CAN-ACN

CANADIAN ASSOCIATION FOR NEUROSCIENCE
ASSOCIATION CANADIENNE DES NEUROSCIENCES

The Royal Ontario Museum (ROM)

Toronto, ON

May 21 – 24, 2025 Toronto
Sheraton Centre Hotel

TUESDAY, MAY 20, 2025

6:30 – 8:45 pm

CAN 2025 PUBLIC LECTURES

Neuroscience of memory and tools for making our memories stronger

Speakers: Sheena Josselyn, Hospital for Sick Children
Morgan Barense, Rotman Research Institute at Baycrest Hospital
Hosted by Iva Zovkic, University of Toronto Mississauga

DAY WEDNESDAY, MAY 21, 2025

9:00 am – 4:00 pm
Various locations

CAN SATELLITE SYMPOSIA

5:00 – 5:15 pm
Grand Ballroom

WELCOME AND OPENING REMARKS

Melanie Woodin, President of the Canadian Association for Neuroscience

5:15 – 6:15 pm
Grand Ballroom

PRESIDENTIAL LECTURE

Gina Turrigiano, Brandeis University

Keeping your brain in balance: homeostatic tuning of network function

6:15 – 8:00 pm
Sheraton Hall

NEURON10
Krembil Relentless. SickKids

OPENING RECEPTION

Check the back of your badge for your complimentary drink ticket.

DAY THURSDAY, MAY 22, 2025

8:30 – 10:15 am
Grand Ballroom

Fondation
Brain Canada
Foundation

PLENARY SYMPOSIUM 1

Fear, threat and aversive learning

Chair: Mihaela Iordanova, Concordia University Speakers:
Maithe Arruda-Carvalho, University of Toronto, Scarborough
Josh Johansen, RIKEN Institute, Japan Susan Sangha,
Indiana University, USA

10:15 – 10:25 am
Grand Ballroom

UPDATE ON BRAIN CANADA INITIATIVES
Viviane Poupon, President and CEO, Brain Canada

10:25 – 10:45 am
Sheraton Hall

COFFEE BREAK

10:45 – 11:00 am
Grand Ballroom

BRAIN STAR AWARD WINNER TALK
Justine Hansen, McGill University

CIHR | Institute of Neurosciences,
IRSC | Mental Health and Addiction
Institut des neurosciences, de la
santé mentale et des toxicomanies

11:00 am – 12:00
Grand Ballroom

FEATURED PLENARY SPEAKER 1
Kerry Ressler, McLean Hospital Harvard University, USA
Translating the neuroscience of fear to understanding PTSD

12:00 – 1:30 pm
City Hall

SCIENCE ADVOCACY SESSION

Navigating regulatory frameworks for research models in Canada
(pre-registration required)

1:30 – 3:00 pm
Grand West

SYMPOSIUM 1

Gut Microbiome-brain interaction on host health

Symposium Chair: Frank Duca, University of Arizona

Speakers:
Lesley MacNeil, McMaster University
Role of the gut microbiome in neural aging
Frank Duca, University of Arizona
Bacterially-derived metabolites impact hypothalamic regulation of metabolic homeostasis via Aryl Hydrocarbon Receptor
Tony Lam, Toronto General Hospital Research Institute
Small intestinal microbiota impacts nutrient-induced gut-brain signaling pathways that regulate glucose homeostasis
Premysl Bercik, McMaster University
The Brain-gut-microbiome axis and irritable bowel syndrome

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1:30 – 3:00 pm
Grand Centre

SYMPOSIUM 2

New insights of noradrenaline in neural diseases: From neurodevelopment to neurodegeneration

Symposium co-chair: Xuming Yin, University of Ottawa &
Qi Yuan, Memorial University of Newfoundland

Speakers:
Xuming Yin, University of Ottawa
Abnormal Spatiotemporal dynamics of noradrenaline release during motor learning in 16p11.2 deletion mice of autism
Oxana Eschenko, Max Planck Institute for Biological Cybernetics
Noradrenergic transmission promotes flexibility of spatial behavior and sleep-dependent memory consolidation
Bruno Giros, McGill University
Noradrenergic control of the resilient shift following chronic stress
Qi Yuan, Memorial University of Newfoundland
Locus coeruleus neuronal vulnerability in a pretangle tau rat model

1:30 – 3:00 pm
Grand East

SYMPOSIUM 3
A genomics approach towards understanding sex differences in cognition, mental health, and neurodevelopment

Symposium chair: Giannina Descalzi, University of Guelph

Speakers: Iva Zovkic, University of Toronto Mississauga
Sex specific effects of histone H2A.Z on normal and pathological memory
Marija Kundakovic, Fordham University
Single-cell insights into gene regulation in the mouse brain across the estrous cycle and sex
Silvia De Rubeis, Icahn School of Medicine at Mount Sinai
Sex differences in the developmental functions of the autism risk gene DDX3X
Deena Walker, Oregon Health & Science University
Sex-specific transcriptional mechanisms of substance use disorder

1:30 – 3:00 pm Osgoode East	**SYMPOSIUM 4** Mechanisms of stress regulation in complex behaviors Symposium co-chair: Jaideep Bains, University of Calgary Speakers: Nuria Daviu, University of Guelph Survival optimization: Role of PVN-CRH neurons in innate escape execution Mijail Rojas-Carvajal, University of Calgary Exercise erases the behavioral and synaptic consequences of stress Thomas Kash, University of North Carolina at Chapel Hill Probing the role of lateral septum peptide signaling in aversion and reward Jamie Maguire, Tufts University Biased information routing through the BLA mediates behavioral deficits following chronic stress
3:15 – 3:45 pm Grand West	**TRAINEE POWER PITCH SESSION**
3:45 – 5:15 pm Sheraton Hall	**POSTER SESSION 1 & EXHIBITS** (coffee served from 3:45 PM – 4:15 PM)
5:15 – 5:45 pm Grand Ballroom XENON	**NEW INVESTIGATOR AWARD LECTURE** Mark Cembrowski, University of British Columbia Cell types for reverse engineering learning and memory in the brain
5:45 – 6:45 pm Grand Ballroom THE BRAIN PRIZE Lundbeck Foundation	**BRAIN PRIZE LECTURE** Larry Abbott, Columbia University Modeling the navigational circuitry of the fly
7:30 – 9:30 pm	**CAN STUDENT SOCIAL** Ballroom Bowl: 145 John St, Toronto, ON, M5V 2E4

8:30 – 10:15 am
Grand Ballroom

PLENARY SYMPOSIUM 2

Visualizing the brain
Chair: Jibran Khokhar, Western University Speakers: Istvan Katona, Indiana University Bloomington, USA Isabelle Boileau, CAMH, University of Toronto Michele Desjardins, Université Laval

10:15 – 10:25 am
Grand Ballroom

INMHA address
Sam Weiss, Scientific Director of INMHA Nina Cluny, Associate Director of INMHA

10:25 – 10:45 am
Sheraton Hall

COFFEE BREAK

10:45 – 11:00 am
Grand Ballroom

BRAIN STAR AWARD WINNER TALK
Chien Chou, McGill University

CIHR IRSC — Institute of Neurosciences, Mental Health and Addiction
Institut des neurosciences, de la santé mentale et des toxicomanies

11:00 am – 12:00 pm
Grand Ballroom

FEATURED PLENARY SPEAKER 2

Yulong Li, Peking University, China
Spying on neuromodulator dynamics in vivo by constructing multi-color genetically-encoded sensors

12:00 – 1:00 pm
Grand West

CIHR CANADIAN NATIONAL BRAIN BEE SHOWDOWN

1:15 – 2:45 pm
Grand West

SYMPOSIUM 5:

Diversity and flexibility in motor cortical control

Symposium chair: Jonathan Michaels, York University

Speakers:
Jonathan A. Michaels, York University
Sensory expectations shape neural population dynamics during reaching
Shreya Saxena, Yale University
Constrained models of neural dynamics induce generalizability and interpretability

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Matthew G. Perich, Université de Montréal

Motor cortical dynamics evoked by closed-loop modulation of spinal sensory pathways

Emily Oby, Queen's University

Dynamical constraints on neural population activity

1:15 – 2:45 pm
Grand Centre

SYMPOSIUM 6

Opioid drugs and the brain: Research insights to understand the opioid epidemic

Symposium chair: Gaspard Montandon, University of Toronto

Speakers:
Bernard Le Foll, CAMH

Regulation of fatty acid amide hydrolase (FAAH) in the brain of subjects using opioid drugs
Tuan Trang, University of Calgary

A brain to spinal cord neurocircuit in opioid withdrawal
Anna Taylor, University of Alberta

Protracted opioid withdrawal and the gut microbiome
Gaspard Montandon, University of Toronto

Neural mechanisms mediating opioid-induced respiratory depression

1:15 – 2:45 pm
Grand East

SYMPOSIUM 7

Connectomics across scales: from Synapses to systems

Symposium chair: Per Jesper Sjostrom, McGill University

Speakers: Mei Zhen, Lunenfeld-Tanenbaum Research Institute; University of Toronto

Form of developmental plasticity: insights and reflection from the C. elegans connectomics study
Paul De Koninck, Université Laval

Brain-wide functional and structural circuit development in larval zebrafish

Jesper Sjöström, McGill University
Principles of mouse visual cortex excitatory microcircuit organization
Kathryn Manning, University of Calgary
The impact of prenatal maternal distress upon the developing human connectome

1:15 – 2:45 pm Osgoode East	**SYMPOSIUM 8** Circuit, synaptic and neuromodulatory mechanisms underlying basal ganglia function Symposium chair: Corey Baimel, Dalhousie University Speakers: Talia Lerner, Northwestern University Feinberg School of Medicine Plasticity of striatal dopamine circuits and neuromodulatory mechanisms during motor skill learning Nicolas Tritsch, Douglas Research Institute, McGill University Revealing dopamine's contributions to motor vigor Meaghan Creed, Washington University School of Medicine Synaptic mechanisms of reward seeking in the ventral pallidum Corey Baimel, Dalhousie University Subregion specific processing of reward cues in the nucleus accumbens
3:00 – 3:30 pm Grand West	**TRAINEE POWER PITCH SESSION**
3:30 – 5:00 pm Sheraton Hall	**POSTER SESSION 2 & EXHIBITS** (coffee served from 3:30 PM – 4:00 PM)
5:00 – 6:00 pm Grand Ballroom	**KEYNOTE LECTURE** Paul Frankland, University of Toronto Developmental critical periods for episodic memory

6:00 – 7:00 pm
Grand Ballroom

Economic barriers to trainee achievement, retention and funding opportunities

Event organized by the CAN Equity, Diversity and Inclusion committee
Moderators: Dr. Paul Sheppard, postdoctoral fellow, Western and
Olivia Reshmi Ghosh-Swaby, PhD student, Western).

Presentation by Support of Science Representative
Panelists: Dr. Sam Weiss, Professor at University of Calgary, Scientific Director of the CIHR Institute of Neurosciences, Mental Health and Addiction
Dr. Sarah MacFarlane, Professor at University of Calgary, Director of the REALISE Career Development Program
Dr. Maithe Arruda-Carvalho, Associate Professor at University of Toronto, mid-career researcher
Dr. Annemarie Dedek, Assistant Professor at University of Waterloo, early-career researcher
Dr. Haley Vecchiarelli, Postdoctoral fellow, Trembley lab, University of Victoria
Adiia Stone, CGSD-holding PhD student, Murray lab, University of Guelph

DAY   SATURDAY, MAY 24, 2025

8:30 – 10:15 am
Grand Ballroom

PLENARY SYMPOSIUM 3

External influences on neurodevelopment

Chair: Deborah Kurrasch, University of Calgary
Speakers: Jessica Rosin, University of British Columbia Catherine Lebel, University of Calgary Armen Saghatelyan, University of Ottawa

10:15 – 10:25 am
Grand Ballroom

PARTNER UPDATE

10:25 – 10:45 am
Grand Ballroom

COFFEE BREAK

10:45 – 11:00 am Grand Ballroom	**BRAIN STAR AWARD WINNER TALK** Sergio Crespo-Garcia, University of Montreal

CIHR IRSC | Institute of Neurosciences, Mental Health and Addiction
Institut des neurosciences, de la santé mentale et des toxicomanies

11:00 am – 12:00 pm Grand Ballroom	**FEATURED PLENARY SPEAKER 3** Yasmin Hurd, MSSM, USA Cannabis — The Good, The Bad and the Ugly
12:00 – 1:30 pm Grand Ballroom	**CAN-ACN ANNUAL GENERAL MEETING (AGM)** Career Networking event / Lunch on own
1:30 – 2:00 pm Grand Ballroom	**TRAINEE POWER PITCH SESSION**
2:00 – 3:30 pm Sheraton Hall	**POSTER SESSION 3 & EXHIBITS** (coffee served from 2:00 PM – 2:30 PM)
3:30 – 5:00 pm Grand West	**SYMPOSIUM 9** Sleep, stress and sex differences in development and aging: Translational impact in health and disease Symposium chair: Haung (Ho) Yu, University of Toronto Speakers: W. Haung (Ho) Yu, University of Toronto Sex differences in proteostasis and pathology in response to chronic stress and sleep impairments in AD pathology Ciarán Murphy-Royal, Université de Montréal Astrocyte glucocorticoid receptors mediate sex-specific changes in activity following stress: Interactions with sleep-regulating orexinergic neurons Ksenia Kastanenka, Harvard Medical School Neural network dynamics and microglial activation during sleep Mayuko Arai, Simon Fraser University The impact of trazodone administration on sleep in the APPNL-F mouse model of Alzheimer's disease

3:30 – 5:00 pm
Grand Centre

SYMPOSIUM 10

Therapeutic applications for focused ultrasound in the treatment and diagnosis of Alzheimer's and Parkinson's disease

Symposium chair: Joanne Nash, University of Toronto

Speakers: Isabelle Aubert, Sunnybrook Research Institute

MR-guided-focused ultrasound mediated permeabilization of the blood brain barrier to deliver gene therapies: Progress in the treatment of Alzheimer's disease
Joanne Nash, University of Toronto

MR-guided-focused ultrasound mediated delivery of AAV9. SIRT3-myc is neuroprotective in a rat model of Parkinson's Disease
Samuel Pichardo, University of Calgary, Hotchkiss Institute

Tremor reduction using a multi-focus transcranial ultrasound stimulation method targeting the thalamus: Preliminary results
Oury Monchi, Université de Montréal

Non-invasive forms of neuromodulation in the treatment of cognitive symptoms of Parkinson's disease

3:30 – 5:00 pm
Grand East

SYMPOSIUM 11

Models of inhaled cannabis exposure: effects on behaviour and brain across the lifespan of rodents

Symposium chair: John Howland, University of Saskatchewan

Speakers: John Howland, University of Saskatchewan

Long-term effects of gestational cannabis exposure to cannabis smoke on behaviour and cortico-limbic brain circuits in the offspring
Hakan Kayir, University of Guelph

Impact of adolescent exposure to vaporized cannabis on adult rat behaviour and brain connectivity
Ryan McLaughlin, Washington State University

Clouded judgement: long-term cellular and behavioral changes in a rodent model of adolescent cannabis use

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Cassie Moore, Johns Hopkins University School of Medicine

Behavioral and neurobiological consequences of chronic vaporized Δ9-tetrahydrocannabinol (THC) self-administration in rats

3:30 – 5:00 pm
Osgoode East

SYMPOSIUM 12
The function of catecholamines in learning and decision-making
Symposium chair: Robert Rozeske, University of Toronto, Scarborough

Speakers:
Robert Rozeske, University of Toronto, Scarborough

Prefrontal dopamine dynamics during context fear learning and discrimination

Laura Corbit, University of Toronto

Locus coeruleus activity increases in response to omission of an expected reward

Michael Baratta, University of Colorado, Boulder

Prefrontal dopamine reveals sex differences in coping with stress

Kate Wassum, University of California, Los Angeles

Dopamine release in the basolateral amygdala facilitates reward learning and prediction

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PLENARY TALKS

PRESIDENTIAL LECURE
Keeping your brain in balance: homeostatic tuning of network function

Gina Turrigiano, Brandeis University

Neocortical networks must generate and maintain stable activity patterns despite perturbations induced by learning and experience- dependent plasticity. Here I will discuss how network stability is maintained through the expression of homeostatic plasticity mechanisms that adjust synaptic and neuronal properties, and how this plasticity enables networks to operate within an optimal computational range. I'll discuss recent work on the role of sleep and wake states in gating homeostatic plasticity, and present evidence that naturalistic learning can re-set homeostatic setpoints.

FEATURED PLENARY SPEAKER 1
Translating the neuroscience of fear to understanding PTSD

Kerry Ressler, McLean Hospital Harvard University USA

Neocortical networks must generate and maintain stable activity patterns despite perturbations induced by learning and experience- dependent plasticity. Here I will discuss how network stability is maintained through the expression of homeostatic plasticity mechanisms that adjust synaptic and neuronal properties, and how this plasticity enables networks to operate within an optimal computational range. I'll discuss recent work on the role of sleep and wake states in gating homeostatic plasticity, and present evidence that naturalistic learning can re-set homeostatic setpoints.

NEW INVESTIGATOR AWARD LECTURE
Cell types for reverse engineering learning and memory in the brain

Mark Cembrowski, University of British Columbia

The hippocampus, a brain region critical for learning and memory, is often conceptualized as a serial processor. Here, we examine the output region of the hippocampus, and reveal that excitatory neurons within this brain region can be coherently separated across molecular profiles, morphological structure, and circuit wiring. Functionally, these subtypes have different representational properties and timescales, and can govern parallel aspects of spatial and non-spatial memory. Our results here illustrate highly specialized dissociable pathways for learning and memory in the brain.

BRAIN PRIZE LECTURE
Modeling the navigational circuitry of the fly

Larry Abbott, Columbia University

Navigation requires orienting oneself relative to landmarks in the environment, evaluating relevant sensory data, remembering goals, and convert all this information into motor commands that direct locomotion. I will present models, highly constrained by connectomic, physiological and behavioral data, for how these functions are accomplished in the fly brain.

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FEATURED PLENARY SPEAKER 2
Spying on neuromodulator dynamics in vivo by constructing multi-color genetically-encoded sensors

Yulong Li, Peking University

The human brain consists of billions of neurons, most of which communicate with each other by releasing different kinds of neuromodulators through chemical synapses, and therefore is able to control different physiological functions like perception, motion, learning and memory. To dissect the mechanism underlying how brain take part in different physiological functions and pathological conditions, it's important to monitor the dynamics of neuromodulators in vivo. In the past few years, we and others have developed a series of multi-color GPCR-activation—based (GRAB) sensors for monitoring extracellular neuromodulator dynamics with high sensitivity, specificity, and spatial-temporal resolution in living animals. In this report, I will share our recent progress in developing sensors for monitoring monoamines, nucleotides, neurolipids and neuropeptides. With these GRAB sensors, we have monitored the dynamics of neuromodulators in mice in a wide range of physiological processes (sleep-wake cycle, motion, etc.) and pathological conditions (epilepsy, etc.).

KEYNOTE LECTURE
Developmental critical periods for episodic memory

Paul Frankland, University of Toronto

Memories for events (i.e., episodic memories) formed in early development differ from those in adulthood in at least two regards. First, these memories tend to be less precise than those formed in adulthood (i.e., infantile generalization). Second, they tend to be rapidly forgotten (i.e., infantile amnesia). My talk will focus on the neurobiological mechanisms that account for these different operating characteristics of episodic memory in the developing brain. With respect to infantile generalization, our studies have revealed that maturation of inhibitory microcircuits in the hippocampus is necessary for the formation of adult-like, precise memories for events. With respect to infantile amnesia, our studies have shown that activity-dependent myelination of prefrontal cortical circuits is necessary for the formation of adult-like, enduring event memories. Like developing sensory systems—where cortical circuit refinement occurs during defined windows of heightened brain plasticity known as critical periods—our work suggests that similar refinement of hippocampal and prefrontal cortical circuits underlies the emergence of adult-like episodic memory function.

PLENARY SPEAKER 3
The Good, The Bad and the Ugly

Yasmin Hurd, MSSM, USA

The global use of cannabis and cannabinoid products has risen significantly driven by sociopolitical shifts in recent decades that led to their decriminalization and legalization. While some of the underlying reasons for this shift had important implications for certain groups, many questions continue to mount regarding the potential impact—positive or negative—of these rapid changes in the cannabis landscape on mental health. One of the most critical concerns revolves around developmental exposure to cannabis, especially given the sensitivity of the developing brain—fetal, childhood and adolescence—to environmental conditions that could place individuals at risk for addiction and other psychiatric disorders later in life. This

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presentation will provide insights from translational studies, including molecular and behavioral animal studies as well as human longitudinal investigations, that explore the neurobiological consequences of developmental cannabis exposure and its relevance to psychiatric risk. It is clear that several factors, such as THC potency, can negatively impact mental health. On the other hand, emerging evidence suggest that other cannabinoids may alleviate psychiatric disorders. For example, while THC has been linked to certain negative outcomes associated with cannabis, cannabinoids like cannabidiol appear to have hold potential beneficial properties. This presentation will delve into translational studies on the medicinal potential of cannabidiol for alleviating substance use disorders and related psychiatric illnesses. It is hoped that insights gained from the growing body of research will provide science-based evidence to inform future clinical practices and treatment approaches, ultimately addressing the complex relationship between cannabis and mental health.

PLENARY SYMPOSIA

PLENARY SYMPOSIUM 1: FEAR, THREAT AND AVERSIVE LEARNING
Chair – Mihaela Iordanova, Concordia U

Prefrontal Cortex Modulation of Fear Processing Across the Lifespan

Maithe Arruda-Carvalho, UToronto Scarborough

The medial prefrontal cortex (mPFC) is critical to cognitive and emotional function and underlies many neuropsychiatric disorders, including fear and anxiety disorders. Childhood and adolescence are the predominant age of onset for the majority of mental disorders, and coincide with anatomical and morphological changes within mPFC pathways. Yet, how such changes influence mPFC function and consequently affect behaviour and the onset of mental illness is currently unknown. Using a combination of viral tracing, optogenetic-assisted patch clamping and chemo and optogenetic manipulations during behaviour, we examined how mPFC subregions are differentially engaged in fear processing across ages, with a focus on the timing of mPFC recruitment and contribution to fear encoding, extinction and remote retrieval from infancy to adulthood in mice. Our data show that prelimbic and infralimbic mPFC projections to the basolateral amygdala become necessary for fear encoding and extinction during adolescence and juvenility, respectively, with important consequences for fear expression. We also show important contributions of mPFC input projections to the regulation of remote memory retrieval in adult and infant mice. Our data highlight age-, sex- and pathway-specific contributions within mPFC pathways to fear processing, with crucial implications for early life influences on adult cognitive function.

