and CAF-1 complexes. Recently, a cohort of 16 patients carrying 14 different mono-allelic RBBP4 variants were collected, all of which have developmental delay, intellectual disability and speech delay. Yet, the re-classification of RBBP4 as a novel syndromic gene remains unresolved. We will present an assay where human RBBP4 expression in *Drosophila* can rescue severe loss of function phenotypes arising from loss of its ortholog *Caf1-55*. This assay is being used to test the function of these variants to help establish causality in this novel syndrome. Rare coding variants in PRC2 cause syndromic disorders including Weaver syndrome (EZH2), Cohen-Gibson syndrome (EED), Imagawa Matsumoto syndrome (SUZ12). Yet, establishing genotype-phenotype relationships is challenging and the functional deficit in most variants is unresolved. We will present our work showing the *Drosophila* assays capable of reclassifying VoUS in EED and SUZ12. We use CRIMIC technologies to efficiently engineer the Drosophila ortholog genes *esc* and *Su(z)12* to mimic the human variants. These mimetic variants are then assayed for classical homeotic phenotypes arising from alleles in these genes. To create assays with clinical predictive value, we report that our fly mimetic approach accurately assesses the loss of function of pathogenic variants and â€˜normalâ€™ function of benign variants.

P3-G-488 - Self-supervised deep learning approach for semantic segmentation of a single neuron in 3D

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Semantic segmenting of single neuron two-photon microscopy volumes into its distinct structural components (soma, axon, dendritic branches, and filopodia), is required for single cell growth analytics as well as rapid random access two-photon microscopy acquisition plans. A common approach to a semantic segmentation task is to implement a deep convolutional neural network, which have been proven to be very successful. The problem with here is the requirement for extensive expertly curated labelled ground truth data for training. However, recent advances in the new field of self supervised learning strive to subvert the need for this lengthy and costly process of acquiring ground truth data. With this approach the amount of data that is already in existence can be used immediately without the requirement for lengthy ground truth labelling. In this study a supervised deep convolutional neural network architecture has been modified to become self supervised, allowing it to train on a much larger training set. The self supervised model outperforms the supervised model in a neural semantic

segmentation task, and overall requires less training time because there is no need for labelled ground truth data.

P3-G-489 - Graph neural networks for neuronal populations: modelling dynamics, structure and time-invariant representation of neurons

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Recent advancements in brain recording techniques have led to a significant increase in the availability of large-scale neural datasets in which hundreds of neurons are recorded simultaneously. This necessitates the development of new methods that can model neural population dynamics and extract interpretable and low-dimensional representations. Here, we introduce a novel graph neural network (GNN) architecture designed to model neuronal populations. In this model, a population of neurons is seen as a graph where nodes represent neurons and edges represent the functional connectivity. Within this biologically realistic framework, each neuron, characterized by a learnable embedding, receives information from connected neurons, updates its state, generates a message, and communicates it to the adjacent neurons. A shared decoder at each neuron uses the current state and the time-invariant embedding of that neuron to predict its activity in the next time step. We evaluated the model's performance on calcium imaging data (Bugeon et al., 2022), achieving an average prediction accuracy (measured by Pearsonâ€™s correlation) of 0.31 across all neurons. Moreover, after training the model on the self-supervised task of time-series forecasting, we demonstrate that the learned neuron embeddings can classify excitatory vs. inhibitory cells, achieving a classification performance of 65%, significantly surpassing the chance level. These findings underscore the effectiveness of our proposed model in capturing both the dynamics and time-invariant characterization of neuronal populations.