Prefrontal encoding of an internal model for emotional inference

Josh Johansen, RIKEN Institute, Japan

A key function of brain systems mediating emotion is to learn to anticipate unpleasant experiences. While organisms readily associate sensory stimuli with aversive outcomes, higher-order forms of emotional learning and memory require inference to extrapolate the circumstances surrounding directly experienced aversive events to other indirectly related sensory patterns which weren't a part of the original experience. This type of learning requires internal models of emotion which flexibly track directly experienced and inferred aversive associations. While the brain mechanisms of simple forms of aversive learning have been well studied in areas like the amygdala, whether and how the brain forms and represents internal models of emotionally relevant associations is not known. Here we report that neurons in the rodent dorsomedial prefrontal cortex (dmPFC) encode a flexible internal model of emotion by linking sensory stimuli in the environment with aversive events, whether they were directly or indirectly associated with that experience. These representations form through a multi-step encoding mechanism involving recruitment and stabilization of dmPFC cells which support inference. While dmPFC population activity encodes all salient associations, dmPFC neurons projecting to the amygdala specifically represent and are required to express inferred associations. Together, these findings reveal how internal models of emotion are encoded in dmPFC to regulate subcortical systems for recall of inferred emotional memories.

Neural circuits of safety learning

Susan Sangha, Indiana University

Clinical disorders arising from maladaptive emotion regulation present a large burden on society worldwide and many of these disorders show comorbidity, for example, anxiety disorders with addiction. Even though there has been considerable research on reward and fear processing, the majority of studies have been conducted in parallel, investigating the neuronal circuitries separately. Our lab uses a behavioral paradigm designed to assess how safety cues can regulate fear and reward seeking behaviors in male and female rats. In combination with this behavioral task, we use in vivo single unit electrophysiology, longitudinal single cell calcium recordings, pharmacology and chemogenetics to track biological changes in the brain as the animal is learning about environmental cues signifying safety, fear and reward. Thus far we have identified neuronal correlates of safety in the amygdala and prefrontal cortex and demonstrated that the projection from the infralimbic cortex to the central amygdala is necessary for safety recall. Our goal in investigating how safety, fear and reward circuits integrate their functions to influence behavior, is to better understand and treat disorders resulting from maladaptive emotion regulation.

PLENARY SYMPOSIUM 2: VISUALIZING THE BRAIN

Chair – Jibran Khokhar, WesternU

Nanoscale molecular imaging of cannabinoid and dopamine signaling
Istvan Katona, Indiana University Bloomington

Traditionally used preclinical and clinical imaging modalities offer sufficient quantitative power to measure the overall changes in binding density or protein density at the regional level in brain disorders. However, cell-type, microdomain, and even nanodomain-specific molecular changes are associated with adaptive and maladaptive plasticity processes in specific brain circuits. In this lecture, I will first discuss antibody-based immunolabeling approaches and fluorescent microscopy modalities that enable cell-type-specific and subcellular-compartment-specific molecular imaging. Next, I will introduce PharmacoSTORM, a novel ligand bindingand microscopy pipeline that combines the strengths of ligand-based imaging and immunolabeling. We developed fluorophore-tagged receptor ligands, performed ligand incubation in live brain slice preparations, and then conducted post hoc immunolabeling. Brain sections were imaged using correlated STORM super-resolution imaging and confocal microscopy. I will provide evidence that PharmacoSTORM is useful for visualizing individual drug molecules targeting cannabinoid and dopamine receptors in a cell- and synapse-type-specific manner. I will demonstrate the strength of this approach by introducing the workflow for visualizing and quantifying the nanoscale binding sites of cariprazine, an FDA-approved antipsychotic drug. Our results suggest that the axons of the granule cells in the Islands of Calleja, a relatively understudied brain region in the ventral striatum, represent a major cariprazine binding site. We propose that cariprazine treatment influences how dopamine controls the output of the Islands of Calleja granule cells.

PET Perspectives: Mapping Neuropsychiatric Disorders from Synapses to Systems
Isabelle Boileau, CAMH, University of Toronto

Clinical disorders arising from maladaptive emotion regulation present a large burden on society worldwide and many of these disorders show comorbidity, for example, anxiety disorders with addiction. Even though there has been considerable research on reward and fear processing, the majority of studies have been conducted in parallel, investigating the neuronal circuitries separately. Our lab uses a behavioral paradigm designed to assess how safety cues

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Multimodal neuro-vascular imaging in the aging mouse brain
Michele Desjardins, Université Laval

Human fMRI studies of aging have revealed changes in brain network characteristics which appear related to cognitive decline. However, fMRI is an oxygen-based contrast determined by the interplay of many factors, including neurovascular coupling, cerebrovascular reactivity, cerebral blood flow and volume, all of which are modified during aging. It thus remains uncertain exactly which aspects of brain function are reflected in aging-related fMRI network differences. Preclinical imaging in mice can help disentangle neuronal and vascular changes during aging. This talk will present some of our ongoing efforts to develop and apply imaging and modelling tools to study neuronal and vascular function in mice.

First, I will show how a linear model of fMRI functional connectivity revealed its correlation with neuronal structural connectivity, as expected - but also with regional microvascular structure. Then, I will present some preliminary data from our ongoing longitudinal study in aging mice. This data combines microvascular structure, cortical neuronal and vascular functional connectivity, and behavioral assessments. Finally, I will discuss some biophysical models that we develop in our efforts to mechanistically explore the microvascular origin of fMRI signals.

PLENARY SYMPOSIUM 3: EXTERNAL INFLUENCES ON NEURODEVELOPMENT
Chair – Deborah Kurrasch, University of Calgary

Maternal sleep disruption alters neurodevelopmental programs in the embryo and has long-term impacts on offspring sleep

Jessica Rosin, University of British Columbia

Circadian rhythms are 24-hour cycles that are part of the body's internal clock and align our biological functions with the external environment. The suprachiasmatic nucleus—a bilateral structure located in the hypothalamus—is the central pacemaker of circadian timing and regulates most circadian rhythms in our body. Sadly, misalignment of circadian rhythms is becoming commonplace, as sleep disturbances brought on by shift work, nighttime light pollution from technology, and other factors (e.g., stress, anxiety, etc.) are prevalent in modern society—with circadian disruptions showing strong links to reproductive challenges in women. However, there is a lack of literature examining the impact of maternal circadian disruptions experienced during pregnancy on fetal neurodevelopment. Accordingly, we established a variety of maternal circadian disruption paradigms whereby pregnant mice are either exposed

to a constant light stimulus (i.e., continuous light exposure), an environment lacking light as a visual cue (i.e., constant darkness) or a phase-shift in their light cycle spanning critical periods of neurodevelopment in the embryonic hypothalamus, with the overarching objective to explore how prenatal maternal circadian disruption during fetal neurodevelopment affects the offspring. In my talk, I will share the results of the various physiological and behavioural assessments we conducted on the dams from each maternal circadian disruption model, including sleep patterns, circulating corticosterone, cytokines, chemokines, etc., and the impact these changes have on the developing embryonic hypothalamus. My talk will also highlight the long-term consequences of prenatal maternal circadian disruption on a variety of offspring behaviours, including changes to offspring sleep patterns. With these findings, we hope to further our understanding of the mechanism(s) by which fetal neural cells change their developmental programs in response to maternal sleep disturbances and how these changes may adversely impact the offspring to result in neuropsychiatric consequences.

Prenatal maternal stress and human brain development
Catherine Lebel, University of Calgary

Prenatal maternal stress (e.g., depression, anxiety, stressful life events) is common and can have lasting effects on children, including an increased risk of behavioural and later mental health problems. We have previously shown that prenatal depression and anxiety are associated with altered brain connectivity in children, and that altered connectivity between the amygdala and prefrontal cortex mediates the association between prenatal depression and child behaviour. More recently, we have been studying associations between prenatal stress and child brain in a cohort recruited during the COVID-19 pandemic (Pregnancy during the Pandemic study), a time when pregnant people experienced very high depression and anxiety symptoms. We are again seeing associations between prenatal anxiety/depression and child brain structure and function, and the associations vary by age and sex. We have identified several moderators, such as social support and sleep, that may be good targets for future interventions to support families. Further, we have shown that objective stress (i.e., stressful life events) is an independent contributor to child brain and behaviour outcomes, above and beyond psychological distress. Here, I will talk about our work studying maternal stress and the child brain, with a focus on how the brain may act as a mechanism via which maternal stress leads to child behavioural problems.

Neural stem cells regulation by their neighbors and environment
Armen Saghatelyan, University of Ottawa

Neural stem cells (NSCs) respond to external stimuli and reside in a complex niche micro-environment where they are surrounded by multiple cell types providing numerous molecular signals. Life-long maintenance of NSCs and their proliferative activity is regulated by distinct environmental stimuli and implies that feedback mechanisms from the niche regulate their quiescence/activation dynamics.
To shed light on how stem cells interact with their niche micro-environment and how their proliferative activity is influenced by different external stimuli, we used mouse adult subventricular NSCs niche as a model system and ex vivo and in vivo NSCs imaging. We charted a precise spatiotemporal map of functional responses in NSCs induced by multiple niche cells and used machine learning to predict NSC interactions with specific niche cell types. We revealed a feedback mechanism whereby NSC proliferative state is directly repressed by transient amplifying cells (TAPs), their rapidly dividing progeny. We further revealed that NSCs activation and their interplay with distinct niche cell types is regulated in a sex-dependent manner and is influenced by distinct external stimuli. Our data indicate that

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feedback signaling which is regulated by distinct external stimuli controls stem cell quiescence and activation and reveals how distinct niche cell types in the micro-environment regulate stem cell pools throughout life.

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PARALLEL SYMPOSIA

SYMPOSIUM 1: GUT MICROBIOME-BRAIN INTERACTION ON HOST HEALTH

S01.1 - Role of gut microbiome brain axis in obesity

Frank Duca [1]

[1] University of Arizona

The gut microbiome is a salient contributor to metabolic disease. Interestingly, decent the physiological importance of the small intestine in regard to nutrient digestion, absorption, and gut-brain feedback, the small intestinal microbiome is often overlooked. Furthermore, recent work has highlighted the potential influences of the gut microbiome on brain function and behavior. We have recently highlighted several novel pathways by which the small intestinal microbiome can impact energy homeostasis via impacting neural signaling pathways. First, we have found that the bacteria in the small intestine interact with vagal afferent signaling pathways that regulate meal size via gut peptide signaling. Dietary manipulations of the small intestinal microbiome can restore vagal signaling pathways that are impaired during high-fat diet feeding, leading to reductions in food intake and long-term body weight. Second, we have not discovered a novel signaling pathway, where indoles, bacterially derived metabolites of tryptophan, could potentially directly target the hypothalamus to activate the aryl hydrocarbon receptor (AhR). Knockdown of AhR in the mediobasal hypothalamus leads to robust increases in body weight and adiposity, while ICV treatment with indoles can reduce diet-induced obesity. These studies highlight the importance of the small intestinal microbiome on impacting peripheral and central neural signaling pathways that regulate energy homeostasis.

S01.2 - Using C. elegans to study neuroprotection from the microbiome

Lesley Macneil [1]

[1] McMaster University

Neurodegenerative diseases are complex diseases that are influenced by both genetic and environmental factors. Correlative studies identify differences in microbiome composition between patients and controls in both Parkinson's and Alzheimer's diseases, suggesting the microbiome may have the potential to influence disease progression. *C. elegans* is a tractable model to examine the potential impact of different bacterial species on neurodegeneration. We find that neurodegeneration in *C. elegans* can be dramatically altered by exposure to different bacteria from the human microbiome. From a large-scale analysis of clinical isolates, we identified bacteria that increase or decrease neurodegeneration in models of Alzheimer's disease. We focused our analysis on species that induce neuroprotection and found that different bacteria stimulate different host pathways to induce this protection, suggesting that bacteria have the potential to influence neurodegeneration in many different ways. Genetically, bacteria can influence neurodegeneration by influencing the insulin-like signaling pathway, the p38 MAPK pathway, and many other signaling and metabolic pathways. Our efforts to

characterize how bacterial species from the human microbiome promote neuroprotection will be discussed.

S01.3 - Small intestinal microbiota alters bile acids to impact glucose and energy homeostasis via the brain

Tony Lam [1]

[1] Toronto General Hospital Research Institute

I will present findings indicating that changes in small intestinal microbiome alter circulating bile acid levels and impact brain bile acid receptors to regulate feeding, weight and glucose homeostasis. The studies highlight the therapeutic relevance in targeting brain bile acid receptors to lower weight and glucose levels in obesity and diabetes.

SYMPOSIUM 2: NEW INSIGHTS OF NORADRENALINE IN NEURAL DISEASES: FROM NEURODEVELOPMENT TO NEURODEGENERATION

S02.1 - Disrupted Temporospatial Noradrenaline dynamics in motor cortex underlie motor learning deficits in an ASD mouse model

Xuming Yin [1], **Nathaniel Jones** [1], **Aaron Jumarang** [2], **Tommaso Patriarchi** [3], **Yulong Li** [4], **Simon Chen** [1]

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Children with autism spectrum disorders (ASDs) frequently experience delays in motor development. In the 16p11.2 deletion mouse model, which mimics a common copy number variation associated with ASDs, we previously demonstrated delayed motor learning alongside abnormally elevated neuronal activity in the primary motor cortex (M1). Remarkably, activating locus coeruleus noradrenergic (LC-NA) neurons rescued both circuit deficits and delayed motor learning. In this study, we used in vivo two-photon microscopy to monitor LC-NA calcium axonal activity during motor learning and identified the temporal of LC-NA activity, in which non-behavioral related 'rapid' axonal activity (sub-second duration events) profoundly affect the behavior-induced 'persistent' axonal activity (second duration events). In addition, we performed two-photon imaging of NA sensor in M1, and we further uncovered that behavior-induced NA release is spatially heterogeneous at the scale of local microcircuitry. However, these temporospatial specificities were disrupted in 16p11.2 deletion mice. Intriguingly, pharmacological and closed-loop optogenetic interventions designed to mimic the temporal and spatial NA disruptions observed in 16p11.2 deletion mice were sufficient to induce motor learning delays in WT mice. These findings shed light on previously unrecognized patterns of NA release dynamics within M1 at temporal and spatial scales, underscoring their pivotal role in motor skill learning and their disruption in ASD-related conditions.

S02.2 - Noradrenergic transmission promotes flexibility of spatial behavior and sleep-dependent memory consolidation

Oxana Eschenko [1]

[1] Max Planck Institute for Biological Cybernetics

In my talk, I will review our two recent studies addressing the role of noradrenergic modulation in the 'online' and 'offline' processing of spatial information. The Locus Coeruleus (LC) has been implicated in many cognitive functions, yet its contribution to spatial cognition received less attention. The prefrontal cortex (PFC) is essential for cognitive control and its reciprocal connectivity with the LC suggests a functional role of this pathway in adaptive behavior. In our first study [1], we chemogenetically modulated the activity of projection-specific subpopulations of the LC-NE neurons and tested rat behavior in an 8-arm radial maze. Rats with reduced NE release in the anterior cingulate cortex (ACC), a key node within the executive prefrontal network, were deficient in reorganizing their behavior in a new spatial context. LC-lesioned rats showed a similar behavioral deficit. Our findings highlight the beneficial role of NE for an optimal function of the ACC and point to projection-specific modulation of neural circuits involved in cognitive control. In our second study [2], we pharmacologically reduced the global NE release after learning of a spatial task. Our earlier electrophysiological recordings in the LC during natural sleep in rats revealed a coordinated firing of the LC-NE neurons with cortical slow oscillations, thalamocortical sleep spindles, and hippocampal sharp-wave ripples, all have been causally implicated in sleep-dependent memory consolidation. Reduced noradrenergic transmission was associated with altered sleep microarchitecture, robust suppression of the hippocampal ripples, and memory deficit. Thus, the LC-NE systems promote both 'online' and 'offline' processing of spatial information by facilitating cross-regional interactions within the executive prefrontal and cortico-hippocampal memory-supporting networks.

1. Kabanova, A., L. Fedorov, and O. Eschenko, *The Projection-Specific Noradrenergic Modulation of Perseverative Spatial Behavior in Adult Male Rats*. eneuro, 2024. **11**(8): p. ENEURO.0063-24.2024.

2. Duran, E., et al., *Altered norepinephrine transmission after spatial learning impairs sleep-mediated memory consolidation in rats*. Sci Rep, 2023. **13**(1): p. 4231.

S02.3 - Noradrenergic control of the resilient shift following chronic stress

Elsa Isingrini [1], **Dea Slavova** [1], **Bruno Giros** [2]

[1] Université de Paris-Cité, [2] McGill University

*Stressful life events—job loss, accident, death of a loved one—c*an trigger major depression in one person, but not in another. A deciding factor is resilience, a biological mechanism that determines an individual's capacity to rebound from stressful or traumatic events. The *locus coeruleus*-norepinephrine (LC-NE) system is a critical component in the brain's stress response. However, its role in individual variability in stress responses has been difficult to apprehend given the LC-NE system's anatomical and functional complexity.

We recently showed that NE neurons in the *locus cœruleus* have direct connections within the VTA to Dopamine (DA) neurons that are known to play a key role in mediating stress susceptibility and resilience. Using conditional knockout mice unable to release any NE, and optogenetic stimulation of the LC-VTA pathway in vulnerable control mice, we have been able

to demonstrate the role of these NE neurons in regulating vulnerability against social defeat via a direct inhibitory control of VTA-DA neurons.

To further identify specific genes that may be implicated in this process, we investigated at the molecular and cellular level human post-mortem brain samples of depressed suicides with or without early life adversity (ELA), and whether differential neurobiological mechanisms can be revealed in resilient (RES) individuals.RNA sequencing of laser captured LC-NE neurons highlighted differentially expressed genes, principally in the RES-ELA group.

Overall, functional and molecular characterization of this new neural circuit underlying resilience against chronic emotional stress, provided a rationale for future direction to develop depression treatments targeting NE transmission.

S02.4 - Locus coeruleus vulnerability to Tau Hyperphosphorylation: Age and sex differences in a rat model

Qi Yuan [1], **Tamunotonye Omoluabi** [1], **Zia Hasan** [1], **Jessie Piche** [1], **Abeni Flynn** [2], **Jules Dore** [1], **Susan Walling** [1], **Andrew Weeks** [2], **Touati Benoukraf** [1]

[1] Memorial University of Newfoundland, [2] Nipissing University

The locus coeruleus (LC) is among the first brain regions affected in Alzheimer's disease (AD), accumulating hyperphosphorylated pretangle tau, which precedes neurofibrillary tangle (NFT) formation. LC degeneration is strongly linked to cognitive decline, yet the mechanisms driving tau-induced neuronal vulnerability remain unclear. Here, we investigated the transcriptomic and mitochondrial changes in LC noradrenergic neurons following transduction with pseudophosphorylated human tau (htauE14) in a rat model, focusing on age and sex differences.

Tau hyperphosphorylation led to increased somatic expression of L-type calcium channels (LTCCs), mitochondrial dysfunction, and impairments in spatial and olfactory learning. Notably, gene expression changes in htauE14 rats were sex-dependent, with males and females exhibiting distinct transcriptional responses to tau. Furthermore, mitochondrial mRNA expression showed opposite regulation in adult and aged rats, suggesting an age-specific response to tau pathology.

To determine whether LTCC hyperactivity contributes to these deficits, we administered a chronic LTCC blocker, which rescued behavioral impairments and partially restored mitochondrial gene expression. These findings indicate that LTCC dysfunction is a key driver of LC vulnerability to tau pathology, potentially linking tau-induced calcium dysregulation to mitochondrial decline and neurodegeneration.

By identifying age- and sex-dependent factors that influence LC resilience to tau pathology, this study provides new insights into early AD progression and highlights LTCCs as potential therapeutic targets for preventing tau-driven neurodegeneration in vulnerable neuronal populations.

SYMPOSIUM 3: A GENOMICS APPROACH TOWARDS UNDERSTANDING SEX DIFFERENCES IN COGNITION, MENTAL HEALTH, AND NEURODEVELOPMENT

S03.1 - Sex-specific effects of histone H2A.Z in Alzheimer's disease

Iva Zovkic [1]

[1] University of Toronto, Mississauga

Alzheimer's disease (AD) and existing therapies differentially impact men and women, demonstrating a need to understand sex-specific contributions to the disease. Sex differences in chromatin function are especially promising because chromatin coordinates changes in gene expression that impact all aspects of cell function. Histone variants replace canonical histones in chromatin to impact gene expression and we previously showed that the histone variant H2A.Z.1 impairs memory in a sex-specific manner. Given its role as a memory suppressor, we tested if H2A.Z is dysregulated in post-mortem hippocampi of human AD patients. H2A.Z levels accumulated in male but declined in female AD patients compared to controls. Despite loss of major H2A.Z binding sites, female patients exhibited an increase in H2A.Z binding at minor sites, suggesting that H2A.Z is redistributed in the female AD brain. To test the functional relevance of H2A.Z in AD, we used 5xFAD AD model mice. H2A.Z showed strong accumulation in female, but not male AD model mice at 4 months of age, but levels dropped dramatically by 8 months, indicating that disease progression is associated with a decline in H2A.Z levels in female AD model mice. Viral vector-mediated H2A.Z.1 depletion at 2.5 months of age improved memory and reduced disease pathology in female 5xFAD mice, while impairing memory in male 5xFAD mice at 3 months of age. Beneficial effects of H2A.Z.1 depletion persisted until 8 months of age in females, whereas no differences were observed in male mice from 4 to 8 months of age. These data are the first to characterize histone H2A.Z function in AD and demonstrate sex-specific impact of this histone on disease progression and memory decline, pointing to sex-specific therapeutic relevance of H2A.Z in AD.

S03.2 - Single-cell insights into gene regulation in the mouse brain across the estrous cycle and sex

Maria Tickerhoof [1], **Ayyappa Sista Kameshwar** [2], **Luisa Demarchi** [1], **Laila Ouldibbat** [1], **Masako Suzuki** [2], **Marija Kundakovic** [1]

[1] Fordham University, [2] Texas A&M University

Ovarian hormone shifts are an important contributing factor to the increased prevalence of depression and anxiety disorders in women compared to men, although the underlying molecular mechanisms are underexplored. We showed that anxiety- and depression-related behaviors vary across the estrous cycle in mice. We further linked these estrous cycle-dependent behavioral changes to changes in chromatin and gene expression in neurons of the ventral hippocampus (vHIP), a region critical for emotion regulation. To further address this question, here we characterized gene expression and chromatin accessibility within the mouse vHIP at single-nucleus resolution across sex and the estrous cycle, focusing on diestrus (low estradiol-high progesterone) and proestrus (high estradiol-low progesterone). >55,000 nuclei from 11-week-old diestrus and proestrus females and male C57BL/6J mice (N=6 animals or 3

biological replicates/group) were processed using the 10XChromium Single-Cell Multiome (ATAC+Gene-Expression) kit. Using Seurat, we identified 42 cellular clusters in the vHIP, including subclusters of excitatory and inhibitory neurons, astrocytes, microglia, oligodendrocytes, and endothelial cells. The scProportionTest R package revealed sex- and estrous cycle-dependent changes in cellular proportions among these clusters. In addition, within specific cell types, we identified differential gene expression and chromatin accessibility between the sexes and across the estrous cycle, including genes relevant for neuronal function, stress response, and emotion regulation. We identified the gene encoding transthyretin (*Ttr*), a transporter of thyroxine and retinol, as the most significant differentially expressed gene in the proestrus-diestrus comparison. We then overexpressed *Ttr* in excitatory vHIP neurons to show its functional relevance in behavioral changes across the estrous cycle. These results provide single-cell resolution insights into sex- and estrous cycle-dependent gene regulation relevant to emotion regulation and neuroplasticity. This work critically informs the neurobiology underlying sex bias in depression and anxiety disorders, paving the way for precision medicine approaches taking hormonal state into account.

S03.3 - Sex differences in the developmental functions of the autism risk gene DDX3X

Adele Mossa [1], **Lauren Dierdorff** [1], **Jeronimo Lukin** [1], **Silvia De Rubeis** [1]

[1] Icahn School of Medicine at Mount Sinai

DDX3X is an X-linked RNA helicase that escapes X chromosome inactivation and is expressed at higher levels in female brains. Mutations in *DDX3X* are associated with intellectual disability (ID) and autism spectrum disorder (ASD) and are predominantly identified in females (DDX3X syndrome). Using cellular and mouse models, we show that *Ddx3x* mediates sexual dimorphisms in brain development at a molecular, cellular, and behavioral level. During cortical neuronal development, *Ddx3x* sustains a female-biased signature of enhanced ribosomal biogenesis and mRNA metabolism. Compared to male neurons, female neurons display larger nucleoli, higher expression of a set of ribosomal proteins, and a higher cytoplasm-to-nucleus ratio of ribosomal RNA. All these sex dimorphisms are obliterated by *Ddx3x* loss. *Ddx3x* regulates dendritic arborization complexity in a sex- and dose-dependent manner in both female and male neurons. *Ddx3x* regulates the development of dendritic spines but only in female neurons. Further, ablating *Ddx3x* conditionally in forebrain neurons is sufficient to yield sex-specific changes in developmental outcomes and motor function. Together, these findings pose *Ddx3x* as a mediator of sexual differentiation during neurodevelopment and open new avenues to understand sex differences in health and disease.

S03.4 - Sex-specific transcriptional mechanisms of substance use disorder

Deena Walker [1]

[1] Oregon Health & Science University

The molecular mechanisms underlying sex differences in motivation and reward are likely hijacked by drugs of abuse and serve as candidates underlying well-established sex differences in substance use disorder (SUD). Generally, clinical and preclinical studies suggest that females acquire and escalate use more quickly, display greater craving during abstinence, and exhibit a higher likelihood of relapse. The questions of why males and females become addicted to drugs of abuse at different rates is an elusive and unanswered question that involves several variables including genetics, social pressures, life experience, and hormone

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signaling. Canonically, hormones, when bound to their receptors, are transcription factors that mediate the transcriptional response to drugs of abuse. Most studies investigating sex differences in drug-associated behaviors and transcription have focused on sex-specific neuroendocrine systems like the reproductive axis. However, our lab has identified thyroid hormone system, a regulator of energy and metabolism, as a candidate for regulating sex-specific motivated behaviors. By integrating multiple bioinformatic approaches, we identified the thyroid hormone binding protein Crystallin me (Crym), as a key driver of the sex-specific transcriptional effects of cocaine and showed that overexpression of Crym in the medial amygdala, an established sexually dimorphic brain region in the rodent, enhanced the preference for cocaine and repressed transcription in males but not females. We also found that females have higher expression of thyroid hormone receptor beta (Thrb) mRNA in the medial amygdala than males. Given that THRb is less likely to engage in transcriptional repression than other thyroid hormone receptors, we hypothesized that THRb may be critical to blocking the rewarding properties of drugs of abuse. To test this, we treated male and female mice with the CNS-specific THRb agonist, Sob-AM2, during the 2-bottle choice test for alcohol, a model of alcohol drinking in rodents, to determine if activating THRb would suppress alcohol drinking. We found that treatment with Sob-AM2 significantly reduced alcohol intake and preference in male and female mice, suggesting that activating THRb in the brain can suppress intake of drugs of abuse regardless of sex. Together these data not only suggest that thyroid hormone is a critical regulator of the rewarding properties of drugs of abuse but highlight ways in which sex-specific transcriptional responses to drugs of abuse can be leveraged to identify potential therapeutic targets for substance- and alcohol-use disorder.

SYMPOSIUM 4: MECHANISMS OF STRESS REGULATION IN COMPLEX BEHAVIORS

S04.1 - Keeping up with danger: Hypothalamic CRH neurons sustain defensive escape

Kathryn Simone [1], **Tamás Füzesi** [2], **Jaideep Bains** [3], **Nuria Daviu** [4]

[1] University of Waterloo, [2] Hotchkiss Brain Institute, [3] University Health Network, [4] University of Guelph

Escape from a predator is a rapid process that integrates a series of specialized stages to execute an appropriate response. The stages include threat detection, decision-making, and culminate in the execution of a specific motor response. In recent years, in an effort to disentangle the escape components, the escape initiation mechanism and the cognitive map of shelter location have been identified. Meanwhile, one of the key questions that is still unknown is whether the escape initiation is a momentary decision leading to a point of no return, or if the drive needs to be sustained continuously throughout the maneuver until safety is reached. Here, we show that the animals maintain a representation of the threat throughout the escape maneuver. Our findings reveal that animals can complete the escape maneuver targeted to the shelter, regardless of the presence of the stimulus. The escape execution in the absence of a threat was accompanied by stimulus-independent CRH activity throughout the entire escape. Disrupting the CRH-PVN activity during the execution time results in an aborted maneuver that prevents the animal from reaching the shelter. Using single-cell imaging in freely moving

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animals, we uncovered a bimodal recruiting process in CRH-PVN neurons. Two distinct neuronal pools are recruited before and after escape initiation, suggesting an independent, obligatory role of CRH neurons in the escape execution phase. We propose that the activity of CRH neurons during escape serves as a neural representation of the threat, which is crucial for the animal's ability to execute a successful escape maneuver in response to a predatory threat.

S04.2 - Exercise counters stress effects on CRH-PVN neurons and anxiety without affecting fear recall

Mijail Rojas-Carvajal [1], **Dinara Baimoukhametova** [1], **Tamás Füzesi** [2], **Nuria Daviu** [3], **Sarah Cook** [2], **Jaideep Bains** [4]

[1] University of Calgary, [2] Hotchkiss Brain Institute, [3] University of Guelph, [4] University Health Network

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. However, contextual fear memory recall was not affected by exercise. 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.

S04.3 - Probing the role of lateral septum peptide signaling in aversion and reward

Thomas Kash [1]

[1] University of North Carolina at Chapel Hill

High-intensity alcohol drinking during binge episodes contributes to the socioeconomic burden created by alcohol use disorders (AUDs), and nociceptin receptor (NOP) antagonists have emerged as a promising intervention. To better understand the contribution of the NOP system to binge drinking, we found that nociceptin-containing neurons of the lateral septum (LS^{Pnoc}) displayed increased excitability during withdrawal from binge-like alcohol drinking. LS^{Pnoc} activation promoted active avoidance and potentiated binge-like drinking behavior, whereas silencing of this population reduced alcohol drinking. LS^{Pnoc} form robust monosynaptic inputs locally within the LS and genetic deletion of NOP or microinjection of a NOP antagonist into the LS decreased alcohol intake. LS^{Pnoc} also project to the lateral hypothalamus and supramammillary nucleus of the hypothalamus, and genetic deletion of NOP from each site reduced alcohol drinking. Together, these findings implicate the septo-hypothalamic

nociceptin system in excessive alcohol consumption and support NOP antagonist development for the treatment of AUD.

S04.4 - Biased information routing through the BLA mediates behavioral deficits following chronic stress

Pantelis Antonoudiou [1], **Najah Walton** [1], **Grant Weiss** [1], **Jamie Maguire** [1]

[1] Tufts University

The basolateral amygdala (BLA) is an emotional processing hub that plays a critical role in both positive and negative valence processing. Subpopulations of neurons within the BLA and distributed networks throughout the brain, referred to as ensembles, have been identified based on their specific coding of positive or negative valence. Further, our lab and others have shown that specific oscillatory states within and between the medial prefrontal cortex (mPFC) and BLA drive valence specific behavioral outcomes. Recent work from our laboratory demonstrates that unique oscillatory states recruit unique BLA ensembles and downstream circuits to subserve either positive or negative valence processing. This process represents a novel neural computation involved in valence processing. Interestingly, our lab has demonstrated that chronic stress, a major risk factor for psychiatric illnesses, corrupts oscillations in the BLA involved in valence processing and induces long-term deficits in behavioral states. Further, we demonstrate that chronic stress biases information routing through the BLA, facilitating the activity of neurons in the BLA projecting to brain regions involved in negative valence processing and suppressing the activity of neurons projecting to brain regions involved in positive valence processing. Conversely, positive experiences, such as enriched environment, facilitate the activity of neurons in the BLA projecting to brain regions involved in positive valence processing and suppress the activity of neurons projecting to regions involved in negative valence processing. These data demonstrate that previous experiences influence information processing in the BLA, altering the recruitment of ensembles and downstream brain regions, biasing information routing to drive divergent behavioral outcomes. These studies inform the cellular and molecular mechanisms through which previous experiences can exert long-term impacts on emotional processing which may be relevant to psychiatric illnesses given that altered valence processing is a core feature shared across disorders. Finally, these findings may also represent a mechanism whereby salient experiences exert long-term impacts on mood and may contribute to negative bias, or the tendency to attend to negative stimuli, which has been observed in patients across psychiatric illnesses.

SYMPOSIUM 5: DIVERSITY AND FLEXIBILITY IN MOTOR CORTICAL CONTROL

S05.1 - Sensory expectations shape neural population dynamics in motor circuits

Jonathan Michaels [1]

[1] York University

The neural basis of movement preparation has been extensively studied during self-initiated actions where motor cortical activity during preparation shows a lawful relationship to the parameters of the subsequent action. However, movements are regularly triggered and

constantly corrected based on sensory inputs caused by disturbances to the body or environment. Since such disturbances are often predictable and since preparing for disturbances would make movements better, we hypothesized that expectations about sensory inputs also influence preparatory activity in motor circuits. Here we show that when humans and monkeys are probabilistically cued about the direction of a future mechanical perturbation, they incorporate sensory expectations into their movement preparation and improve their corrective responses. Using high-density neural recordings, we establish that sensory expectations are widespread across the brain, including the motor cortical areas involved in preparing self-initiated actions. The geometry of these preparatory signals in the neural population state is simple, scaling directly with the probability of each perturbation direction. After perturbation onset, a condition-independent perturbation signal shifts the neural state leading to rapid responses that initially reflect sensory expectations. Based on neural networks coupled to a biomechanical model of the arm, we show that this neural geometry emerges through training, but only when the incoming sensory information indicating perturbation direction coincides with – or is preceded by – a condition-independent signal indicating that a perturbation has occurred. Thus, motor circuit dynamics are shaped by future sensory inputs, providing clear empirical support for the idea that movement is governed by the sophisticated manipulation of sensory feedback.

S05.2 - Constrained models of neural dynamics for generalization and insights

Shreya Saxena [1]

[1] Yale University

Our ability to record large-scale neural and behavioral data has substantially improved in the last decade. However, the inference of quantitative dynamical models for cognition and motor control remains challenging due to their under-constrained nature. Here, we incorporate constraints from anatomy and physiology to tame machine learning models of neural activity and behavior.

How does the motor cortex achieve generalizable and purposeful movements from the complex, nonlinear musculoskeletal system? I will introduce a deep reinforcement learning framework that trains recurrent neural network controllers to generate purposeful movements in anatomically accurate macaque and mouse musculoskeletal models. This framework mirrors biological neural strategies and aids in predicting and analyzing novel movements. In the second part, I will discuss ongoing work on integrating region-specific constraints in models of the cortico-basal ganglia-thalamic loop during timing tasks to gain insights into pathway-specific computations. Through these projects, we show that a constraints-based modeling approach allows us to predictively understand the relationship between neural activity and behavior.

S05.3 - A cortical subspace enables the flexible integration of unexpected inputs for behavioral error correction

Matthew Perich [1]

[1] Université de Montréal

Behavior is generated from neural activity spanning a large number of anatomically distinct brain areas. While each area certainly has a specific and important function, they should not be considered as isolated components; the processes that shape motor output ultimately arise from the interactions between these areas. This talk will explore how sensory and motor regions dynamically interact to drive flexible behavioral output. Indeed, primate behavior relies on continuous influx of sensory information about the body, and the motor cortex must integrate somatic feedback to accurately reach and manipulate objects. A hallmark of the cortical control of movement is the production of robust and predictable dynamics that drive muscle contractions in the face of many layers of variability. Yet, flexible motor output requires rapid responses to unexpected inputs, a need that is at odds with the desire for robustness. We studied how the motor cortex can balance robustness and flexibility by simultaneously recording neural population activity in motor and somatosensory cortex from four monkeys performing a naturalistic object interaction behavior resulting in occasional errors. While we observed robust and predictable dynamics on normal trials, we saw that the motor cortex was strikingly input-driven surrounding behavioral error correction. Intriguingly, these input-driven dynamics were isolated to a subspace of the population activity that putatively captured somatosensory feedback. Using electrical stimulation of ascending somatosensory tracts, we causally verified that this feedback subspace captured peripheral inputs to cortex. Our results demonstrate that cortical activity is compartmentalized within distinct subspaces, enabling flexible integration of salient inputs for robust behavior.

S05.4 - Dynamical constraints on neural population activity

Emily Oby [1], **Alan Degenhart** [2], **Erinn Grigsby** [3], **Asma Motiwala** [2], **Nicole Mcclain** [3], **Patrick Marino** [3], **Byron Yu** [2], **Aaron Batista** [4]

[1] Queen's University, [2] Carnegie Mellon University, [3] University of Pittsburgh, [4] Department of Bioengineering, University of Pittsburgh; Pittsburgh, USA

The manner in which neural activity unfolds over time is thought to be central to sensory, motor, and cognitive functions in the brain. Network models have long posited that the brain's computations involve time courses of activity that are shaped by the underlying network. A prediction from this view is that the activity time courses should be difficult to violate. We leveraged a brain-computer interface (BCI) to challenge monkeys to violate the naturally occurring time courses of neural population activity that we observed in motor cortex. This included challenging animals to traverse the natural time course of neural activity in a time-reversed manner. Animals were unable to violate the natural time courses of neural activity when directly challenged to do so. These results provide empirical support for the view that activity time courses observed in the brain indeed reflect the underlying network-level computational mechanisms that they are believed to implement.

SYMPOSIUM 6: OPIOID DRUGS AND THE BRAIN: RESEARCH INSIGHTS TO UNDERSTAND THE OPIOID EPIDEMIC

S06.1 - Brain circuits mediating opioid-induced respiratory depression

Gaspard Montandon [1]

[1] University of Toronto

Opioid drugs are widely used as pain therapies but can be abused and lead to overdose. In fact, opioid drugs, such as fentanyl, present severe side-effects including respiratory depression that can be lethal with overdose. Neural circuits in the medulla are critical for the generation of breathing and express various neuropeptides including substance P or somatostatin. Although these medullary cells mediate respiratory depression by opioid drugs, the neurochemical identity of the neurons mediating this depression have not been identified. Our research aims to determine whether substance P and/or somatostatin neurons mediate respiratory depression by the opioid drug fentanyl. We used transgenic mice, optogenetics, respiratory recordings and behavioral profiling to determine the role of various cell populations in opioid-induced respiratory depression and analgesia. First, we generated knockout mice lacking μ-opioid receptors (encoded by the gene *Oprm1*) in substance P (encoded by the gene Tac1) or somatostatin (encoded by *Sst*) cells. We found that respiratory depression by fentanyl was absent in *Tac1/Oprm1*$^{-/-}$ knockout mice, suggesting that substance P neurons fully mediate respiratory depression by fentanyl. Consistent with these results, *Tac1* mRNA was highly expressed in medullary neurons and was co-expressed with *Oprm1* mRNA. Importantly, respiratory depression by fentanyl was reversed when *Tac1* medullary neurons were selectively stimulated by optogenetics in mice, suggesting that substance P neurons could be targeted to prevent respiratory depression. Interestingly, medullary neurons expressing somatostatin, which are highly expressed in respiratory circuits, did not mediate respiratory depression by fentanyl. These results suggest that somatostatin neurons are sparred by opioid drugs. In conclusion, our results reveal the identity of the brain circuits mediating opioid-induced respiratory depression and may suggest new directions to prevent or alleviate respiratory depression by opioid drugs.

S06.2 - Regulation of fatty acid amide hydrolase (FAAH) in the brain of subjects using opioid drugs

Bernard Le Foll [1]

[1] Campbell Mental Health Research Institute

Fatty acid amide hydrolase (FAAH), the anandamide-catabolizing enzyme, is a key determinant of endocannabinoid metabolism and a growing target of interest for the treatment of several neurological conditions. Preclinical and clinical findings suggest reduced FAAH is associated with altered addiction risks, mood, and pain sensitivity. In our recent positron emission tomography (PET) studies utilizing FAAH radioligand [^{11}C]CURB, reduced FAAH was implicated in cannabis and alcohol use disorder. However, the status of FAAH in subjects using opioid drugs and its relationship with clinical symptoms is unclear. Our work aims to determine whether endocannabinoid metabolism is altered in patients using opioid drugs, and to elucidate whether the development of opioid use disorder (OUD) would play a role in the observed changes in endocannabinoid metabolism via FAAH. In adult patients taking prescription opioids for chronic non-cancer pain and age/sex matched healthy controls, we found FAAH activity in the brain to be trending higher in patients with a history of OUD compared to healthy individuals and those taking opioids without history of OUD. The current trend suggests difference in brain FAAH levels that is in contrast with the original hypothesis that FAAH is lower in substance use disorders. The observed variability may be driven by complex interactions of underlying pain pathophysiology and its treatments. The clinical significance of these variabilities, whether it relates to opioid dosing, pain intensity, or mood

and anxiety symptoms as well as how these findings implicate future pharmacological development will be explored.

S06.3 - A critical brain to spinal cord neurocircuit in opioid withdrawal

Tuan Trang [1]

[1] University of Calgary

People on long-term opioid medications can experience withdrawal symptoms when attempting to reduce or stop their use. Withdrawal is a major problem in the opioid crisis because it impacts people on chronic opioids, including those who are prescribed opioids for therapeutic control of pain or those who are misusing or abusing opioids. Interventions that reduce withdrawal can break the cycle of opioid use. We uncovered a mechanistic explanation for the aberrant autonomic output, which underlies many of the debilitating withdrawal symptoms. The locus coeruleus (LC) is a key autonomic centre with projections originating from this site containing norepinephrine that sends important inputs to the spinal cord. Both the LC and spinal cord are implicated in withdrawal, but the interaction between these anatomical sites for opioid action is not well defined. Using a combination of advanced behavioral, cellular, chemogenetic, and electrophysiological techniques, we demonstrate a specific top-down LC to spinal circuit that is crucial for the physical and aversive sequelae of opioid withdrawal. We establish that aberrant spinally projecting LC output critically requires microglial pannexin-1 channel activation, providing a microglia-to-neuron link in opioid withdrawal.

S06.4 - The gastrointestinal control of opioid induced hyperalgesia

Anna Taylor [1]

[1] University of Alberta

Adverse side effects, including tolerance, hyperalgesia, and addiction, are serious clinical challenges for chronic pain management and opioid deprescribing. Opioid receptors are expressed throughout the gastrointestinal (GI) tract, and opioid use both influences gut motility and alters the gut microbiome. This talk will present evidence that exposure to chronic morphine produces long lasting hyperalgesia that persists for at least 1 week following drug cessation. This time course correlates with an altered gut microbiota composition measured with 16s gene amplicon sequencing and cellular markers of central inflammation. Interventions targeting gastrointestinal composition, motility and permeability are effective at mitigating signs and symptoms of hyperalgesia. These results indicate that strategies that target the gut microbiome or GI motility may improve clinical outcomes in prescribing and deprescribing opioids.

SYMPOSIUM 7: CONNECTOMICS ACROSS SCALES: FROM SYNAPSES TO SYSTEMS

S07.1 - Principles of mouse visual cortex excitatory microcircuit organization

Per Jesper Sjostrom [1], **You Chien Chou** [2], **Hovy Ho-Wai Wong** [1], **Connie Guo** [1], **Shawniya Alageswaran** [1], **Haley Renault** [1]

[1] McGill University, [2] liV Medical Education Agency

Understanding cortical microcircuit function requires synapse-specific investigation, as connectivity patterns and short-term plasticity vary appreciably by synapse type. The state of the art for synapse-specific measurements has long been paired electrophysiological recordings. Although powerful, this method is slow, leading to a throughput problem. To overcome this, we therefore implemented optomapping — an approximately 100-fold faster 2-photon optogenetic method — which we validated against paired-recording data. Using optomapping, we tested 30,454 candidate excitatory inputs to find 1,790 connections onto pyramidal, basket, and Martinotti cells in mouse primary visual cortex, V1. We measured connectivity, synaptic weight, and short-term dynamics across the V1 layers. We found log-normal synaptic strength distributions, even in individual inhibitory cells, a previously unrecognized phenomen. We reproduced the canonical circuit for pyramidal cells but found surprising and differential microcircuit structures, with excitation of basket cells concentrated to layer 5, and excitation of Martinotti cells dominating in layer 2/3. Excitation of inhibitory cells was denser, stronger, and farther-reaching than excitation of excitatory cells, which promotes stability and difference-of-Gaussian connectivity. Our excitatory short-term plasticitome showed that synaptic dynamics is determined by both target cell type and presynaptic cortical layer, underscoring the complexity of synaptic signaling in V1. Peak depolarization latency in pyramidal cells also emerged as more heterogeneous, suggesting heightened sensitivity to redistribution of synaptic efficacy. Optomapping additionally revealed high-order connectivity patterns including shared-input surplus for interconnected pyramidal cells in layer 6. Optomapping not only resolved the throughput barrier but also uncovered novel organizational principles of cortical microcircuits, offering a fresh perspective on excitatory fine structure. Building on these insights, we will next apply optomapping to additional cortical regions to explore whether these principles generalize across the brain. We also aim to leverage optomapping to uncover how synaptic and connectivity phenotypes contribute to neuropathology.