P3-G-490 - MesoPi: Open-source camera system for capture of mouse neural activity during a skilled water reaching task

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Open-source cameras are increasingly popular in biological imaging due to their affordability and adaptability. However, the onboard image signal processor often modify images generated, which may

distort data through non-linear transformations, color distortions, and pixel value degradation. These distortions are particularly problematic in custom setups involving various lenses, filters, and lighting conditions. Here, we present an innovative and cost-effective system designed to bypass these limitations by extracting raw, unprocessed RGB (Bayer Pattern) or monochrome data, making it highly suitable for detailed biological analyses. To demonstrate the effectiveness of our system, we were capable of recording widefield cortical activity of mice with green fluoresce tetO-GCaMP6s x CAMK tTA or Thy1-GCaMP6s and red fluorescent Thy1-jRGECO during a skilled water reaching task. Moreover, the system can be used in laser speckle contrast imaging to quantify lesion sizes and blood flow, effectively. Lastly, the system was also capable of capturing single unit Purkinje cell dendrite activity in mice executing skilled water-reaching tasks. Our system offers a cost-effective (<$200 per unit), versatile, and robust solution for quantifying widefield and single-cellular image signals in biological research. It stands out in its ability to provide high-quality, unprocessed data, essential for accurate biological analysis.

P3-G-491 - Investigating the impact of human herpesvirus 6 on neurons and glia derived from human pluripotent stem cells

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Viral infections of the human central nervous system have historically been difficult to study due to limited access to human tissue. However, pluripotent stem cells now offer an abundant source of human neurons and glia for these investigations. Human herpesviruses have long been implicated in neuropathology, but the effects on the CNS of Human Herpesvirus 6 remain elusive. HHV6 is the causative agent of childhood Roseola, which can lead to febrile seizures and even encephalitis. HHV6 is also speculated to contribute to the pathogenesis of neurological disorders including multiple sclerosis and Alzheimerâ€™s disease. Thus, to understand how HHV6 infection impacts the brain, we generated human iPSC-derived neurons and glia, including the resident immune cells of the brain, microglia, using inducible CRISPR-engineered iPSC lines and established differentiation methods. We then infected these cell types to establish the selective tropism of the virus and measure transcriptional response. Further, we generated 3D co-cultures containing astrocytes, oligodendrocytes, neurons, and microglia and infected these organoids with HHV6. We then performed single cell RNA-sequencing on these samples to capture cellular crosstalk that may drive inflammation and mediate tissue response to infection. We have concluded that microglia are uniquely permissive to infection by HHV6, and rapidly die once exposed. Conversely, neurons are resilient to similar viral loads. We therefore propose that indirect neurotoxicity from reactive glia underlies the mechanisms of neurological injury by HHV6.

P3-G-492 - Effects of tACS on electrophysiological signals are task-dependent

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Transcranial alternating current stimulation (tACS) is a non-invasive technique that delivers low-intensity alternating currents intending to affect neural activity and behavior. Recent research has shown that the effects of tACS are often inconsistent and not replicable. In this study, we investigated the effects of 10Hz alpha (vs 41Hz gamma) stimulation on alpha oscillations during a vigilance-oddball paradigm.

Participants (n=38) underwent occipital alpha (10Hz) and gamma (41Hz) stimulation, on separate days, where they performed three blocks of a vigilance-oddball task: pre-stimulation, alpha or gamma stimulation (STIM), and post-stimulation. During the task, they responded to one of 2 color changes to the fixation cross (DEFAULT for 80% and ODDBALL for 20% of the trials). Half the participants used their left index finger for the DEFAULT color change and the other half their right. Simultaneous EEG is recorded from 256 electrodes.

We observed that enhancement in alpha power (Î” = POST - PRE) was greater for alpha stimulation than gamma stimulation but only for the contralateral electrodes ($t(37)=-2.55$, $p=0.015$) to the dominant response hand and not for the ipsilateral electrodes ($t(37)=1.45$, $p=0.156$).

Our findings reveal a lateralized effect of tACS though our tACS electrode montage wasn't lateralized. This implies that the effect is likely driven by the motor planning aspects involved during the task paradigm. Thus suggesting that the effects of tACS on electrophysiological signals depend on the nature of the task being performed.

P3-G-493 - Generating synthetic interview transcripts in a psychiatric setting using a double-language model approach

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Training data is a key part of producing and improving large language models (LLMs). Narrative interviews form the basis of mental health treatment plans, and comprehensive history-taking is an ideal application for LLMs. However, the use of history-taking training data is often restricted by privacy and legal considerations.