S07.2 - Brain-wide structure-function in larval zebrafish: translational model for exposome influence

Antoine Légaré [1], **Mado Lemieux** [1], **Patrick Desrosiers** [1], **Paul De Koninck** [1]

[1] Université Laval

The establishment of a properly wired nervous system to support all of its functions is astonishingly complex. In addition to extremely elaborate genetic programming, the exposome—the sum of all exposures the developing nervous system encounters—plays a highly influential and sometimes detrimental role. Addressing the impact of the exposome on brain connectivity ideally requires the ability to resolve synaptic across the brain, quantify brain-wide functional connectivity, and integrate these measures with whole-body interactions. A strategic vertebrate model for this challenge is the larval zebrafish, offering outstanding access to monitor circuit formation and connectivity under the microscope, from the synaptic level to the whole brain and body. On the other hand, an important consideration in adopting a model is its translational potential to humans. We explored the structure-function relationship

of larval zebrafish brain by adapting methodologies inspired by human magnetic resonance imaging studies, using network-theoretical approaches and whole-brain calcium imaging at cellular resolution, combined with neuronal morphologies and spatially resolved gene expression profiles from recent atlas datasets.

We found that brain-wide functional connectivity is a robust measure that captures the individuality of larvae and that is strongly coupled with structural connectivity. Using visual stimuli and tail monitoring, we identified a functional network gradient that maps onto the sensorimotor axis of brain regions. We observed a modular architecture within the structural network that constrains the shape of co-activation patterns across brain regions, as well as a spatially resolved transcriptomic barcode predicting functional connectivity. Hence, the larval zebrafish brain exhibits numerous similarities with that of larger mammals, offering a strategic model for advancing our understanding of brain-wide circuit function in health and disease. In this context, we will discuss how manipulations of the exposome alter functional connectivity of the developing larval brain, integrating body-wide interactions, with the aim to examine their long-term impacts.

S07.2 - Developmental strategies for wiring plasticity: insights from nanoscale connectomics

Ben Mulcahy [1], **Mei Zhen** [2]

[1] Mount Sinai Hospital, [2] Lunenfeld Tanenbaum Research Institute

During development, neural circuits underline coordinated structural and functional maturation. Nanoscale connectomes, using volume electron microscopy, remain the gold standard for mapping structural wiring. We optimized an imagine pipeline to acquire the connectomes of many individuals from *C. elegans*, at sequential developmental timepoints, and as they make developmental decisions in response to environmental challenges. This talk shares insights on strategies of developmental plasticity, revealed by examining how structural and functional wiring are coordinated as the *C. elegans* matures.

S07.3 - The impact of prenatal maternal distress upon the developing human connectome

Kathryn Manning [1], **Aliza Jaffer** [1], **Claire Donnici** [1], **Lianne Tomfohr-Madsen** [2], **Gerry Giesbrecht** [1], **Nicole Letourneau** [1], **Catherine Lebel** [1]

[1] University of Calgary, [2] University of British Columbia

Most imaging studies tend to focus on a single modality at a time but considering the co-development of the structural and functional connectome, there are advantages in incorporating multiple measures. Prenatal maternal depression and anxiety can directly alter the in-utero environment, but the impacts to child brain development and long-term behaviour and mental health remain poorly understood. In this study we utilize rich longitudinal maternal survey data gathered through the Pregnancy during the Pandemic Study, and behavioural and imaging data from a subset of participants' children. We seek to understand the relationship between prenatal maternal distress and child brain and behavioural development, as well as the role of possible moderators or interventions in that relationship. We observed significant associations between prenatal maternal distress and infant amygdala-prefrontal structural and

functional connectivity. Importantly, the relationship with functional connectivity was moderated by social support, where maternal distress was associated with weaker amygdala-prefrontal functional connectivity only when self-reported quality of social support was rated low. Looking more broadly at functional networks and structural connectomics, we have also observed associations with prenatal distress and behaviour that evolve across early development. Overall, these findings demonstrate the differential effect of prenatal maternal distress upon the functional and structural architecture of the developing brain. Finally, we will present results from a pilot study examining a video-feedback parenting intervention that not only improves symptoms of maternal postnatal depression but supports healthy development of the child brain.

SYMPOSIUM 8: CIRCUIT, SYNAPTIC AND NEUROMODULATORY MECHANISMS UNDERLYING BASAL GANGLIA FUNCTION

S08.1 - Subregion specific responses to reward predicting cues in the nucleus accumbens

Corey Baimel [1]

[1] Dalhousie University

Recognizing and responding to cues that predict rewarding outcomes help guide our decisions and actions in daily life. Reward cues are processed by many areas of the brain, and this information converges in the nucleus accumbens, where it can be translated into goal-directed actions. The nucleus accumbens is a heterogeneous brain region made up of multiple cell types distributed across distinct subregions, such as the medial and lateral shell. It is becoming increasingly clear that at the level of neural circuits, these two subregions are anatomically and functionally distinct, and that they integrate different streams of information to promote and support different aspects of motivated behaviour. Here we focus on how reward cue information is relayed to and processed by these subregions. To do this, we use dual site fiber photometry in mice undergoing Pavlovian reward conditioning. We record how the nucleus accumbens medial and lateral shell respond to reward predicting cues across several modalities, including dopamine dynamics and the recruitment of medium spiny neurons. We also relate responses in the nucleus accumbens to presynaptic inputs from the basolateral amygdala. With the addition of anatomical tools and slice electrophysiology, we demonstrate that distinct populations of basolateral amygdala neurons target the nucleus accumbens medial and lateral shell, and that they are differently engaged in Pavlovian reward conditioning. Our findings suggest that across several modalities, cues that drive motivated behaviours are differentially routed to and processed by distinct location within the nucleus accumbens.

S08.2 - Adolescent stress alters amygdala-midbrain circuitry to promote compulsive reward-seeking

Talia Lerner [1]

[1] Northwestern University Feinberg School of Medicine

In humans, a history of adolescent stress leads to a lifelong elevated risk of developing a substance use disorder. To evaluate the neural mechanisms mediating this risk, we modeled adolescent stress in mice using a chronic unpredictable stress paradigm. We then evaluated the tendency of these mice to develop compulsive reward-seeking – a defining feature of substance use disorders – in an operant task. We found that mice with a history of adolescent stress display robust increases in compulsive reward-seeking. Next, using slice electrophysiology, fiber photometry, and genetic manipulations of cellular excitability, we identified changes in amygdala-midbrain circuitry evoked by adolescent stress that likely contribute to the change in behavior by elevating dopamine release in the tail of the striatum. Ongoing work is evaluating causal mechanisms and identifying points for potential intervention to prevent or reverse the developmental changes in this circuit incurred by stress.

S08.3 - Revealing dopamine's contributions to motor vigor

Nicolas Tritsch [1]

[1] Douglas Research Institute, McGill University

Ever since the discovery that the degeneration of midbrain DA neurons (mDANs) projecting to the striatum underlies bradykinesia (i.e., slowness of movement) in Parkinson's disease (PD), DA has become synonymous with motor vigor. However, the mechanisms through which DA contributes to the speed and amplitude of individual voluntary movements are still debated. Initial investigations suggested a somewhat slow or permissive role for DA, but recent experiments in rodents proposed a stronger and faster role for DA in the dynamic control of the gain of motor commands. In this presentation, I will describe our latest attempts at better understanding how dopamine contributes to motor vigor through the study of release patterns, lesions, and optogenetic and pharmacological manipulations. Our findings call into question the widely held view that phasic fluctuations in extracellular dopamine control the vigor of ongoing movements, constraining the kinds of mechanisms and timescales that dopamine likely acts on to modify behavior.

SYMPOSIUM 9: SLEEP, STRESS AND SEX DIFFERENCES IN DEVELOPMENT AND AGING: TRANSLATIONAL IMPACT IN HEALTH AND DISEASE

S09.1 - Sex differences in proteostasis and pathology in response to chronic stress and sleep impairments in AD pathology

Darcy Wear [1], **Christopher Morrone** [2], **Haung (Ho) Yu** [1]

[1] University of Toronto, [2] Centre for Addiction and Mental Health

Background: Sleep loss is a potential risk factor for the development of Alzheimer's disease. There are several factors that can play into these events, including dysregulation in sleep centres prodromal to disease pathology. There are also likely sex differences that can influence the progression of disease from sleep-regulating subcortical regions like orexinergic neurons to AD-related cortical areas (e.g., hippocampus).

Methods: We examined the impact of chronic sleep disruption on App^{NL-G-F} and identified unique signatures that are reminiscent of changes seen in Alzheimer's disease. We used a 2-

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week chronic uninterrupted stress model where variable frequency/tone and light signals reduced the ability of mice to sleep.

Results: Significant sex differences were seen in their sleep recovery response. These mice had increased levels of p62 in TH-positive orexinergic neurons, as well as neuronal loss. There is evidence of compromised proteostasis in a sex-dependent manner that resulted in increased pathology in both sexes. In addition, as orexinergic neurons are a stress-regulating area, we noted elevated corticosterone in female mice. Overall, these changes may be informative of early subcortical changes in Alzheimer's disease.

Significance: We believe these are early sleep impairment events that occur prodromal to disease and involve altered pathways in areas regulating sleep and that this is an important risk modifier for Alzheimer's disease and related disorders.

S09.2 - Astrocyte glucocorticoid receptors mediate sex-specific changes in activity following stress. Interactions with sleep-regulating orexinergic neurons

Ciaran Murphy-Royal [1]

[1] Université de Montréal

Alterations in circadian and motivated behaviours form a hallmark of stress-related neuropsychiatric disorders. To understand the underlying mechanisms, we focused our attention on the lateral hypothalamus, a brain region in which neuron-glia interactions are essential to maintain circadian activity. I will discuss our recent work where we reveal that glucocorticoid signalling in astrocytes prompts divergent effects of stress on activity levels in male and female mice.

S09.3 - Neural network dynamics and microglial activation during sleep

Ksenia Kastanenka [1]

[1] Harvard Medical School

Background: Alzheimer's disease is a progressive neurodegenerative disorder that is a major cause of dementia. In addition to memory impairments, Alzheimer's patients exhibit sleep disruptions. They have difficulty falling asleep and staying asleep. Alzheimer's patients spend less time in non-rapid eye movement (NREM) sleep and more time awake. They also experience disruptions of their sleep-dependent brain rhythms, slow oscillations, prevalent during deep NREM sleep. These sleep deficits are recapitulated in a mouse model of amyloidosis, APP/PS1 (APP) mice. APP mice spend less time in NREM sleep and more time awake. These animals also exhibit disruptions in slow oscillations. However, until recently it was not known whether sleep-dependent impairments are symptomatic of the disease or whether these sleep impairments actively contribute to Alzheimer's progression. **Methods:** We used optogenetics to target either cortical excitatory neurons, inhibitory neurons or astrocytes to rescue slow oscillations, which were monitored with widefield imaging of voltage sensors through cranial windows in APP mice. The effects of chronic optogenetic stimulation (continuously for 2-4 weeks) were determined on neuropathophysiology, sleep and memory function of APP mice. AD-related neuropathophysiology, amyloid deposition and neuronal calcium homeostasis, was monitored through cranial windows using high-resolution multiphoton microscopy. Sleep was monitored using fully implantable sleep (EEG/EMG) telemetry system followed by automated sleep

scoring to minimize experimenter biases. Sleep-dependent memory consolidation was assessed using the fear conditioning paradigm. **Results:** Optogenetic stimulation of either excitatory neurons, inhibitory neurons or astrocytes restored slow oscillations in APP mice. Chronic optogenetic treatment halted amyloid plaque deposition and normalized neuronal calcium levels. Furthermore, slow wave rescue restored sleep and sleep-dependent memory consolidation. **Conclusion:** These results suggest that APP mice exhibit sleep deficits and deficits in sleep-dependent brain rhythms. Optogenetic targeting of three distinct elements of the circuit, excitatory neurons, inhibitory neurons or astrocytes, slowed Alzheimer's progression, rescued sleep and memory. Thus, sleep-dependent impairments actively contribute to Alzheimer's progression and could be targeted with therapeutics to slow Alzheimer's disease.

S09.4 - Effects of chronic trazodone administration on sleep and neuropathology in the APP NL-F mouse model of Alzheimer's disease

Mayuko Arai [1], **Emad Shams** [1], **Jefferey Yue** [1], **Cody Stevens** [1], **Brianne Kent** [1]

[1] Simon Fraser University

A majority of patients with Alzheimer's disease (AD) experience some form of sleep disruption, often characterized by reduced slow-wave sleep (SWS, defined by low-frequency oscillations in the electroencephalogram (EEG)). Research suggests that sleep disturbances contribute to the clinical presentation and pathological manifestations of AD and that treating sleep disorders in this population may target basic mechanisms of the disease. Trazodone, an antidepressant with sleep-promoting properties, is frequently prescribed off-label to improve sleep. Notably, it is well-tolerated in elderly individuals and has been shown to specifically enhance SWS. Given its potential benefits, trazodone has been proposed as an intervention for AD. However, research on its effects beyond its antidepressant properties remains limited. This study aimed to evaluate the effects of chronic trazodone administration in a mouse model of AD, marking the first assessment of its potential as a disease-modifying therapeutic for AD.

We developed a novel translationally focused protocol for orally administering trazodone to C57BL/6J mice and demonstrated that acute trazodone administration dose-dependently enhances NREM sleep and increases delta power during NREM sleep. Using this validated protocol, we administered trazodone (60mg/kg) daily to APP NL-F mice for 60 days to evaluate its effects on sleep and AD pathology. Two age groups were selected to represent early (9 months) and late (14 months) stages of neuropathology development. Preliminary analysis revealed that trazodone consistently enhances slow oscillation (0.5–1 Hz) and delta (0.5–4 Hz) power during NREM sleep over a 60-day treatment period ($p = 0.0040$, comparing baseline with 1, 4, and 8 weeks of treatment in the 9-month-old cohort). The slow oscillation power was also significantly higher compared to the vehicle control, indicating a notable treatment effect ($p = 0.0246$, comparing the treatment and control groups at 1, 4, and 8 weeks). Importantly, preliminary neuropathological analysis reveals that 60 days of trazodone treatment leads to a reduction in Aβ pathologies, particularly in male mice. In male APP NL-F mice treated with trazodone from 14 to 16 months of age, the treatment significantly reduced amyloid plaques in the dorsal hippocampus ($p = 0.0174$) and fibrillary plaques in the parietal cortex ($p = 0.0495$). Furthermore, trazodone treatment in the male mice showed a downward trend in insoluble Aβ42 levels ($p = 0.0555$). Taken together, these findings suggest that trazodone has the potential to serve as a novel disease-modifying therapeutic for neurodegenerative diseases by improving sleep and slowing the progression of underlying neuropathology.

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SYMPOSIUM 10: THERAPEUTIC APPLICATIONS FOR FOCUSED ULTRASOUND IN THE TREATMENT AND DIAGNOSIS OF ALZHEIMER'S AND PARKINSON'S DISEASE

S10.1 - MR-guided-focused ultrasound mediated delivery of AAV9.SIRT3-myc is neuroprotective in a rat model of Parkinson's Disease

Joanne Nash [1], **Dennison Trinh** [1], **Kate Noseworthy** [2], **Madeline Mencher** [1], **Elfin Akteke** [1], **Ahmad Israwi** [1], **Ivy Pham** [1], **Sheng-Kai Wu** [2], **Rikke Kofoed** [2], **Isabelle Aubert** [2], **Kullervo Hynynen** [2]

[1] University of Toronto, [2] Sunnybrook Research Institute

Background:
Mitochondrial dysfunction and α-synuclein pathology are key contributors to Parkinson's disease (PD) pathogenesis. We have shown that levels of Sirtuin 3 (SIRT3), a mitochondrial deacetylase are inversely correlated with α-synuclein pathology in human subjects with PD. Furthermore, in pre-clinical rat models of PD, we have previously shown that intranigral infusion of AAV1.SIRT3-myc has both neuroprotective and rejuvenative effects. This suggests that increasing SIRT3 expression may provide disease-modifying effects in PD. MR-guided-focused ultrasound (MR-g-FUS) mediated permeabilization of the blood brain barrier offers a non-invasive approach to enhance SIRT3 delivery. Here, we investigate whether FUS-mediated delivery of AAV9.SIRT3-myc expression can mitigate neurodegeneration and behavioral deficits in the virally over-expressing mutant α-synuclein rat, a unilateral model of PD that recapitulates dopaminergic loss, α-synuclein pathology, and behavioral impairments.

Methods:
AAV-SIRT3 was delivered intravenously with and without FUS to brain regions affected in PD (substantia nigra (SN), striatum and hippocampus). Two weeks later, rats were intra-nigrally infused with AAV-α-synuclein to induce PD-like pathology. Behavioral outcomes were assessed using the cylinder test and RotoRod. Dopaminergic cell survival and α-synuclein pathology were assessed postmortem using unbiased stereology and immunohistochemistry.

Results:
Following IV infusion of AAV9.SIRT3-myc, MRgFUS resulted in a 2.8-, 2.5-, and 1.5-fold increase in SIRT3 expression in the striatum, hippocampus and SN respectively. In parkinsonian rats, elevation of SIRT3 prevented motor dysfunction in the forelimb asymmetry test (A53T+control:43.0±8.7% vs. A53T+SIRT3:18.2±6.1%). In parkinsonian rats, stereology showed a 56.4±15.7% decrease in dopaminergic cells compared to non-parkinsonian rats. This loss of dopaminergic neurons was prevented by MRgFUS-mediated delivery of AAV9.SIRT3-myc.

Conclusion:
These findings demonstrate that FUS-enhanced SIRT3 delivery provides robust neuroprotection and restores motor function in a preclinical PD model, highlighting its potential as a non-invasive therapeutic strategy. This approach may pave the way for targeted mitochondrial therapies in PD and other neurodegenerative disorders.

S10.2 - Blood-brain barrier modulation with focused ultrasound: Preclinical and clinical updates for the treatment of Alzheimer's disease

Isabelle Aubert [1]

[1] Sunnybrook Research Institute

Our collaborative research program is developing therapies for neurodegenerative disorders. We use focused ultrasound (FUS) combined with microbubbles to modify the blood-brain barrier (BBB), promote neural plasticity, and deliver therapeutics to the brain.

MRI-guided FUS-BBB modulation provides millimetric precision for the safe treatment of small or large brain volumes, from mice to humans. In animal models of Alzheimer's disease, the biological effects of FUS-BBB modulation alone have been shown to reduce pathology, trigger regenerative events, and improve cognitive function. Repeated treatments with intravenous therapeutics and FUS-BBB modulation for brain delivery significantly improve treatment outcomes in animal models of Alzheimer's disease and in patients. Gene therapy offers the potential for a one-time delivery, providing long-term benefits to treat neurodegenerative conditions.

Gene therapy is progressing rapidly, with advances in bioengineering and the use of recombinant adeno-associated viruses (AAVs) as efficient and safe carriers for human gene delivery. Image-guided FUS technologies can meet the need for minimally invasive gene delivery to targeted regions of the central nervous system (CNS). We tested the delivery of serotypes AAV1, 2, 5, 8, 9, PHP, HBKO, administered intravenously or intracisterna magna, to the CNS using MRI-guided FUS in rodents. Data suggest that several factors influence the transduction efficiency of AAV in different cell types. These include the route of administration, the degree of FUS-BBB modulation, the targeted brain regions, the properties of the serotypes (e.g., tropism and diffusion peripherally and centrally), and the animal strain or species.

In summary, in animal models of Alzheimer's disease and in patients, FUS-BBB modulation is controlled, localized, and transient. Treatment efficacy increases when FUS-BBB modulation is used in combination with intravenous therapeutics. In animal models, FUS-BBB modulation has been shown to acute proinflammation, resolving into a permissive regenerative environment that can be harnessed for drugs, genes and cell therapies. FUS offers a promising modality for fundamental research and clinical applications for neurological disorders.

S10.3 - Tremor reduction using a multi-focus transcranial ultrasound stimulation method targeting the thalamus: Preliminary results

Samuel Pichardo [1], **Alan Coreas** [1], **Shirshak Shrestha** [2], **Janet Adeoti** [2], **Georgia Peacock** [2], **Catherine Swytink-Binnema** [1], **Nishaad Sheth** [2], **Ana Arantes** [2], **Conrad Rockel** [3], **Davide Martino** [4], **Bruce Pike** [2], **Zelma Kiss** [2]

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Tremor reduction using a multi-focus transcranial ultrasound stimulation method targeting the thalamus: Preliminary results

Background. Transcranial ultrasound stimulation (TUS) is a non-invasive technique that focuses mechanical energy deep in the brain to modulate neural activity. However, targeting errors due to neuronavigation tracking imperfections can hinder TUS effectiveness, especially with deep brain targets. This study reports a multi-focus TUS strategy to target thalamus for tremor reduction in patients with essential (ET) and Parkinson's tremor (PD).

Methods. Fourteen patients (9 ET, 5 PD) underwent TUS targeting the ventrointermediate (Vim) using a 128-element phased array operating at 250 kHz in an open label study. TUS was first delivered at 7 locations arranged in a circular disc pattern with inhibitory parameters (100 Hz pulse repetition frequency, 10% duty cycle, intensity at situ of 10 W/cm^2, initially 30 s durations, 10.1016/j.brs.2024.04.005). BabelBrain software (10.1109/TUFFC.2023.3274046) was used for planning the TUS delivery using subject 1 mm-isotropic T1- and T2-weighted MR imaging and diffusion tensor imaging (DTI) for tractography of the dentato-rubro-thalamo-cortical tract (DRTT) to identify its termination in Vim thalamus. Skull effects in ultrasound propagation were considered using CT (0.45 mm-pixel resolution, 0.63 mm-slice thickness, 120 kVp, 170 mA, BONEPLUS kernel) or pseudo-CT imaging derived from 1mm-isotropic Zero Echo Time MR imaging. The tremor was measured using accelerometry before and after stimulation (0 min and 5 min). The 7 locations were split into two groups, and the group showing the most tremor reduction was further explored with individual location targeting with 2 min sonications.

Results. Figure 1 shows the experimental setup, sonication strategies, patient workflow, simulation of acoustic fields and summary of accelerometer readouts. TUS inhibitory protocol reduced tremor in 9 (ET=6, PD=3) /14 patients. The mean reduction (in dB scale, +/- s.d.) compared between the last accelerometer measurement to baseline was -11.1 (+/- 9) dB and p=0.0012 (Wilcoxon signed rank test, 2-tailed). When considering only ET patients (n=9), the mean reduction was -14 (+/- 8.5) dB and p=0.004. When considering only PD patients (n=5), the mean reduction was -6 (+/- 7.7) dB and p=0.312). The ability to focus on the Vim target with 15 mm axial coverage provided flexibility for multiple focusing. The procedure was well tolerated (took ~2h) with no side effects. However, cumulative effects made the selection of optimal targets challenging.

Conclusions. Multi-focus TUS improved targeting by identifying optimal stimulation locations, enhancing tremor reduction compared to baseline and over time. Because tremor seemed to remain suppressed after initial sonications, it suggests that there may be a cumulative effect of TUS. A major limitation is that this is open-label; future work will execute a sham-controlled, blinded protocol. Nevertheless, these initial findings are encouraging to justify new studies on TUS on movement disorders indications such as ET and PD.

S10.4 - Exploring the potential of transcranial focused ultrasound to modulate pain sensitivity in Parkinson's disease

Oury Monchi [1], **Ali Ak Zadeh** [2], **Pierre Rainville** [1], **Samuel Pichardo** [3]

[1] Université de Montréal, [2] University of Calgary, [3] University of Calgary, Hotchkiss Brain Institute

Parkinson's disease (PD) is the fastest-growing neurological disorder, affecting more than 100,000 Canadians. Chronic pain has emerged as the most prevalent non-motor symptom of

PD (estimated between 40 and 85% and significantly higher than in the general population), yet it remains an underdiagnosed and undertreated symptom despite being one of the most bothersome symptoms of PD.

There is increased interest in using transcranial focused ultrasound (tFUS) as a tool to modulate pain sensitivity in the general population. Existing studies indicate that tFUS stimulation of distinct regions of the pain network might affect pain processing in healthy individuals differently. In this presentation, we will review these studies (e.g. [1]), including ongoing studies in our laboratory, that have or are investigating the effect of tFUS stimulations of distinct regions on pain sensitivity measures. Following studies by Shamli Oghli et al. [2], and others by our team [3], we will also argue for the importance of parameter selection when using tFUS for behavioural modulation, including pain processing.

We will propose new research avenues to explore the potential of tFUS to modulate pain processing in PD specifically and potentially be used as a treatment complement to alleviate chronic pain in the disease. We will argue that they require a better understanding of the neural origins of pain processing in the disease.

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SYMPOSIUM 11: MODELS OF INHALED CANNABIS EXPOSURE: EFFECTS ON BEHAVIOUR AND BRAIN ACROSS THE LIFESPAN OF RODENTS

S11.1 - Parallel symposium 11: Models of inhaled cannabis exposure: Effects on behaviour and brain across the lifespan of rodents

John Howland [1], **Hakan Kayir** [2], **Ryan Mclaughlin** [3], **Catherine Moore** [4]

[1] University of Saskatchewan, [2] University of Western Ontario, [3] Washington State University, [4] Johns Hopkins University School of Medicine

The legalization of cannabis has resulted in increased use for some Canadians and concern regarding the effects on brain and behaviour. As most Canadians continue to consume cannabis products through inhalation, animal models of this mode of delivery are critically needed to understand the specific effects of cannabis on brain circuits. Speakers in this symposium will summarize findings regarding the development and implementation of models of inhaled cannabis use in rodents. Dr. Howland will detail findings related to the effects of

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gestational exposure to cannabis smoke on offspring behaviour. Dr. Kayir will describe the long-term effects of adolescent cannabis vapour exposure on behaviour and brain connectivity. Dr. McLaughlin will share findings related to altered decision making in adulthood following adolescent cannabis exposure. Dr. Moore will summarize work developing a vapor self-administration model in adult rats and present findings on the effects of long-term administration of Δ9-tetrahydrocannabinol vapor on anxiety-like behavior and neurobiology. Taken together, these presentations will highlight how inhaled cannabis exposure at different developmental stages alters brain circuits involved in emotional behaviours, cognition, and motivation.

S11.2 - Impact of adolescent exposure to vaporized cannabis on adult rat behaviour and brain connectivity

Jaiden Smith [1], **Patrick Mccunn** [2], **Pedro Marinho** [2], **Jibran Khokhar** [2], **Hakan Kayir** [3]

[1] University of Guelph, [2] Western University, [3] University of Western Ontario

Cannabis use during adolescence has surged with the popularity of vaping, yet the long-term neurobehavioral and neurobiological consequences of adolescent exposure to different cannabis strains remain unclear. Here, we investigated how daily vapor exposure to high-THC, high-CBD, or balanced THC/CBD contained cannabis flowers during adolescence (postnatal days 28–42) affects adult behavior, cognition, and brain connectivity in male and female Sprague-Dawley rats. Blood cannabinoid levels and cannabinoid tetrad tests (locomotion, analgesia, body temperature, catalepsy) confirmed adequate cannabis exposure. In adulthood, rats underwent Pavlovian autoshaping, active avoidance, and prepulse inhibition (PPI) testing, followed by diffusion and functional MRI at 9.4T.

Males exposed to high-THC or high-CBD cannabis displayed reduced lever-directed (sign-tracking) behavior during autoshaping compared to control and balanced-exposed groups. All three cannabis-exposed male groups showed significant impairments in active avoidance task, suggesting impaired operant-based aversive learning, whereas PPI remained intact. In contrast, female rats were predominantly sign-trackers regardless of cannabis strain, and cannabis exposure did not disrupt active avoidance relative to controls.

Neuroimaging revealed that, in males, adolescent cannabis exposure produced distinct functional and structural connectivity changes. Resting-state fMRI identified altered connectivity in sensory (somatosensory and visual) and subcortical (pontine reticular and raphe nuclei) networks. Diffusion MRI similarly highlighted structural differences in key regions regulating emotion, memory, and sensory integration (e.g., striatum, ventral thalamus, entorhinal cortex, pontine nuclei). In females, only minimal network-level connectivity changes were observed.

Collectively, these findings indicate that adolescent cannabis vapor exposure has lasting, sex-dependent effects on reward-related behavior and brain circuitry, with males showing pronounced vulnerability. Moreover, the distinct THC/CBD content of cannabis strains differentially modulated behavioral outcomes and connectivity patterns, underscoring the need for further research to elucidate how specific cannabinoid profiles impact adolescent brain development.

S11.3 - Functional, structural, and behavioral impacts of adolescent cannabis vapor exposure in adult male and female rats

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Cannabis is the most used drug among adolescents, with a lifetime prevalence of nearly double that of all other illicit drugs combined. This is alarming as the long-term neurobehavioral consequences of adolescent cannabis use remain poorly understood. Recently, we reported that vaporized cannabis self-administration in adolescence led to long-lasting impairments in medial prefrontal cortex (mPFC)-dependent cognitive flexibility. Parvalbumin interneurons (PV) mediate cognitive flexibility as their inhibitory function tightly regulates mPFC output neurons, and PV interneuron function is supported by perineuronal nets (PNNs), which preferentially surround this cell type. Thus, we hypothesized that exposure to vaporized cannabis during adolescence induces long-lasting aberrations in PV function in the mPFC, possibly by altering PNNs, thereby leading to cognitive inflexibility. To test this hypothesis, adolescent male and female Sprague-Dawley rats received daily non-contingent vaporized cannabis extract (63.9% delta-9-THC; extract diluted to 150 mg/ml) or vehicle (polyethylene glycol-400) exposure from postnatal day (P) 35-55 (3-s 'puff' every 2 min for 60 min). On ~P58, rats received bilateral microinfusions of a PV enhancer virus (AAV.PHP.eb-S5E2-dTom-nlsdTom; 300nl/side) into the prelimbic subregion of the mPFC. After a two-week washout period encompassing recovery from surgery, cognitive flexibility testing began on ~P70 using an operant-based attentional set-shifting task. After behavioral testing, the brains of littermates were either collected for immunohistochemistry (IHC) or whole cell patch clamp slice electrophysiology to record from viral-mediated fluorescently tagged PV cells in the mPFC. Our findings indicate that cannabis-exposed rats of both sexes were impaired in the set shifting but not reversal learning component of the task, requiring significantly more trials to reach criterion, and had reduced fluorescent intensity of PNNs surrounding PV cells in the mPFC compared to vehicle-exposed rats. Interestingly*, ex vivo* electrophysiology studies revealed that PV cells from cannabis-exposed females were more excitable (increased spiking and lower rheobase) than PV cells from vehicle vapor-exposed females, with no significant differences observed in males. These findings support the hypothesis that adolescent vaporized cannabis exposure impairs mPFC-dependent cognitive flexibility in adulthood and that this coincides with increased intrinsic excitability of PV interneurons and alterations in PNNs that surround them. Thus, normalizing PNNs and PV cell function may be promising targets to alleviate adolescent cannabis-induced mPFC dysfunction.

S11.4 - Behavioral and neurobiological consequences of chronic vaporized Δ9-tetrahydrocannabinol (THC) self-administration in rats

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Vaping of cannabis and cannabis extracts containing Δ-9-tetrahydrocannabinol (THC, the primary psychoactive constituent of cannabis) is on the rise, but little is known about the behavioral and neurobiological consequences of chronic vaporized THC. Sprague-Dawley rats (N=96; 12 per sex/group) underwent place conditioning to THC vapor (3 dose conditions) or vehicle vapor (VEH: 100% propylene glycol). Following testing for conditioned place preference, rats were trained to self-administer either THC vapor (50 mg/ml) or vehicle vapor; groups were counterbalanced by pre-exposure condition. Following acquisition of responding under a fixed ratio 1 (FR1) schedule, the FR was increased from 1 to 5 and then the concentration of THC in the e-liquid was varied (5-200 mg/ml) to obtain a dose-effect curve for responding. Following chronic (6 months) voluntary administration of THC vapor, subsets of rats were tested for anxiety-like behavior in the open field test (N=20) and euthanized for *ex vivo* Diffusion Tensor Imaging (DTI; N=7). We saw significant conditioned place preference to the THC vapor-paired chamber: males showed CPP to the 2 highest THC vapor dose conditions and females for the highest dose condition only. Self-administration data were analyzed for rats whose self-administration condition was the same as pre-exposure (i.e., Vehicle-Vehicle or THC-THC). Under an FR1 schedule of reinforcement, there was no difference in number of puffs obtained between THC and Veh vapor groups. When the effort required to obtain vapor puffs increased from FR1 to FR5, female THC animals defended their intake (i.e., increased their responses made) compared with females self-administering vehicle ($p < 0.05$ vs Veh at FR4-FR5). In males, both THC and vehicle groups increased responses made as the FR requirement increased (Sex x FR x Drug interaction; $F_{(4, 124)} = 3.91$, $p < 0.01$). As the THC concentration was adjusted from 5-200 mg/ml, THC animals titrated the number of puffs obtained, self-administering a greater number of puffs at lower drug concentrations (Drug x Dose interaction; $F_{(5, 155)} = 8.24$, $p < 0.001$; $p < 0.05$ for 5 and 10 mg/ml vs. training dose of 50 mg/ml). Females in the THC group responded more at lower dose conditions (5 and 25 mg/ml) and less in the 200 mg/ml compared with females in the vehicle group. Rats who self-administered THC vapor displayed increased anxiety-like behavior in the open field test when assessed 24-hrs post-session ($t(18)=2.99$, $p < 0.01$). Analysis of DTI metrics revealed decreased microstructure and impaired diffusivity in major white matter tracts, including the corpus callosum, in rats that self-administered THC vapor compared with VEH vapor (fractional anisotropy, FA; Mean ±SD: Control = 0.63 ±0.02, THC= 0.57 ±0.03, p=0.015). These results demonstrate that THC vapor maintained self-administration behavior in female rats. Further, chronic THC vapor self-administration resulted in increased anxiety-like behavior and reduced white matter integrity. Translational preclinical models of vaporized THC self-administration are important for informing our understanding of the effects of cannabis constituents and lasting consequences.

SYMPOSIUM 12: THE FUNCTION OF CATECHOLAMINES IN LEARNING AND DECISION-MAKING

S12.1 - Prefrontal dopamine activity during a context fear discrimination task

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BACKGROUND: Learning that an environment is safe or dangerous is critical for our survival and impairments in this process are a central criterion of post-traumatic stress disorder (PTSD). To

develop PTSD treatments, understanding the neural activity that supports fear expression in threatening and neutral environments is a necessary first step.

METHOD: We investigated context fear discrimination in male and female mice using fiber photometry that measured dopamine signaling in the medial prefrontal cortex (mPFC) during a context retrieval task. Mice were threat conditioned inside a cylindrical LED screen to generate threat conditioned freezing behaviour. The following days, the specificity of threat learning was tested by measuring freezing behaviour in response to different visual-audio contexts, while mice remained in the same physical cylindrical space. Throughout the threat conditioning protocol, we used GRAB-DA to measure mPFC dopamine signaling during weak and strong conditioning protocols. Dopamine measures were also collected when we "teleported" mice between neutral context B and threatening context A across multiple testing days.

RESULTS: We report four primary findings: (1) In stronger conditioning protocols, mouse fear discrimination improved across testing days. Overall, male and female mice expressed similar fear discrimination. (2) Conditioning intensity dose-dependently increased mPFC dopamine. (3) Stronger conditioning protocols produced greater mPFC dopamine during context transitions. (4) As fear discrimination improved across context transition trials and testing days, mPFC dopamine was reduced. Trends in sex differences of mPFC dopamine during context transitions will be discussed. Together, these results suggest that mPFC dopamine signaling is altered during context fear memory encoding and retrieval.

CONCLUSIONS: These findings illustrate that mPFC dopamine dynamics are associated with context fear discrimination learning and suggest that mPFC dopamine may be a therapeutic target to reduce fear generalization and anxiety in individuals with PTSD.

S12.2 - The role of the locus coeruleus in learning about reduced reward

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Predictive learning is highly adaptive; it allows us and other animals to use information in the environment to anticipate and prepare for biologically significant events. Equally important is the ability to update previous learning when environmental conditions change, and the organism's predictions about impending events are no longer accurate. I will describe recent experiments that examined the role of the locus coeruleus (LC) in two types of learning that occur when the experienced reward is less than expected based on previous learning. Rats were first trained in a discriminated operant task where three distinct stimuli predicted the availability of food reward if a lever-press response was performed. Once stable responding was established, the rats underwent further training sessions where expected reward was either omitted (extinction) or was less than expected (overexpectation). Optogenetic stimulation of LC neurons during extinction trials, improved the later expression of extinction. In contrast, pharmacological inhibition of the LC with the a_{2A}-adrenergic receptor agonist clonidine during extinction lead to rapid reacquisition, consistent with weakened extinction. To assess whether these effects were specific to extinction, we also examined whether the LC contributes to another example of learning driven by a negative prediction error; overexpectation, where reinforcement is still delivered, but is smaller than anticipated based on the stimuli presented. The LC was inhibited during overexpectation trials where two stimuli were presented together, thus predicting two rewards, but only a single reward was delivered. While control animals learned to decrease responding to the stimuli, in line with the

now smaller reward, those that received LC inhibition failed to selectively update responding. These data demonstrate that activity of the LC is important for learning to reduce responding in both extinction and overexpectation paradigms. The overexpectation results are of particular interest because this procedure provides an opportunity to reduce the influence of predictive stimuli under circumstances were omitting the outcome entirely may not be possible or practical (e.g. in cases of obesity or eating disorders where the individual must continue to eat). Understanding how the brain updates previously learning to reduce the acquired power of predictive stimuli is important for developing more effective therapies that aim to reduce the influence of such stimuli and to do so with more lasting effects. Discovery that LC activity contributes to learning to reduce responding in both extinction and overexpectation paradigms suggests that enhancing noradrenaline during related therapeutic interventions could enhance the benefits of both.

S12.3 - Sex-specific mesocortical regulation of stress outcomes

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The consequences of exposure to adverse events are not determined solely by the physical nature of the event, but rather by complex cognitive factors such as coping ability. In male rats, instrumental control over stress recruits a corticostriatal system involving the prelimbic cortex (PL) and dorsomedial striatum (DMS) that potently blunts a number of stress outcomes. In contrast, the stress-buffering effects of control are completely absent in females, and the mechanisms underlying this absence are not understood. I will present recent data that identify a role for mesocortical dopamine in biasing the acquisition of instrumental control over stress towards the striatal 'habit system' in female rats, thereby preventing the protection typically afforded by coping. Reduced benefit from a resilience factor, rather than enhanced responding to a vulnerability factor, may represent a novel approach for understanding sex-specific mechanisms underlying mood disorders.

S12.4 - Dopamine in the basolateral amygdala supports reward learning and prediction

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To make adaptive decisions, we build an internal model of the associative relationships in an environment and use it to make predictions and inferences about specific available outcomes. Detailed, identity-specific cue–reward memories are a core feature of such cognitive maps. By using fiber photometry measurements of dopamine release, cell-type and pathway-specific optogenetic manipulation, Pavlovian cue–reward conditioning and decision-making tests in male and female rats, we have found that ventral tegmental area dopamine projections to the basolateral amygdala drive the encoding of identity-specific cue–reward memories and the cued reward predictions necessary for adaptive decision making.

POSTER SESSION 1

A-DEVELOPMENT

P1-A-01 - CA3 inputs to CA1 drive the emergence of inhibitory signaling and memory specificity

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A bias towards memory generalization has been observed in both human children and juvenile mice. Children are more likely than adults to mistake a similar lure item for an identical target, while juvenile mice generalize contextual fear memories to similar, but distinct, contexts where an adult would exhibit fear memory only in the original training context. Although there are differences in these tasks and the rate of development, this developmental phenomenon appears to be conserved in altricial mammals and provides an opportunity to study potential mechanisms underlying how our memory system develops. In particular, the hippocampus undergoes a critical period during the transition to greater memory specificity. This critical period has been associated with the maturation of interneurons in the CA1 of the hippocampus and is hypothesized to occur in an activity-dependent manner. Computational modelling also proposes that CA3 to CA1 projections are critical for the formation of specific episodic memories. The present study seeks to investigate the development of memory specificity in both human children and juvenile mice. We show that 1) human children and juvenile mice both show an increase in memory specificity with age, although at a different rate 2) CA3 projections to CA1 are immature in juvenile mice, particularly in their connection to interneurons 3) in mice, the development of memory specificity can be accelerated or delayed by manipulating the activity of CA3 to CA1 projections. These findings suggest that the development of memory specificity relies on the activity-dependent maturation of CA1 interneurons.

P1-A-02 - Early-life bisphenol A exposure triggers anxiety-like behaviors and pyroptotic death of nerve cells in juvenile and adult male rats via the NF-κB/IL-1β/NLRP3/Caspase-1 pathway

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IntroductionBisphenol A (BPA), a common endocrine disruptor, is vastly used in plastics industry. BPA was reported to disrupt hormonal and metabolic pathways, while limited data were available about its effects on neurodevelopment, especially exposure to it during early-life stages. We hypothesize that postnatal BPA exposure causes pyroptosis and activates autophagy that thought to be linked with the development of psychiatric conditions later in life.MethodsEarly BPA exposure began from postnatal day (PND) 18 to PND 60 and to PND 95 at dosages of 50 and 125 mg/kg/day. We started with behavioral tasks, including open field, elevated plus- and Y-maze, performed on young and adult rats. Molecular docking analysis was done in addition to neuroinflammatory and autophagic protein markers measured by ELISA and

immunohistochemistry in the prefrontal cortex (PFC) and hippocampus.ResultsThe in vivo and molecular docking analyses revealed that BPA significantly activated the NF-κB/IL-1β/NLRP3/Caspase-1 pathway and enhanced autophagic markers, in addition to causing unique neurodegenerative histopathological hallmarks in the PFC and hippocampus.DiscussionOur results provide new clues to the underlying mechanism of postnatal BPA exposure to induce neuroinflammation and enhanced autophagic flux leading to activation of pyroptosis and development of long-term anxiety-like behaviors.

P1-A-03 - Role of Glypican 6 on the development of the embryonic mouse neocortex

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

P1-A-04 - How adolescent neurodevelopment is affected by different patterns of cannabis use

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Introduction: This project aims to investigate the effects of varied patterns of vaporized cannabis inhalation on adolescent rodent neurodevelopment and behaviour.Methods: Adolescent (P34) male and female rats were split into four usage cohorts: 1) control non-users, 2) weekly users, 3) daily users, and 4) high frequency (HF) users (daily exposure up to up to 3 times/day). Magnetic resonance imaging (MRI) scans were taken pre- and post-vapour exposure, to quantify individual volumetric differences in corticolimbic regions of interest (ROI). Rats were also subject to three behavioral tests: 1) light-dark box (LDB), 2) fear conditioning, and 3) novel-object-context-mismatch (NOCM).Results: MRI: In some of the ROIs analysed, HF-exposed rats had significantly more volumetric growth than controls, and the daily and weekly exposed rats had significantly less growth than controls. Behaviour: During LDB, the weekly- and daily-exposed rats showed significantly less anxiety-like behaviour than controls and HF. During FC extinction retrieval, the daily- and weekly-exposed rats had a stronger association with the fear memory than controls.

During NOCM, there were no significant effects of treatment.Conclusions: Here we present structural and behavioural evidence for a biphasic effect of cannabinoids, where at low frequencies cannabis had one effect, and at higher frequencies cannabis had different effects.

P1-A-05 - Delays in infant neurodevelopment: Examining the interaction between genetic risk and environmental stressors

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Previous work has unveiled that early adversity characteristics such as poverty and maternal depression can affect infant neurodevelopment, as measured through electroencephalography (EEG). However, relatively few studies explore the role of polygenic risk score (PRS) for depression. PRS is stable across the lifespan and can interact with aspects of early adversity, affecting infant development. This study includes 116 samples from Boston Children's Hospital and Children's Hospital Los Angeles to test how maternal depression and environmental stressors interact with PRS to predict neurodevelopment, particularly frontal alpha asymmetry (FAA), in two-month-old infants. Linear regression analyses will be conducted to test the associations between PRS, environmental stressors (i.e. maternal depression and socioeconomic variables), and 2-month FAA. A moderation analysis will test whether PRS acts as a moderator between environmental risk and neurodevelopment. We expect that environmental risk will predict higher levels of FAA, PRS will predict higher levels of FAA, and PRS will act as a moderator between environmental risk and FAA. The current study bridges current gaps in the literature by exploring how genetic and environmental risk can interact early in infancy, holding implications for early detection of who may be at risk for depression later in life.

P1-A-06 - Astrocyte-mediated regulation of inhibitory interneuron development in the somatosensory cortex: Implications for autism spectrum disorder

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The refinement of primary sensory systems depends on experience-driven plasticity in the brain, which relies on coordinated changes among various cell types within neural circuits during development. This complex interaction between sensory development, neural circuit refinement, and plasticity forms the basis for understanding the mechanisms underlying numerous neurological conditions. Research from our lab and others has established that basket cells (BCs) play a critical role in the development of autism spectrum disorder (ASD), and disruptions in protein expression in BCs have been linked to the absence of astrocytes. However, the specific spatiotemporal relationship between the development of astrocytes and BCs remains unknown. To address this gap, we have employed (1) immunohistochemical techniques to analyze astrocyte branching, protein expression in BCs, and their developmental timing, and (2) calcium imaging of astrocytes and BCs to monitor early synchronous activity, and identify the cessation of this activity, which marks the conclusion of the initial plasticity period in brain development. Our data suggest that delay in astrocyte morphological development is correlated with delay in maturation of BCs in a mouse model of autism.