Our tool uses two LLMs to create a synthetic interview transcript in a clinical intake setting (the double-model approach). This is to counteract the effects of generating an interview from one model (the single-model approach), where biases and context size limitations narrow the diversity of patient characteristics and assistant responses.

We implement the double-model tool in Python, creating two instances of ChatGPT 3.5 with the OpenAI API. The user can customize a question bank and create patients with randomized attributes. Additionally, we embed edge case situationsâ€“for example, if the patient neglects to provide their medication dosage, the assistant should ask an appropriate follow up question. This diversity of patient personas and edge case embedding creates more robust and efficient model behavior.

We evaluate the quality of the synthetic interviews using metrics of interview completeness, accuracy, and safety using established LLM benchmarks. We also compare the performance of a model trained on our interview dataset against an untrained model to evaluate how consistently it performs in responding to edge cases. Our double-model approach can be applied for generating additional conversational datasets and investigating LLM behavior.

P3-H-494 - Coping with Costs: Analyzing GoFundMe Financial Aid Requests from Brain Tumor Patients in Ontario

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Background

Brain tumors present a global health challenge, impacting individuals, families, and societies globally. Patients often suffer the financial responsibility for treatments like oral chemotherapy due to inadequate coverage by healthcare systems, leading to complex insurance navigation or out of pocket payments. This study aims to analyze GoFundMe data, investigating the additional direct and indirect financial costs associated with brain tumor diagnoses.

Methods

Utilizing a qualitative descriptive design drawing on thematic analysis, GoFundMe data from individuals diagnosed with brain cancer between 2014-2021were examined. Ontario, Canada, with its diverse population, posed unique financial burdens for patients. The focus on GoFundMe campaigns in Ontario, generated a final dataset of 154 requests subjected to analysis, selected through a screening process from an initial pool of 9025.

Results

Results revealed additional direct and indirect financial strain from the loss of income experienced by patients and their caregivers. Requests highlighted 1) lack of awareness on available financial, psychosocial, and medical support; 2) not knowing who to contact for assistance in finding support services; 3) concerns over the long-term financial well-being of bereaving family; 4) more public awareness on the financial burden and emotional distress experienced by of those impacted and 5) funding to support life changes.

Conclusions

These GoFundMe requests highlight a connection between financial burden, emotional distress, and an overall lack of awareness of where/how to seek financial and emotional support. These results will serve as the foundation to advocate and raise awareness of the assistance needed by brain tumor patients and their families.

P3-H-495 - seed2STEM: An innovative program for increasing indigenous youth engagement in neuroscience

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Despite growing awareness of the need for diversity in science-technology-engineering-math (STEM), Indigenous youth remain underrepresented in neuroscience. To address this issue, the seed2STEM program at the University of British Columbia (UBC) (www.icord.org/issp/) provides Indigenous high school students with paid neuroscience research experience. Students in grades 9-12 engage in 6-week internships, combining lab research, interactive learning, field trips, and guest lectures. Supervisors receive cultural sensitivity training to foster an inclusive environment. The program culminates in a student research symposium and program alumni can return as mentors, promoting a sustainable community of Indigenous scholars in neuroscience. seed2STEMâ€™s success is evaluated through formal evaluation in consultation with an external advisory panel which includes 5 members of local Indigenous communities as well as ongoing student and supervisor feedback. Since 2018, seed2STEM has successfully engaged 33 Indigenous students in neuroscience research, with >90% expressing

increased interest in STEM careers. The initiative has also increased awareness among non-Indigenous students, staff, and faculty about Indigenous perspectives in science. seed2STEM demonstrates a successful engagement model for Indigenous youth in neuroscience, highlighting the importance of hands-on experience, mentorship, and cultural support. This novel approach offers a framework for similar programs, aiming to bridge the diversity gap in neuroscience and foster a more inclusive scientific community.