P1-A-07 - Maternal high-fat diet impairs offspring's cognition and alters synaptic plasticity and transmission

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Introduction: Maternal exposure to high-fat diets (mHFD) is associated with cognitive impairments in offspring, along with molecular and structural changes in the hippocampus. However, it remains unclear whether these behavioral deficits derive from alterations in synaptic plasticity and/or transmission.Methods: Female mice were fed a maternal high-fat diet containing 60% of calories from fat, starting one month before pregnancy and continuing throughout gestation until the end of lactation. Offspring's memory was assessed using novel object recognition and object location memory tests. Electrophysiological recordings were performed in the CA1 region of the hippocampus to evaluate the synaptic efficacy of pyramidal neurons. All experiments were carried out during the juvenile stage, between postnatal days 30 and 45.Results: Offspring exposed to a mHFD showed impaired cognition. We also observed impairment in the excitatory long-term potentiation (LTP) and an increased inhibitory synaptic transmission in hippocampal CA1 pyramidal neurons.Discussion: Our findings show that maternal high-fat diet exposure during critical developmental periods impairs offspring cognition, likely through disruptions in hippocampal synaptic plasticity and excitatory/inhibitory balance. These results suggest synaptic alterations may contribute to cognitive deficits linked to maternal obesity, highlighting potential targets for therapeutic intervention.

P1-A-08 - Decoding neuronal differentiation: the emerging role of splicing order in alternative splicing regulation

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Neuronal differentiation is a complex process that integrates signals driving the engagement of pluripotent stem cells into differentiated neurons. This process relies on precise alternative RNA splicing, which generates diverse mature messenger RNAs from a single gene. Alternative splicing is critical for cell fate decisions, neuronal migration, axon guidance, and synaptogenesis. However, certain aspects of its regulation remain poorly understood, and this study aims to shed light on its mechanisms. The introns flanking alternative exons are frequently excised after their neighbors1. Our hypothesis is that this delayed intron excision is critical for correct alternative splicing decisions to be made. We tested this hypothesis using the induced-neurogenin (iNGN) differentiation model2. We first identified 390 splicing events that are highly regulated during neuronal differentiation using RNA sequencing and validated 13/13 candidates via quantitative RT-PCR. Their intron excision order was then analyzed by droplet digital PCR. Some alternative exons exhibit delayed excision of their flanking introns throughout differentiation, while for other exons, the delay is restricted to a specific stage. We next determined whether partially spliced RNAs accumulate on chromatin to be processed before export. We finally examined chromatin accessibility and its impact on splicing order. Our findings highlight the importance of splicing order during neuronal differentiation and open the door to potential regulatory defects in neurological disorders.

P1-A-09 - Sexually dimorphic long-term effects of acute high-dose edible cannabis consumption in adolescence

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Cannabis use legalization has increased availability of cannabis products, particularly edible cannabis. In adolescents, whose brains are still developing, incidences of edible cannabis-induced intoxication are on the rise, though its long-term effects are unknown. We aim to investigate the long-term effects of acute high-dose edible cannabis consumption (AHDECC) in adolescence.Juvenile Sprague-Dawley rats received either nutella (Nutella group) or nutella-tetrahydrocannabinol (THC; 20 mg/kg) mixture (AHDECC group) and underwent cannabis tetrad tests over 14 days. Seven weeks after Nutella or AHDECC, rats were subdivided into Nutella/Air, Nutella/Cannabis vapor, AHDECC/Air, and AHDECC/Cannabis vapor groups and exposed to air/cannabis vapor for 6 days (3x daily). After last air/cannabis vapor exposure, tetrad tests, somatic withdrawal signs recordings, and sucrose preference tests were performed over 2 days. AHDECC reduced locomotor activity and rectal temperature for 8 h and decreased pain sensitivity for 14 days in male and female rats. Seven weeks after AHDECC, male rats continued to show reduced pain sensitivity. Before chronic cannabis vapor exposure, AHDECC did not affect somatic signs of withdrawal-like behavior in either sex. However, after chronic cannabis vapor exposure, AHDECC increased locomotor activity and further decreased pain sensitivity in male rats, and caused anhedonia and decreased activity in female rats.Our findings suggests that a single adolescent high-dose THC exposure may have long-term negative consequences persisting into adulthood.

P1-A-10 - miR-216/217 cluster affects neuronal differentiation from human pluripotent stem cells

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IntroductionThe human miR-216/217 cluster is associated with tumor suppression across several cancers. No prior studies have linked this cluster to neuronal differentiation from human pluripotent stem cells (hPSC) or stemness regulation. A study analyzing miRNA expression during neural differentiation identified miR-217 as one of the top 20 upregulated miRNAs in neural stem cells (NSC). This work aims to clarify the role of this cluster during neuronal differentiation of hPSC. MethodsWe differentiated H9 human embryonic stem cells (hESC) into NSCs and neurons (NEU). We conducted a differential expression analysis to identify miRNAs with stage-specific expression patterns and compared with published RNA-Seq datasets. The expression profile of this cluster was validated in an induced pluripotent stem cell line (hiPSC). To test its functional impact, we used exogenous molecules to over-express (mimic) or inhibit (inhibitor) miR-217 expression in hPSC and NSC. ResultsThis cluster was expressed exclusively in NSC and NEU. Overexpression of miR-217 in hPSC increased the G1-phase population without affecting cell viability. Either overexpression or inhibition of miR-217 in NSC did not alter cell cycle distribution. Gene target analysis showed that miR-217 overexpression decreased mRNA levels of OCT-4 and GRIA3 in hESC and WEE1, SIRT1, and CYCLIN D1 in hiPSC. This genes are associated with stemness and proliferation. DiscussionOur results suggest that the miR-216/217 cluster, particularly miR-217, may participate in the regulation of the early stages of neural specification from hPSC.

P1-A-11 - Regulation of neural progenitor cell proliferation by Dachshund family transcription factor 1 in the developing mouse neocortex

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The neocortex is responsible for higher cognitive functions in mammals and consists of neurons generated from neural progenitor cells (NPCs) during development. The abundance and proliferative capacity of NPCs, which can be regulated by external signalling molecules, thus determines the number of cortical neurons that can be generated. Preliminary findings in our lab determined that the placental hormone growth differentiation factor 15 (GDF15) promotes NPC proliferation, and identified Dachshund family transcription factor 1 (Dach1) as a downstream effector of GDF15. This project aims to determine the role of Dach1 in NPC behaviour in the developing mouse neocortex. We performed in utero electroporation to inject Dach1 overexpression vector into the brain ventricles of wildtype and GDF15 knockout C57BL/6N mice at embryonic day (E) 13.5. The brains were dissected at E15.5 and E18.5 and examined using immunofluorescence and confocal microscopy. The abundance and proliferative capacity of NPCs and the abundance of cortical neurons in control and Dach1-overexpressed mouse neocortices were quantified to determine how Dach1 affects NPC proliferation, and ultimately neocortex development. The findings from this study will contribute to an enhanced understanding of the regulatory effects that placental factors and their downstream targets have on cells of the developing brain. The knowledge obtained from this project can help reveal how proper brain structure and function is established and potentially be used to advance therapeutic approaches for neurodevelopmental disorders.

P1-A-12 - Brain myeloid cells regulate oligodendrogenesis and myelination in the developing central nervous system

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Myelin is the insulating membrane surrounding axons which is formed by mature oligodendrocytes starting in the second trimester in humans and in the early postnatal period in mice, and is crucial for central nervous system (CNS) function. However, there is still lack of understanding of the fundamental mechanisms contributing to developmental myelination. CNS-resident myeloid cells are a heterogeneous group, encompassing microglia, border-associated macrophages (BAMs) in the meninges, the choroid plexus and the perivascular space. Together, these cells are promising candidates as their depletion impairs oligodendrogenesis and myelination; nonetheless, the specific myeloid cell subtype driving these processes is unclear. Our recent work using a mouse model specifically lacking microglia indicated that these alone are not required for developmental oligodendrogenesis and myelination. Here, we uncover the contribution of BAMs to myelin development. We identified that BAM numbers increase coinciding with oligodendrogenesis and myelination in mouse and human developing brains. By developing two new transgenic models of specific depletion of BAMs, we observed reduced oligodendrogenesis at early postnatal ages. Overall, our study reveals a novel cellular interaction facilitating oligodendrogenesis and myelination, highlighting that different phases of myelination are regulated by distinct CNS myeloid cell subsets.

P1-A-13 - Investigation of cerebrovascular deficits in animal and cellular models of SYNGAP1 deficiency

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The health of blood vessels is critical for brain maturation, as they supply growth factors, nutrients and oxygen, and protects brain cells from pathogens. Early deficits in the cerebrovasculature may cause lifelong neuronal impairments. We have shown that endothelial cell (EC) dysfunction contributes to functional changes in models of 16p11.2 deletion syndrome, associated with autism spectrum disorders. However, the role of EC dysfunction in other neurodevelopmental disorders (NDDs) has yet to be investigated, for instance, in SYNGAP1 intellectual disability (SYNGAP1-ID). SYNGAP1-ID is caused by mutations in the SYNGAP1 gene, leading to cognitive impairments, seizures and communication deficits. Here, we hypothesize that deficiency in SYNGAP1 protein alters EC structure/function. To address this knowledge gap, we are using both in vitro and in vivo approaches. Preliminary data obtained with SYNGAP1-deficient human-induced ECs (hiECs), differentiated from patient-derived pluripotent stem cells (iPSCs), suggest altered distribution of EC-specific adhesion molecule VE-cadherin, with increased junctional protrusions and gaps. We also measured altered angiogenic activity in Matrigel® with SYNGAP1-deficient hiECs compared to healthy controls. We will now examine the impact of EC-specific SYNGAP1 deletion in vivo (Syngap1ΔEC mice) on cerebrovascular maturation, as well as EC morphology and function in vitro using primary mouse brain ECs. This project will provide novel insight into the role of SYNGAP1 in ECs, broadening the implications of cerebrovascular health in NDDs.

P1-A-14 - Sex-dependent modulation of the lateral hypothalamus-dorsal raphe nucleus pathway following acute stress

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Acute stress affects neural activity and emotional regulation, affecting circuits that maintain mood stability. Orexinergic inputs to the dorsal raphe nucleus (DRN) play a critical role in modulating serotonin-driven stress responses, which are essential for emotional balance. Using foot shock, a model of acute stress, we examined its effects on social, stress coping and anxiety-like behaviours in male and female C57Bl6 mice. Mice received 10 shocks (2 seconds each), followed by behavioural tests that assess socioemotional regulation. In vivo calcium imaging of orexinergic inputs to the DRN in foot-shocked mice revealed a significant increase in neuronal activity of this pathway, and a threat-induced alteration in orexin activity. Ex vivo optogenetic stimulation of orexinergic terminals in the DRN, infused with light-activated opsins (ChR2), produced altered DRN firing patterns in shocked mice compared to control mice. Overall, our findings indicate that acute foot-shock disrupts orexin inputs to the DRN, and may result in dysregulation of orexins, which regulate sleep, appetite, and emotion, and is linked to disorders such as depression and anxiety, making this pathway promising target for treatment. These findings also highlight that orexins contribute to sex differences in stress responses and subsequent mental health phenotypes, emphasizing the need to understand sex-dependent mechanisms underlying emotional dysregulation for the development of effective treatment strategies.

P1-A-15 - Disruption in serotonin modulation of the cerebellum in a mouse model of Autism spectrum disorder

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Autism spectrum disorders (ASD) are neurodevelopmental conditions characterized by socioemotional dysfunction, motor coordination deficits, and impaired cognitive function. Traditionally considered a motor control center, the cerebellum is now recognized for its role in cognition and social behaviour. Postmortem analysis of ASD brain tissue reveals a significant reduction in the number and size of cerebellar Purkinje cells (PCs), which are critical for cerebellar function. During development, serotonin receptors in cerebellar cells modulate synaptic plasticity and the maturation of PCs. As serotonin dysregulation is a hallmark of ASD, its impact on PCs and associated behavioural outcomes remains unclear. In this study, we hypothesized that disruption in serotonin-induced modulation of the cerebellum contributes to ASD-induced behavioural dysfunction. Using a mouse model lacking the Engrailed-2 transcription factor (En2 KO mice), which exhibits cerebellar serotonin deficits and ASD-like phenotypes, we investigated the link between cerebellar serotonin signaling, neurophysiology, and behaviour. Whole-cell patch-clamp electrophysiology of cerebellar PCs revealed a disrupted excitatory response to serotonin in En2 KO mice compared to WT controls. Fiber photometry demonstrated impaired serotonin release in the cerebellum during motor, social, and cognitive tasks. Behavioural analysis has also revealed significant sex differences, with social deficits being more pronounced in male En2 KO mice. These findings underscore a previously unrecognized role of cerebellar serotonin dysregulation in driving ASD-related behaviours. Understanding the mechanisms underlying these disruptions provides a framework for developing novel, targeted therapies for ASD.

P1-A-16 - Evaluating the risk of air pollution nanoparticles in developing human brain using human cortical organoids

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Autism Spectrum Disorder (ASD) is a complex neurodevelopmental condition characterized by impaired social communication, speech, and motor skills. The global prevalence of ASD is rising, now affecting 1 in 36 children. Recent epidemiological studies suggest that exposure to air pollution, particularly carbon black nanoparticles (CBNPs) been potentially linked to onset of ASD. Prenatal exposure to CBNPs has been shown to adversely affect early brain development along with persistent effects in animal models, although the underlying mechanisms in human brain remain unclear. To investigate the toxic effects of CBNPs on physiological and structural features of the developing human brain, we generated cortical organoids through guided differentiation of human embryonic stem cells. We found that CBNPs exposure was associated with a general reduction in size and circularity of cortical organoids in a dose- and time-dependent manner. The impact of CBNPs exposure on brain cell differentiation will be analyzed through immunostaining for various cell types, including neurons, astrocytes, and neural stem cells. We anticipate that CBNPs exposure will lead to alterations in cell type distribution and dysregulation of ASD risk genes, accompanied by changes in chromatin accessibility. Our current findings and expectations support the potential role of air pollution in negatively

impacting brain development and provide critical molecular insights into the toxic effects of nanoparticles on early human brain development and their potential role in neurodevelopmental disorders.

P1-A-17 - Impact of a paternal diet high in fat and sugar on offspring brain structure volumes

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Studies in our lab show that parental consumption of a high-fat, high-sugar Western diet (WD), leads to changes in offspring neurodevelopment. We investigated the paternal contribution to this outcome by feeding only the fathers the WD. Four-week-old C57Bl6/J mice were placed on either a WD or low-fat low-sugar control diet (CD) for an eight-week acclimation period. Female mice were fed CD over the same time frame. Animals were brought together for breeding over a three day period; males were then removed from the cages and dams continued on CD during gestation and lactation. Offspring were weaned onto CD at postnatal day (P) 21. At P42, offspring brains were harvested for ex vivo MRI. Brains were scanned with a 7-Tesla 306-mm horizontal bore magnet, scanned with a 3D T2 weighted sequence. Images were segmented with the Multiple Automatically Generated Templates algorithm and existing atlases. Relative structure volumes were fitted with a linear mixed effects model, significance determined with a 10% false discovery rate (FDR). Sires on the WD gained significantly more weight than those on the CD. However, paternal WD consumption did not cause significant weight differences in offspring, nor did it result in significant changes to offspring brain structure. Trends of WD-induced changes were observed in 12 brain structures; however, none were considered significant after FDR correction. We acknowledge that our results cannot rule out small changes in offspring caused by paternal diet, that were undetectable here. However, our findings suggest paternal WD consumption alone may not cause substantial neurodevelopmental changes in offspring, especially as compared to offspring effects observed previously and attributed to maternal diet effects.

P1-A-18 - The developmental trajectory of a unique and sparse cell type in the cortex

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Deep within the neocortex lies a sparse and often-ignored excitatory cell type within layer 6b that is critical for neocortical development. In contrast to all other excitatory neurons in the neocortex, layer 6b neurons are derived from a precursor population known as the subplate, and are the first neuronal population to differentiate. Interestingly, these neurons have a continuous transition to the deepest part of the claustrum, and both these structures are known to share developmental features. As such, we were motivated to examine the developmental trajectories of the subplate and claustrum. To accomplish this, we conducted single-cell spatial transcriptomics on the cortex across embryonic stages. This comprehensive dataset revealed that the subplate and claustrum have subtypes with unique marker gene expression across development. Notably, some subplate and claustral subtypes share similar gene expression profiles, while others are largely different. This suggests that these two structures may share a

developmental origin and diverge into transcriptomically unique structures later in development. Furthermore, subplate cells demonstrate a distinct developmental trajectory, where early embryonic subplate cells are transcriptomically unique from subplate cells at later timepoints, suggesting a convergence into a transcriptomically similar profile. This study provides insight into the developmental trajectories of the subplate and claustrum, which can provide an understanding of how perturbations during this critical window may contribute to neurodevelopmental disorders.

P1-A-19 - Constitutive targeting to the autophagosome may limit netrin-3 secretion

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Although netrin-1 is essential for normal neural development, its close paralogue, netrin-3, has remained largely unstudied. Like netrin-1, netrin-3 is expressed by neurons in the developing and adult CNS. Compared to netrin-1, netrin-3 expression has a more restricted distribution and begins later in embryogenesis. Recent findings have identified de novo single nucleotide mutations in the coding sequence of human netrin-3 in individuals with developmental intellectual disorder and features of autism. We are investigating the hypothesis that these mutations disrupt netrin-3 function, affecting neural development. The literature considers netrin-3 to be a functionally equivalent netrin-1 paralogue. In contrast, my studies identified substantial differences between the two proteins. Secretion of netrin-3 appears to be limited compared to netrin-1. Immunolabeling of cells double-transfected to express ectopic netrin-1 and netrin-3 show the two proteins localized to different subcellular compartments. Furthermore, subcellular fractionation provides evidence that secretion of mutant netrin-3 proteins is severely compromised, consistent with loss-of-function. However, the intracellular trafficking and ultimate destinations of wild-type and mutant netrin-3 proteins remain unclear. Preliminary data suggests netrin-3 may be degraded by the autophagosomal-lysosomal pathway. Ongoing studies are investigating the cellular half-life and secretory processing of netrin-1 and netrin-3 to determine how disruption of its function may contribute to neurodevelopmental disorders like ASD.

P1-A-20 - Investigating how Netrin-1 regulates hippocampal dendritic spine morphology in the developing and aging brain

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Glutamatergic terminals primarily synapse onto dendritic spines. Changes in spine structure correlate with synaptic strength, with mature, mushroom-type spines containing high levels of AMPARs, and filopodia-like, immature thin-type spines exhibiting lower levels. Activity-dependent LTP increases the volume of immature dendritic spines and promotes the trafficking of AMPARs into the spine head, suggesting that the maturation of thin-type spines is crucial for activity-dependent enhancement of excitatory synaptic transmission. Netrin-1 secretion is driven by NMDA receptor activation at synapses. In cultured neurons, netrin-1 contributes to dendritic spine maturation by increasing the density of dendritic filopodia. To determine if netrin-1 regulates dendritic spine volume, we used time-lapse confocal imaging of tertiary apical dendrites from CA1 pyramidal neurons in organotypic hippocampal slice cultures. Bath

application of netrin-1 selectively increased the volume of thin-type spines, but not mushroom-type spines, suggesting that netrin-1 promotes the maturation of thin-type spines. Netrin-1 continues to be expressed in the mature brain. To investigate the consequences of a lack of neuronal netrin-1 expression in the aged brain, we generated CaMKII-Cre/Ntn1fl/fl (cKO) mice, which exhibit deficits in spatial memory and attenuated LTP. Preliminary findings from Golgi-Cox staining of aged cKO mice revealed altered spine density and morphology. Our ongoing studies aim to investigate the impact of netrin-1 on dendritic spine morphology during maturation and aging.

P1-A-21 - Is cerebral white matter development linked to somatic height growth?

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Diffusion MRI shows clear patterns of cerebral white matter (WM) microstructural development typically attributed to increasing myelination and axon packing. Children in more advanced stages of puberty tend to be in more advanced stages of WM development than age-matched peers. However, axons are pruned peripubertally such that energy formerly used by the brain can be allocated for the pubertal somatic growth spurt. Thus, healthy children with earlier growth spurts have undergone more WM pruning than age-matched peers and may appear to have "less mature" WM development. To test this hypothesis, we analyzed diffusion MRI data from 1,726 participants of the Adolescent Brain Cognitive Development (ABCD) study. We computed diffusivity and kurtosis values, which are complementary measures of tissue microstructure, in ten large WM tracts. We assessed a main effect of parent-reported growth spurt status on diffusion metrics using a Kruskal-Wallis test in each tract, and assessed diffusion differences between each growth spurt category using a Wilcoxon rank-sum test. Our results show that children with earlier growth spurts exhibit higher diffusivity and lower kurtosis, which are associated with a less advanced WM developmental trajectory. Hence, earlier onset of the somatic growth spurt in height appears to be associated with a less advanced WM trajectory. This study therefore supports our hypothesis and challenges the interpretation of WM developmental trajectories as reflecting insulated monotonic growth.

P1-A-22 - Identifying molecular alterations in astrocytes from a 16p11.2 deletion mouse model of autism

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Dysfunction in the neurovascular unit during critical periods of development was linked to neurodevelopmental disorders (NDDs), as our lab has shown in the 16p11.2 deletion syndrome (16pDel). Since astrocytes are central hubs within the NVU, it is imperative to ask if, and how, the 16pDel may alter their molecular profile. Our hypothesis is that 16p11.2 haploinsufficiency leads to molecular changes in astrocytes. Using an established 16pDel mouse model, intracellular metabolites extracted from primary astrocytes were quantified using liquid chromatography-mass spectrometry (LC-MS). In males, a reduction of Adenosine and an increase in both alpha-ketoglutaric acid and ribose-5 phosphate were found. In females, there was a decrease in alpha-ketoglutaric acid and an increase in Fructose 1-6 biphosphate.To identify potential genetic

correlates to these changes, gene expression was assessed in 16pDel vs. wild-type astrocytes using bulk RNA sequencing. 33 differentially expressed genes (DEGs) were identified in 16pDel male astrocytes, with 4 genes upregulated and 29 downregulated (including 18 related to excised locus). Upregulated genes appeared associated with inflammatory responses, cytoskeletal organization and cell migration. In female 16pDel astrocytes, 24 DEGs were identified (3 upregulated and 21 downregulated); all downregulated genes were expected 16p11.2 locus genes. Upregulated genes associated with cell morphological changes.Overall, results suggest different molecular signatures in 16pDel astrocytes, including sex differences.

P1-A-23 - Regulation of gene expression by growth differentiation factor 15 in the developing neocortex

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The evolution of the neocortex, specifically its expansion, is the key advancement that is thought to make higher cognitive abilities possible. The expansion of the neocortex reflects increased and prolonged activity of neural progenitor cells (NPC), which give birth to neurons. Cell extrinsic signal, growth differentiation factor 15 (GDF15), has been found to be involved in regulating cell proliferation among other cellular processes. It has also been found in drastically high concentrations in the placenta relative to the rest of the human body. So, a key question is how does GDF15 regulate NPC proliferation in the developing neocortex. In this study, we manipulated GDF15 levels to understand its effects on potential downstream targets, including Dachshund Family Transcription Factor 1 (DACH1), Glypican 6 (GPC6), Semaphorin 5A (SEMA5A), Shroom Family Member 3 (SHROOM3); all of which have been reported to have roles in neurodevelopment. Our findings are consistent with the notion that GDF15 regulates NPC proliferation through these downstream targets, allowing for the expansion of the neocortex.