P3-H-496 - Canvas of care: An exploration of the availability of art therapy for patients living with Parkinson's disease in northern, rural and remote British Columbia

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Parkinsonâ€™s disease (PD) is a hypo-kinetic neurological disorder characterized by the degeneration of dopaminergic neurons that is increasing in prevalence throughout the world (Pringsheim et al., 2014). The incorporation of art therapy into the treatment approach has been studied for its utility in improving both the motor and non-motor symptoms of PD (Ba & Pfeiffer, 2021). This project utilized a mixed-methods approach to evaluate and improve the quantitative accessibility of art therapy programs in Northern, rural and remote communities. A compilation was made from publicly available sources to determine the scope of current art-based programs. These resources were then mapped using the provincial health authority designations to highlight trends in geographical accessibility. The quantification of available PD-specific art therapy resources in British Columbia (B.C.) found a higher density of programs available within the Fraser, Vancouver Coastal and Vancouver Island regions. Conversely, the Northern and Interior health regions showed a distinct scarcity of services despite these regions having a similar population percentage of PD prevalence as more densely serviced areas. Given the increasing prevalence of PD and the marked benefit that can be seen with access to art and movement therapy, we hope that the resource inventory compiled by our team will encourage funding and provider support for the expansion of PD-specific art therapy programs in Northern and rural communities in B.C.

P3-H-497 - Neuroscience research in the social media sphere: A current ethical review and future prospectus evaluating the societal impacts of using social media to recruit and track subjects for neuroscientific studies

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Are neuroscience researchers using social media to recruit/track subjects ethical and altruistic, or violating privacy and betraying the Belmont Report?

Social media has clear research recruitment pros: it promotes new neuroscience therapies; destigmatizes disease; reaches many; generates statistical significance due to high enrollment; links to online informed consent; targets wanted subjects; and tracks them through longitudinal design.

Cons are confidentiality concerns and vague rules lacking governmental guidelines for *all* biomedical research using social media. Vulnerable cohorts mustnâ€™t feel coerced by personalized research ads or feel their safe online space invaded. Subjects must be wary of social mediaâ€™s open interface, not post misinformation or too much to unblind studies. They must know liking a post is public. Research is not synonymous with treatment; social media recruitment ads mustnâ€™t enforce therapeutic misconception.

This review evaluates social mediaâ€™s societal benefits/harms in neuroscientific studies then presents solutions. Social media recruitment requires universal congruency, or institutions will have unequal protocols with confounding variables and incomparable data amongst studies. Who will implement rulesâ€“governments, IRBs, or researchers? Why are rules currently only in select universities? This review propels framework for needed future policy debate. Interdisciplinary engagement between researchers, MDs, ethicists, governments, the NIH, and social media hosting sites is vital to safeguard neuroscientific research recruitment using social media.

P3-A-498 - Regulation of Drosophila neuronal lipid droplets by lipid droplet- and triglyceride-associated genes

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Triglyceride is the main form of stored fat in eukaryotes and is stored within a specialized organelle called a lipid droplet. While many studies have identified genes that regulate triglyceride and lipid droplets in tissues such as the adipose tissue and liver, less is known about triglyceride and lipid droplets in neurons. To address this knowledge gap, we expressed a lipid droplet-targeted GFP (GFP-LD) specifically in neurons to visualize *Drosophila* neuronal lipid droplets. By co-expressing RNAi transgenes targeting different triglyceride- and lipid droplet-associated genes with GFP-LD in neurons, we were able to assess how RNAi-mediated knockdown of these genes influenced neuronal lipid droplet number. We targeted six triglyceride- and lipid droplet-associated genes. These genes are involved in processes such

as triglyceride synthesis (*CG34348*), lipid droplet formation (*seipin*), lipid droplet transport (*klarsicht*), and neuronal *de novo* fatty acid synthesis (*lactate dehydrogenase, Basigin*, *Sterol regulatory element binding protein*) genes. We found that neuronal loss of multiple genes led to significant changes in the number of neuronal lipid droplets. Together, our data identify a new role for multiple triglyceride- and lipid droplet-associated genes in regulating neuronal lipid droplets under normal physiological conditions.