P1-A-24 - Disentangling fat and sugar in the Western diet: Parental consumption of high fat alters offspring brain development

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Maternal Western diet consumption is a precursor for metabolic conditions such as obesity and is associated with increased offspring risk for neurodevelopmental disorders (NDDs). We examined which components of Western diet (i.e., high-fat or high-sugar) contribute to changes in offspring neurodevelopment. Cohorts of five-week-old C57Bl/6J mice were fed either control diet (CD), high-sugar diet, 43% high-fat diet, 60% high-fat diet, or high-fat/high-sugar diet for 6 weeks and then through breeding, gestation, and lactation. All offspring were fed CD post weaning. Male and female offspring (avg n=15/sex/diet) were assessed for differences in body weight and developmental milestone achievement, and their brains were imaged at postnatal day (P) 65 using magnetic resonance imaging to compare structure volumes.Offspring of the three high-fat diet groups, but not the high-sugar-only group, showed delayed righting reflex success (avg -45.1% success vs CD) and heavier weight at weaning (avg +0.7 g), $p < 0.05$. Weight normalized post weaning, but impacts on brain structure were still observed in early adulthood (P65), including decreased volumes of cingulate cortex area 30 (avg -3.7%) and corpus callosum

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(avg -1.9%), q<0.1.In conclusion, high-fat-only and combined high-fat/high-sugar diets altered offspring brain structure, but high-sugar-only diet did not, indicating the changes mainly resulted from high fat consumption. These findings point to a modifiable NDD risk factor, and future studies could use this mouse model to identify underlying mechanisms and test ameliorative measures.

P1-A-25 - Characterizing the subventricular zone in a 16p11.2df/+ mouse model of autism

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Autism spectrum disorders (ASD) are associated with deficits in behavior, social interactions, motor skills and cognition. The 16p11.2 deletion is a common mutation associated with ASD, leading to haploinsufficiency of ~30 genes on chromosome 16 in humans. Our lab has recently demonstrated that 16p11.2-deficient (16p11.2df/+) mice display cerebrovascular abnormalities leading to behavioral changes. While research on neurodevelopment in 16p11.2 deletion syndrome is ongoing, characterization of neurogenic niches in 16p11.2df/+ mice has been overlooked. Here, we aimed to study the subventricular zone (SVZ) from 16p11.2df/+ mice and their WT littermates. Newborn mice were used to investigate whether the SVZ displays differences between 16p11.2df/+ and WT littermates at birth (i.e., postnatal day, P0). Following cryostat sectioning, immunohistochemistry was used to stain the vasculature (CD31) as well neural progenitors (SOX-2) and mature neurons (NeuN, MAP-2). Images were analyzed using ImageJ to assess key differences in regions of interest. While results are preliminary at this stage, the SVZ appeared different between WT and 16p11.2df/+ mice at P0. Lateral ventricles appeared larger in 16p11.2df/+ animals; and SOX2-, MAP2- and NeuN-positive neurons were less densely populated around the ventricles in 16p11.2df/+ mice versus WT littermates. These differences may have implications in neurogenesis and neurovascular development in the postnatal brain of 16p11.2df/+ mice. This project paves the road for studies assessing neurogenic niches postnatally in ASD mouse models.

P1-A-26 - Reliable multimodal brain signatures predict mental health trajectories in children

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Background: Inter-individual brain differences likely precede the emergence of mood and anxiety disorders, however, the specific brain alterations remain unclear.Methods: In we applied data-driven linked independent component analysis to a population-based cohort of children from the Adolescent Brain Cognitive Development (ABCD) study (N>10K) to identify linked variations in cortical structure and white matter microstructure that together predict mental health trajectories. In a sub-sample of twins, we examined if brain patterns differed depending on the presence of at-risk behaviours.Results: Two multimodal brain signatures at age 9-10y predicted mental health trajectories from 9-12 years of age and replicated across two independent split-halves. The first involved cortical variations in association, limbic, and default mode regions linked with peripheral white matter microstructure that together predicted stable-high or decreasing trajectories of depression and anxiety symptoms. Linked variations of subcortical gray matter structure and projection tract microstructure variably predicted behavioural inhibition, sensation seeking, and psychosis symptom severity in male, but not female,

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participants. There were significant differences in these brain patterns between pairs of twins discordant for self-injurious behaviour.Conclusions: Our results demonstrate reliable, multimodal brain patterns in childhood, before mood and anxiety disorders tend to emerge, that lay the foundation for mental health trajectories and offer targets for early identification of children at-risk.

P1-A-27 - The maternal microbiome shapes development of the hypothalamic suprachiasmatic nucleus, leading to lasting deficits in circadian behaviors in adulthood

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Sleep disruptions occur in nearly 90% of individuals with a neurodevelopmental disorder (NDD) and in 30% of typical developing individuals. Emerging studies link the gestational gut microbiome dysbiosis to disrupted social behaviors, including in NDDs; however, the impact of the maternal microbiome during gestation on offspring circadian rhythms is poorly characterized. Here, using mice housed in control (SPF) or germ-free (GF) environments, I test the hypothesis that gestational microbiome deletion in the pregnant dam impacts the development of the brain region that serves as the master circadian rhythm pacemaker – the suprachiasmatic nucleus (SCN) of the hypothalamus – leading to long-term sleep and circadian disturbances later in life. My preliminary data shows a dramatic disruption in the development of SCN neurons embryonically, with no changes observed in other cellular types in the adjacent nuclei. These data are supported by our scRNA-seq findings showing downregulation of several specific transcription factors driving specification of SCN neurons. Behavioral experiments in adult offspring of GF dams revealed a disrupted circadian rhythm manifested by significantly altered circadian offset, overall activity, days needed to entrain to jetlag, and free running period compared to SPF offspring. Together, our findings suggest that the maternal microbiome during gestation might shape the development of the fetal SCN and stabilize the circadian behaviors in adulthood.

B - NEURAL EXCITABILITY, SYNAPSES, AND GLIA: CELLULAR MECHANISMS

P1-B-28 - Neuronal cholesterol turnover influences synapse maturation and function

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Cholesterol turnover by CYP46A1 is the primary mechanism through which the brain removes excess cholesterol. CYP46A1 converts cholesterol into 24-hydroxycholesterol, which can diffuse through the blood-brain barrier. Excitatory neurotransmission leads to CYP46A1-mediated cholesterol loss in synaptic membranes. However, it is yet to be fully understood how CYP46A1 regulates synaptic plasticity. We propose that transient changes in synaptic cholesterol can lead to structural remodeling of dendritic spines in response to neuronal activity. To investigate the role of cholesterol turnover, we depleted CYP46A1 in primary hippocampal neurons using RNA interference and investigated the effects on spine density and plasticity. Our results show that CYP46A1 depletion significantly reduced spine density, particularly the more mature mushroom

spines. Neurons depleted of CYP46A1 also showed reduced calcium signals in the cell body following chemical LTP, indicating decreased excitability or changes in signaling. Additionally, dendritic spine calcium influx, AMPA receptor exocytosis, and the activity-induced enlargement of the spines were reduced in CYP46A1 knockdown neurons. Overexpression of CYP46A1 also reduced spine density, suggesting that a precise regulation of CYP46A1 is required for dendritic spine maturation. Together, these findings suggest that CYP46A1 could influence synaptic activity. Further studies are necessary to relate these functional effects to CYP46A1-induced changes in membrane cholesterol.

P1-B-29 - Melatonin regulates diurnal dopamine release in the striatum through nicotinic receptors

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Melatonin a neurohormone known to regulate circadian rhythms and influences retinal dopamine (DA) release, but its role in other dopaminergic systems is unclear. As striatal DA release follows circadian patterns modulated by cholinergic interneurons, we hypothesize that melatonin regulates this process via nicotinic acetylcholine receptors.We used fast-scan cyclic voltammetry to measure evoked DA release in acute striatal slices from melatonin proficient or deficient mice. Slices were prepared at two time points corresponding to nadir (ZT10) and peak (ZT22) endogenous melatonin levels. Exogenous melatonin was perfused to assess its effects on DA release dynamics.Our findings indicate:Melatonin modulates diurnal fluctuations of striatal DA, with significantly higher DA release at ZT10 compared to ZT22 in melatonin-proficient mice.Exogenous melatonin affects DA release in melatonin-proficient mice but has no effect in melatonin-deficient mice. This lack of response is attributed to the deficiency of endogenous melatonin affecting receptor sensitivity.Nicotinic acetylcholine receptors mediate melatonin's effect on DA release, as DHβE perfusion abolishes the melatonin-induced changes in DA release.These findings shows that melatonin modulates DA release in the striatum. This regulation, mediated by cholinergic interneurons, highlights melatonin's role in DA rhythms and suggests a potential relevance for treating dopamine-related pathologies.

P1-B-30 - Cannabinoid type 1 receptors regulate neuroinflammation-impaired lactate metabolism in astrocytes

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Introduction Astrocytes sustain neurons by providing lactate via glycolysis, but neuroinflammation disrupts this process, contributing to neurotoxicity. While the endocannabinoid system, particularly Cannabinoid Type 1 Receptors (CB1R) are key to brain energy balance, their role in astrocytic lactate dynamics is unclear. Our purpose is to determine if cannabidiol (CBD) modulates lactate shuttling and inflammation via CB1R. Methods We used cortical glia-neuron co-cultures from wild-type, CB1R-knockout, and DN22 mice (lacking mitochondrial CB1R). Before experiments, we treated cultures with vehicle, liposaccharide (LPS), CBD, or LPS + CBD for 24 hours. Lactate dynamics were monitored in real-time using a

fluorescent lactate biosensor in astrocytes, and we measured GFAP, STAT3, p-STAT3, and GAP43 by immunofluorescence. Results LPS treatment induced an increase in intracellular lactate, but not in CB1R-KO astrocytes. CBD significantly rescued the metabolic abnormalities induced by LPS, which required CB1 receptor expression. Also, the expression levels of GFAP, STAT3, p-STAT3, and GAP43 were increased in astrocytes following LPS exposure, while CBD treatment was able to revert this LPS-mediated effect.Discussion Our data suggest that inflammatory conditions rewire astrocyte metabolism, with CB1 receptors critically involved in regulating lactate dynamics and mediating CBD's metabolic effects on astrocytes. This suggests a promising strategy for downregulating neuroinflammation and consequently diminishing negative neurological outcomes.

P1-B-31 - Enhancement of astrocytic Glu/GABA exchange by multiple mechanisms is effective against convulsive and non-convulsive seizures

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Astrocytes are considered promising targets for new epilepsy treatments due to their pivotal role in regulating ion and neurotransmitter homeostasis. We previously revealed that the uptake of Glu during seizures triggers GABA release from astrocytes through GAT-3 transporter, and this negative feedback mechanism effectively suppresses epileptic seizures. Since astrocytic GABA is synthesized from putrescine (PUT), we opted to initially enhance the Glu/GABA exchange pathway by applying exogenous PUT. We observed by multiple approaches that PUT indeed shortens seizures in the low-[Mg2+] in vitro epilepsy model by increasing desynchronization. Furthermore, PUT significantly reduced the duration of seizures in vivo in WAG/Rij rats, a genetic model of absence epilepsy. Even more importantly, inhibiting the conversion of PUT to spermidine, therefore increasing the astrocytic pool of PUT for GABA synthesis, completely blocked seizure generation in vivo. In addition, we also investigated whether Glu/GABA exchange can be enhanced by the FDA-approved drug levetiracetam whose mechanism of action is poorly understood. We revealed that the Levetiracetam-induced increase in the surface expression of astrocytic GABA transporters leads to additional astrocytic GABA release that activates extrasynaptic GABA receptors. In summary, we propose several pathways by which intensifying the Glu/GABA exchange mechanism can effectively suppress seizure generation in both convulsive and non-convulsive seizure models.This work was supported by the National Research, Development, and Innovation Office grant OTKA K124558.

P1-B-32 - Food deprivation leads to compulsive-like grooming and hyperactivity in female mice

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Anorexia nervosa (AN) is an eating disorder defined by a low caloric intake that leads to a low body mass index. Most individuals also display hyperactivity and an uncontrolled fear of gaining weight. AN has a lifetime prevalence of 0.4-1% among adolescents and young adult, with 90% of those affected being women. Among all eating disorders, AN has the highest mortality rate at ~6. Additionally, within eating disorders, AN has the highest comorbidity with obsessive-compulsive disorder, reaching 19% throughout the lifetime. I aim to investigate the role of the blood-brain

barrier (BBB) and glial cells in AN. To do so, I am using a mouse model, called activity-based anorexia (ABA), where mice are socially isolated, food deprived and are allowed to do physical exercise with a running wheel. Behaviour tests evaluating anxiety-like, depressive-like and obsessive-compulsive-like behaviours of animals are used in my project. The effects of food restriction and access to a running wheel are evaluated in this paradigm. I plan to correlate behavioural measures with biological, functional and morphological changes in the brain.

P1-B-33 - Investigating the role of cortical microglia in viral infection-induced epilepsy

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Microglia, resident immune sentinels in the brain, are crucial in responding to tissue damage, infection via pathogens, damage signals, and cellular debris. It is currently unknown how microglial reactivity devolves and contributes to seizure development following Theiler's Murine Encephalomyelitis Virus (TMEV) infection. Previously, our group has demonstrated that purinergic signaling in microglia is disrupted in the hippocampus of TMEV-infected mice. However, whether reactive cortical microglia also exhibit changes in purinergic signaling, cytokine levels and purinergic receptors are unknown. We seek to evaluate region-based differences in microglial reactivity in the TMEV model. We employed a triple transgenic mouse line expressing tdTomato and GCamp6f and exogenously applied ATP/ADP to acute brain slice preparations from TMEV-infected mice and PBS controls. Interestingly and in contrast to what is observed in hippocampus, we observed that despite microglial reactivity in the cortex, microglia can respond to purinergic damage signals and engage in calcium signaling pathways, comparable to PBS controls. Using a cytokine panel, we also found that pro-inflammatory cytokine levels (TNF-α, IL-1α and IFN-γ) are brain-region dependent in mice infected with TMEV and their levels increased in the order of PBS controls, cortices and hippocampi of TMEV mice. Using RNAScope-FISH, we observed increases in expression of purinergic receptors responsible for microglial motility (P2Y12R) and inflammation (P2X7R). Collectively, our results suggest that following TMEV infection, region-based differences show that cortical microglia can respond to purinergic damage signals, the cortex has a higher production of pro-inflammatory cytokines and expression of certain purinergic receptors as compared to PBS controls.

P1-B-34 - Linking age changes in human neuronal microcircuits to impaired brain function and EEG biomarkers

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Human brain aging involves a variety of cellular and synaptic changes, but how these changes affect brain function and signals remains poorly understood due to experimental limitations in humans, meriting the use of detailed computational models. We identified key human cellular and synaptic changes with age (inhibitory cell loss, NMDA receptor loss, and spine loss), and integrated them into our previous detailed human microcircuit models. Our simulations of middle-age and older microcircuits linked the altered mechanisms to reduced spike rates and impaired signal detection as seen previously in aging. We then simulated resulting EEG signals and showed that the aging mechanisms can explain key EEG power spectral biomarkers seen in

human aging, including reduced aperiodic offset, exponent, and periodic peak center frequency. We further determined the specificity of the EEG biomarkers for deriving the underlying cellular changes. Our results overcome challenges in linking cellular aging mechanisms with impaired cortical function and candidate biomarkers in brain signals that may serve to improve diagnosis in human aging.

P1-B-35 - Investigating cellular and circuit deficits in fragile X syndrome using human cortical and thalamic organoids as models

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Evidence from clinical and rodent studies implicate thalamocortical (TC) circuit dysfunction in the pathophysiology of fragile X syndrome (FXS), the leading monogenic cause of autism spectrum disorder. Although the onset of TC projections and synapses occurs during embryonic neurodevelopment, most research has focused on postnatal TC impairments, leaving a gap in our knowledge of brain abnormalities during fetal/embryonic development. To elucidate the developmental brain region-specific deficits linked to TC circuit disruptions, we generated human embryonic stem cell-derived cortical and thalamic organoids. Single cell RNA sequencing on 2-month-old control and FMR1-/- cortical organoids revealed dysregulated progenitor cell dynamics and excitatory and inhibitory neuron populations as vulnerable cell-types. Immunohistochemistry of cortical organoids at early (1-month) and late (3-month) timepoints validated deficits in neurogenesis and excitatory neuron populations. To examine neural function, we infected 4-month-old cortical organoids with a genetically encoded calcium indicator (AAV-hSYN-GCAMP6f) and found increased spontaneous neuronal activity in the FMR1-/- group. Finally, preliminary immunohistochemistry analysis of control and FMR1-/- thalamic organoids revealed reductions in MAP2+ neurons, and increased SOX2+ progenitors, suggesting that cellular deficits in the cortex may also occur in the thalamus. We will further explore thalamus-specific deficits with ongoing functional experiments and assembloids to identify circuit-linked impairments. Ultimately, our study has revealed cell-type specific developmental cellular and functional deficits which we will further, to gain mechanistic insight that will provide a basis for therapeutic approaches.

P1-B-36 - Comparative analysis of intrinsic electrophysiological and morphological properties of fast-spiking and pyramidal neurons in the dorsolateral prefrontal cortex and primary visual cortex of non-human primates

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Parvalbumin neurons (PV) are crucial inhibitory interneurons in the brain's cortex that maintain the excitation-inhibition balance. While their electrophysiological characteristics are thought to be preserved across cortical areas, their density varies between sensory and association regions like the primary visual cortex (V1) and the dorsolateral prefrontal cortex (dlPFC), particularly in primates.We used whole cell patch clamp to study PV interneurons and pyramidal neurons (PN)

in macaque V1 and dlPFC (pPV: n=54; PN: n=70). Using a classifier trained on mouse genetically labeled neurons, we identified putative PV cells in primates and compared their morphology across species.PN exhibits higher excitability in V1 compared to dlPFC. Conversely, pPV neurons in the dlPFC displayed higher input resistance (Rin) than those in V1. Interestingly, dlPFC pPV neurons had significantly higher Rin than PN, while V1 PN had higher Rin and lower rheobase than pPV cells.PV neuron size increased progressively from mouse to macaque to human. In primate association areas, neurons showed larger dendritic fields but shorter dendrites and fewer primary branches. These changes likely adapt PV neurons to the sparse cell density and expanded cortex of dlPFC versus V1.These results indicate that PV neurons are finely tuned to the specific functional demands of different cortical areas. V1 neurons are optimized for signal detection, while dlPFC neurons prioritize signal selectivity. This study highlights distinct electrophysiological and morphological features of PV neurons across V1 and dlPFC in non-human primates, supporting the existence of a gradient of intrinsic properties along sensory and association areas.

P1-B-37 - Sex differences and estrogen's role in modulating microglial response to cerebral microbleeds in a mouse model

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Microglia, the brain's resident immune cells, exhibit sex differences in development, gene expression, morphology, and function. These differences manifest as sex-related variations in disease prevalence, symptomatology, and treatment responses in conditions like stroke, multiple sclerosis, and Alzheimer's disease. The neuroprotective effects of sex hormones, particularly estrogen, have been linked to increased vulnerability to neurodegenerative diseases after menopause. To investigate the role of sex hormones in this phenomenon, we subjected male and female mice to gonadectomy with or without hormone replacement. Using a tamoxifen-inducible Cre mouse line, we imaged microglial motility, morphology, phagocytosis, and fatigue following microbleeds in vivo. Our findings show that the percentage of mobile microglia is higher in female gonadectomy mice than in sham controls, with estradiol treatment reversing this effect. Male microglial mobility remained unaffected by these interventions. These findings directly implicate estrogen as a key signal in constraining the mobilization of microglia following injury. Given the diverse effects of estrogen signaling on cells throughout the body, current experiments are examining whether a microglia-specific knockdown of estrogen receptor alpha (ERα) is required for these sex-related differences. Collectively, these experiments shed new light on the role of sex hormones in microglial responses to injury, with the potential to promote sex-specific therapeutic strategies in the context of brain injury and neurodegenerative diseases.

P1-B-38 - Assessing sex-specific nanoscopic synaptic changes in response to chronic stress

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Previous studies show that the cortico-accumbal and tegmental circuits, critical for regulating the emotional response to stress, exhibit a sex-specific shift toward increased excitation in the

E/I ratio under chronic stress. Under similar conditions, evidence also suggests morphological and functional changes in SST and PV interneurons, key regulators of the E/I balance. To explore the potential link between these E/I shifts and the reported interneuron alterations, we studied the morphological properties of synapses the aforementioned pathways following chronic stress.Experiments were done on PV-cre and SST-flp male and female mice. The cortico-accumbal and tegmental pathways were labeled with mCherry via a trans-sectional viral approach, while SST or PV neurons were tagged with GFP using conditional viral expression. Mice underwent 21 days of chronic variable stress. STED microscopy with adaptive optics was used to characterize the morphology of synaptic protein nanodomains. To analyze structural synaptic features, we developed multidimensional machine learning techniques. We've established unsupervised clustering as a first step to highlight patterns in synaptic dysregulation.As a next step, using an autoencoder, we seek to integrate both electrophysiological and STED imaging data in a shared latent space to capture the diverse synaptic features that could be associated with changes in synaptic plasticity in the context of chronic stress at the cortico-accumbal and tegmental pathways.

P1-B-39 - 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 have demonstrated that netrin-1 is secreted following long-term silencing of activity induced by tetrodotoxin (TTX). Furthermore, we have shown that netrin-1 is required for the increase in miniature excitatory post-synaptic current (mEPSC) amplitude that follows long-term TTX exposure. Ongoing investigations are examining the necessity of DCC signaling for TTX-induced upscaling. These findings aim to identify a novel molecular mechanism involved in homeostatic plasticity and a convergence point between Hebbian and homeostatic plasticity.

P1-B-40 - The role of oligodendrocyte precursor cells (OPCs) in memory consolidation

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While oligodendrocyte precursor cells (OPCs) differentiate into myelinating oligodendrocytes (OLs) in response to synaptic inputs, they may play critical roles beyond oligodendrogenesis and myelination by directly influencing neural circuit activity. For example, recent studies have shown that OPCs activation leads to the release of gamma-aminobutyric acid (GABA), thereby

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enhancing inhibitory synaptic transmission onto nearby interneurons. Since reduced inhibitory tone may facilitate plasticity mechanisms, we hypothesized that OPC activation may modulate learning and memory. Using a contextual fear conditioning paradigm in mice, our study reveals that optogenetic activation of OPCs in the hippocampus during learning enhances engram formation and memory (assessed at both recent and remote time points). Notably, photo-stimulation alone or photo-activation of OPCs during retrieval did not alter behavior. However, photo-activating OPCs during the consolidation phase further increased freezing behavior, emphasizing their pivotal role in memory consolidation. These findings highlight OPC activation as a key mechanism supporting fear memory consolidation through hippocampal OPC proliferation and engram expansion. Current studies are exploring whether the pro-consolidation effects of OPC activation are mediated via OPC interactions with interneurons.

P1-B-41 - Investigating sex differences in astrocyte-neuronal lactate shuttling in the anterior cingulate cortex in a murine chronic neuropathic pain model

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Chronic pain currently impacts 25% of Canadians over the age of 15, but due to few confirmed details on its underlying molecular mechanisms treatment options are often inadequate. Currently, chronic pain development within the brain is thought to be driven by neuroplasticity occurring in regions implicated in processing the sensorial and affective experiences of pain. The anterior cingulate cortex (ACC) has been identified as a hub for these long-term neuroplastic changes in a model of chronic inflammatory pain. In particular, the activity of the astrocyte-neuronal lactate shuttle (ANLS) within the ACC during chronic pain was found to be increased, suggesting its importance in the development of chronic pain-related neuroplasticity. Here, we investigated these molecular changes in a chronic neuropathic pain model, utilizing the spared nerve injury (SNI) in both female and male mice. We found that while SNI causes robust pain hypersensitivity in both female and male mice, it causes a sex-dependent increase in lactate levels within the ACC. Specifically, male, but not female mice show significant increases in lactate at early timepoints post injury. We also performed western blot assessments of multiple proteins involved in ANLS and neuroplasticity within the ACC. Increased lactate levels within the ACC in a model of neuropathic pain suggests the importance of the ANLS in chronic pain development in a sex-specific manner.

P1-B-42 - Homeostatic gain modulation drives changes in heterogeneity expressed by neural populations

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Diversity exists throughout neurobiology, spanning cells, circuits and system dynamics, and plays important roles in maintaining stability. Recent experiments found reduced neural heterogeneity may accompany pathological states such as epilepsy. While heterogeneity was linked to stability, lost biophysical diversity induced the onset of seizure-like activity, suggesting an important functional role. How such changes in heterogeneity arise remains unknown. Often considered a static metaparameter, heterogeneity is, in fact, a highly dynamic property of biological networks. We consider this through intrinsic plasticity, a form of homeostatic gain modulation where neuron excitability alters in response to statistical input. Using a network of

Poisson neurons endowed with intrinsic plasticity, we computationally investigate the effect of input statistics on cell excitability and network heterogeneity. We find it sensitive to influences on the input statistics, such as connectivity and impinging spike rate. Increased variability in these features yields high amplitude membrane potential fluctuations, promoting heterogeneity. Conversely, if the cell-to-cell membrane potentials are stereotyped, the system excitability homogenizes resulting in unstable dynamics. This bidirectional effect could underlie the pathological alterations to heterogeneity observed in epilepsy. Understanding how input statistics affect neural heterogeneity may provide key insights into brain function resilience, and manipulation of neural diversity through intrinsic plasticity.

P1-B-43 - Progressive overfilling of readily releasable pool underlies short-term facilitation at recurrent excitatory synapses in layer 2/3 of the rat prefrontal cortex

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Short-term facilitation of recurrent excitatory synapses within the cortical network has been proposed to support persistent activity during working memory tasks, yet the underlying mechanisms remain poorly understood. We characterized short-term plasticity at the local excitatory synapses in layer 2/3 of the rat medial prefrontal cortex and studied its presynaptic mechanisms. Low-frequency stimulation induced slowly developing facilitation, whereas high-frequency stimulation initially induced strong depression followed by rapid facilitation. This non-monotonic delayed facilitation after a brief depression resulted from a high vesicular fusion probability and slow activation of Ca2+-dependent vesicle replenishment, which led to the overfilling of release sites beyond their basal occupancy. Pharmacological and gene knockdown (KD) experiments revealed that the facilitation was mediated by phospholipase C/diacylglycerol signaling and synaptotagmin 7 (Syt7). Notably, Syt7 KD abolished facilitation and slowed the refilling rate of vesicles with high fusion probability. Furthermore, Syt7 deficiency in layer 2/3 pyramidal neurons impaired the acquisition of trace fear memory and reduced c-Fos activity. In conclusion, Ca2+- and Syt7-dependent overfilling of release sites mediates synaptic facilitation at L2/3 recurrent excitatory synapses and contributes to temporal associative learning.

P1-B-44 - Systemic nimodipine affects pericyte calcium signaling, resting hemodynamics and neurovascular coupling in healthy mouse brain

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Nimodipine, a L-type voltage-gated calcium channel (VGCC) blocker commonly used in subarachnoid hemorrhage management, has significant effects on brain pericytes, which express L-type VGCCs and play a critical role in regulating cerebral blood flow (CBF) through vasomotion and neurovascular coupling. Our work demonstrates that systemic administration of nimodipine (1 mg/kg; i.p.) reduces calcium transients in all pericyte types, including ensheathing and thin-strand pericytes, across various cerebrovascular regions, as revealed by two-photon microscopy. This reduction leads to local vasodilation near penetrating arterioles but decreases red blood cell (RBC) velocity, suggesting complex hemodynamic consequences. In contrast, topical application of nimodipine increases RBC velocity, highlighting differential

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effects based on the administration route. Furthermore, we show that L-type VGCCs in both pericyte types mediate functional hyperemia by facilitating vasodilation. By blocking these channels, Nimodipine impairs neurovascular coupling at all points in the vascular network. These findings underscore nimodipine's ability to alter cerebrovascular dynamics and pericyte physiology, with distinct outcomes depending on systemic versus topical application, offering critical insights for its clinical use.

P1-B-45 - Galanin receptor 1 mediates the inhibitory effects of galanin on wake-active histaminergic neurons

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Galanin is a key neuropeptide involved in the regulation of sleep and wakefulness. Activation of galanin-expressing neurons in the ventrolateral preoptic area (VLPOGAL) promotes sleep and increases non-rapid-eye-movement (NREM) sleep. Some of the sleep-promoting effects of galanin and VLPOGAL neuron activation may result from their ability to inhibit the wake-promoting histaminergic neurons in the tuberomammillary nucleus (TMN). However, the mechanisms by which galanin influences histaminergic neuron activity remain poorly defined. We used whole-cell patch clamp electrophysiology to characterize the galanin-induced inhibition of genetically identified histaminergic neurons. Whole-cell current clamp recordings were established from histaminergic neurons expressing a red fluorescence protein, using a histidine decarboxylase (Hdc)-cre mouse model. Galanin strongly inhibited the electrical excitability of histaminergic neurons, resulting in a hyperpolarization of the membrane potential and a reduction in the firing rate. A galanin receptor 1 (GAL1R) agonist, but not galanin receptor 2 or galanin receptor 3 agonists, mimicked the galanin-induced inhibition. The GAL1R agonist continued to inhibit histaminergic neurons in the presence of inhibitors of glutamatergic and GABAergic transmission. RNAScope® in situ hybridization performed on TMN hypothalamic brain slices revealed strong expression of Gal1r in histaminergic neurons. These data suggest that the inhibitory effects of galanin involve GAL1R expressed on histaminergic neurons.

P1-B-46 - Molecular mechanisms underlying local inhibitory control over cortico-accumbal and tegmental pathways in stressed male and female mice

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Inhibitory control over excitatory neuronal circuits in the medial prefrontal cortex (mPFC) is crucial for the expression of emotional stress responses. Under chronic stress, this balance is shifted in part by a lost of inhibition over mPFC neurons, including those projecting to the nucleus accumbens (NAc) and the ventral tegmental area (VTA). Here, we assessed the gradual molecular alterations perturbing connectivity between GABAergic subpopulations cortico-accumbal and tegmental neurons in mice exposed to chronic stress.Using trans-sectional viral tracing with electrophysiological recordings in transgenic mice, we labeled mPFC-NAc and VTA projecting neurons and evaluated their communication with GABAergic interneurons. Our analysis revealed morphological and functional changes affecting PV and SST neurons and their capacity to maintain appropriate inhibitory control over both pathways in stressed males and

females. We used snRNAseq to evaluate the molecular mechanisms underlying these effects, revealing transcriptional alterations in several cell types affecting males and females during and after chronic stress. This was associated with changes in intracellular communication cascades, including inflammatory (NF-κB), hormonal (glucocorticoid), and neural (axon guidance) functional pathways, potentially underlying the changes in the inhibitory tone imposed by GABAergic subtypes over mPFC-NAc and VTA projecting neurons.Our results highlight the molecular mechanisms responsible for the gradual lost of inhibitory control over mPFC neurons projecting to subcortical structures.

P1-B-47 - Neuronal excitation and synaptic plasticity require TRPV4 activation in primary hippocampal cultured neurons

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Transient receptor potential vanilloid (TRPV4) is a polymodal bivalent cation channel, sensitive to physiological temperatures (34-41oC), mechanical stretch, as well as endogenous ligands including arachidonic acid (AA) derivatives, 5'-6' epoxyeicosatrienoic acid (EET) and anandamide. TRPV4 is highly expressed in the hippocampus and is localized to the plasma membrane, endoplasmic reticulum (ER), and mitochondria. Previous studies have shown that TRPV4 regulates neuronal excitability at physiological temperatures and mediates calcium release from ER stores. However, little is known of its role in synaptic plasticity and transmission. Chemical-LTP (cLTP) (90mM KCl, 3x1s) was induced in primary hippocampal neurons, significantly increasing post-synaptic expression of GluA1 (146.3±15.9%) and TRPV4 (157±38.9), as well as mitochondria and TRPV4+ve mitochondria. These increases were blocked following exposure to TRPV4 antagonists RN9893 (3μM) and RN1734 (10μM), suggesting a role for TRPV4 in mediating cLTP induced synaptic plasticity. In primary hippocampal cells virally transduced with SynGCaMP6f, TRPV4 antagonists also displayed a dose dependent blockade of spontaneous firing frequency (-99.15±0.15%) and amplitude (-87.01±2.25%) and slowed the decay of KCl-induced calcium release (-64.8±2.3%).These studies suggest that TRPV4 is necessary for calcium signalling and cLTP and likely exerts these roles through regulating calcium dynamics. Studies are currently underway to delineate the role of TRPV4 in other forms of hippocampal synaptic plasticity using electrophysiology.

P1-B-48 - Running exercise sustains pattern separation despite transient changes in hippocampal neurogenesis

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IntroductionAdult hippocampal neurogenesis enhances pattern separation—the ability to distinguish similar stimuli—partly through silencing the dentate gyrus (DG). Exercise increases hippocampal neurogenesis and improves pattern separation. However, after a month of exercise in rodents, DG proliferation returns to baseline, suggesting a transient effect on neurogenesis. This study aims to investigate whether long-term exercise sustains pattern separation improvements and to elucidate the underlying mechanisms.MethodsWe used C57BL/6 mice subjected to short- (1 month) or long-term (2 months) voluntary wheel running. Newborn granule

cells (GCs) in the DG were labeled with BrdU or a retroviral vector (CAG-GFP) at 3 or 30 days of exercise. To assess the differentiation, survival, and morphology of new GCs, the animals were sacrificed 30 days later. Short- and long-term running effects on pattern separation were evaluated using the Novel Object Recognition (NOR) test, and c-fos expression in the DG. ResultsOur results showed that running transiently increases hippocampal neurogenesis. Regardless of duration, running promoted a neuronal phenotype and, in the NOR test, induced faster recognition, reduced activity of mature GCs, and minimal activity in new GCs (<1%). Additionally, long-term running enhanced the dendritic complexity of new GCs.DiscussionOur results suggest that running supports pattern separation by regulating dentate gyrus activity, independent of the number of exercise-induced new GCs, their morphological characteristics, or the running duration.

P1-B-49 - Impact of perinatal insults on cerebellar cortex microarchitecture and Purkinje cell neuronal inputs

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The second half of pregnancy is critical for cerebellar development, involving neurogenesis, neuronal migration, and synaptic maturation. This phase is highly vulnerable to perinatal insults, such as inflammation and hemorrhage, common in preterm infants. These insults may disrupt the balance of excitatory and inhibitory synapses in the cerebellar cortex, leading to neuronal dysfunction and increasing the risk of neurodevelopmental delay.We hypothesize that perinatal insults (CBH and LPS exposure) disrupt Purkinje cells' dendritic structure and organization, leading to an imbalance of inhibitory and excitatory signaling within the cerebellum.Using a transgenic mouse model, we simulated perinatal insults. At postnatal day 2 (P2), mice were exposed to lipopolysaccharide (LPS) to induce inflammation and bacterial collagenase to mimic cerebellar hemorrhage. At postnatal day 15 (P15), we assessed Purkinje cells (PCs) via double immunostaining to evaluate cellular morphology and synaptic inputs, using markers such as GAD67/Gephyrin (inhibitory) and VGLUT1/PSD95 (excitatory).Purkinje cells showed no significant morphological changes, but molecular layer thickness in the right cerebellar hemisphere tended to decrease in the double-insult group (12.2 µm in controls vs. 10.7 µm in Coll-LPS). Dendritic tree volume was also lower in the double-insult group (23.7% vs. 19%). Preliminary analysis indicated reduced GAD67 expression in the molecular layer of the right hemisphere, suggesting altered inhibitory inputs onto Purkinje cells.Conclusion: Preliminary findings suggest perinatal insults affect synaptic inputs on Purkinje cells rather than their morphology. Trends in molecular layer thinning and reduced inhibitory inputs underscore the long-term impact of early-life insults on cerebellar development.

P1-B-50 - Regulation of axonal transport in neurons by S-Acylation of the dynein activator p150glued

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Fast axonal transport of organelles and proteins along axonal microtubules by dynein and kinesin motors is critical for neuronal function. The activity of motor proteins is tightly regulated, and aberrant activity can result in various neuropathies. S-acylation is an important mechanism to

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regulate neuronal protein trafficking. Several kinesin and dynein motor subunits and their activators have been identified in high throughput S-acyl-proteomic studies as being potentially S-acylated. We recently confirmed S-acylation of the dynein activating complex dynactin subunit p150Glued. Dynactin is critical for dynein activation and processivity. p150Glued is S-acylated predominantly in the nervous system on cysteines 617 and 1252 by the ZDHHC12 protein S-acyltransferase. p150Glued is the largest dynactin subunit and mediates dynein complex microtubule binding and processive motility. Due to the importance of p150Glued in dynein-mediated fast axonal transport, I hypothesize the functional role of p150Glued S-acylation is to regulate this process. Interestingly, when S-acylation-resistant p150Glued-GFP is expressed in neurons, less GFP signal is present in distal axons by immunocytochemistry and in the vesicle fraction after biochemical subcellular fractionation than in wild type (WT) expressing neurons, suggesting that S-acylation may regulate association of p150Glued with vesicular cargos and transport of dynein-mediated cargo. Future work will assess the interaction of S-acylation-resistant p150Glued with motor-cargo complex subunits and measure live neural vesicular kinetics.

P1-B-51 - Impact of α-Synuclein aggregation on blood-brain barrier integrity in Parkinson's Disease

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Parkinson's disease (PD) is a neurodegenerative condition marked by the loss of dopaminergic neurons in the substantia nigra. The disease presents differently between sexes: men are at higher risk of developing PD, while women experience more fluctuations in symptoms and higher rates of depression. Despite these differences, the mechanisms behind psychiatric symptoms in PD are poorly understood. The blood-brain barrier (BBB), composed of endothelial cells, astrocytes, and pericytes, plays a critical role regulating brain function and the processing of α-synuclein (αSyn), a key protein in PD. However, the role of BBB components in psychiatric symptoms in PD remains unclear. This study examined neurovascular responses in a mouse model of PD, focusing on αSyn aggregation. We found that male and female mice exhibited distinct depressive-like behaviors. Gene expression analysis revealed differences in BBB function and neurovascular responses depending on sex and brain region. In females, the prefrontal cortex (PFC) showed signs of BBB disruption, with altered expression of endothelial markers, suggesting BBB breakdown. In males, endothelial markers in the PFC were increased, alongside signs of astrocyte activation and minimal changes in pericytes. These findings highlight the complexity of neurovascular changes in PD, suggesting that sex-specific alterations in the BBB may contribute to psychiatric symptoms like depression. Understanding these differences could lead to new therapeutic targets for PD-related mood disorders, providing insights into more personalized treatments.

P1-B-52 - Deriving connectivity from spiking activity in large-scale biophysical cortical microcircuits

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Inferring detailed cortical microcircuit connectivity is essential for uncovering how information is processed in the brain. A common method in vivo uses short-lag spike cross-correlations to derive putative monosynaptic connections, but inactive neurons and correlated firing can hinder the derivation accuracy. Previous studies that developed methods to derive connectivity from cross-correlations using simulated ground-truth spiking data employed simplified or small network models and thus did not address these key confounds of physiological large-scale networks. We tested connectivity derivation methods on ground-truth spiking data from detailed models of human cortical microcircuits in different layers and between key neuron types. We showed that connection derivation was poor in cortical layer 2/3 microcircuits compared to layer 5, due to low firing rates and inactive neurons. General activation strategies for layer 2/3 microcircuits led to only a moderate improvement in derivation performance, due to a trade-off between the proportions of inactive neurons and overactive neurons. We then showed that more refined stimulation paradigms leading to moderate and less correlated response improved derivation accuracy. Our results address key physiological challenges and methods to improve accuracy in deriving connections from spiking activity in large-scale neuronal microcircuits.

P1-B-53 - GATOR1-dependant mitochondrial regulation impacts the metabolic adaptability of astrocytes

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Epilepsy is a neurological disorder affecting 50 million people worldwide. GATOR1 is a protein complex composed of three subunits called NPRL2, NPRL3 and DEPDC5. Genetic mutations in the protein subunits can lead to focal epilepsy with foci located in the frontal cortex and the temporal lobe. GATOR1 helps regulate the balance of protein synthesis and degradation by modulating mTORC1 activity, which governs cellular metabolism, mRNA translation and growth. During amino acid deprivation or starvation, GATOR1 catalyzes RAG-GTP hydrolysis to inhibit mTORC1. When GATOR1 is mutated, mTORC1 activity is maintained even during periods of amino acid starvation. In human brain, GATOR1 defects can result from mosaic mutations, meaning that different parts of the brain can express the mutations that can differently affect specific cell populations, like neurons or astrocytes. The role of GATOR1 in astrocytes, which provide metabolic and neurotransmitter precursors to neurons, has not been established. Here we show that loss of GATOR1 function impacts the morphology of astrocytes and their mitochondria in our genetically modified mouse model and astrocytic cell-line where GATOR1 is impaired. In vivo, GATOR1 defective astrocytes leads to the development of astrogliosis at 5 months of age, with all animals succumbing to death between 6-8 months of age. We find that astrocytes lacking GATOR1 have elevated levels of reactive oxygen species and mitochondrial electron transport chain dysfunctions that are consistent with hyperactive mTORC1-dependent regulation of mitochondrial metabolism.

P1-B-54 - Exploring the impact of MDGA2 haploinsufficiency on synaptic spine density & maturation in a mouse model of autism spectrum disorder

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Synaptic pruning during brain development is essential for refining neural circuits, maintaining excitatory-inhibitory (E/I) balance, and supporting cognitive functions like sensory processing

and behavior. Disruptions in synaptic pruning are hypothesized to contribute to neurodevelopmental disorders, including autism spectrum disorder (ASD), though direct evidence remains limited. MDGA2 (MAM domain-containing glycosyl-phosphatidylinositol anchor 2) regulates synaptogenesis by inhibiting the interaction between pre-synaptic neurexin and post-synaptic neuroligin, modulating excitatory synapse formation. Reduced MDGA2 expression in haploinsufficient (Mdga2+/−) mouse models is associated with increased excitatory synaptic density and signaling in adulthood, but its role in synaptic pruning during critical developmental stages remains unexplored.This study examines how reduced MDGA2 expression disrupts synaptic pruning, focusing on dendritic spine density and maturation in hippocampal CA1 pyramidal neurons across key developmental stages (P14, P21, P28, and P42). In wild-type mice, pruning typically reduces spine density and promotes mature spine morphology over time. We hypothesize that reduced MDGA2 expression in Mdga2+/− mice impairs pruning, resulting in elevated spine density and an accelerated transition to mature spine morphology. Using Golgi-Cox staining, we quantified spine density and assessed morphological parameters to evaluate these developmental disruptions.By investigating how MDGA2 dysfunction impacts synaptic spine properties during a critical period of brain development, this research aims to link the loss of proper synapse regulation to neuronal abnormalities in ASD, advancing our understanding of the cellular basis for neurodevelopmental disorders.

P1-B-55 - GATOR1-dependent mTORC1 hyperactivity drives the development of neuronal communication

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Mutations in GATOR1 complex subunits, NPRL2, NPRL3, and DEPDC5 have been associated with a wide spectrum of familial focal epilepsies and autism spectrum disorders (ASD). GATOR1 controls mTORC1 activation depending of the nutritional status of the cell, particularly intracellular amino acid levels, by function as a GTPase-activating protein complex toward a RAG proteins, and inhibiting mTORC1 activity when amino acid levels is low. We show that disease-linked mutations in the NPRL2 subunit can restrict the function of GATOR1 complex and its inhibitory function on mTORC1, resulting in the hyperactivation of downstream cellular growth signalling. While GATOR1 loss-of-function mutations directly impact the amino acid sensing role of mTORC1, few studies have focused on the impact of these mutations on the homeostatic coordination of mTORC1 regulation with the PI3K/AKT-dependent growth factor signalling pathway. Using a combination of pharmacological tools, we discovered that specific mutations in the NPRL2 subunit of GATOR1 renders cells resistant to mTORC1 inhibition caused by PI3K inhibition, in neuronal and non-neuronal cells. To further investigate the biological contribution of GATOR1 in neuronal development and function, we generated a NPRL2 knockout model using primary neuron culture. Those neurons lacking NPRL2 expression, developed exaggerated neuronal firing activity, reminiscent of seizure activity in vivo. Collectively, our studies show a novel function of GATOR1-dependant amino acid-signalling in the development of hyperactive neural network formations.

P1-B-56 - Cortico-accumbal pathway characterization and sexual differences in stress-induced synaptic plasticity

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The medial prefrontal cortex (mPFC) projection to the nucleus accumbens (NAc) is crucial for the expression of emotional stress responses. Previous studies reported pathway-specific morphological and functional changes induced by chronic stress affecting males and females differently. In the NAc, mPFC inputs target D1 and D2-expressing medium spiny neurons (MSN). However, connectivity patterns defining this pathway remain understudied. Here, we used neuroanatomical and functional approaches to define connectivity patterns at the mPFC-NAc projection and its plasticity in chronic stress conditions.Using an intersectional viral approach, we showed that mPFC projections target an even proportion of D1- and D2-MSNs, with a lower proportion of cholinergic interneurons. We also show a strong preferential innervation of the core, with over 70% of all labelled cells being restricted to this subregion of the NAc. To investigate the impact of stress on synaptic plasticity, we used optogenetics to stimulate cortical terminals in NAc and performed ex vivo patch-clamp on D1 and D2-MSNs. Interestingly, our analysis in females revealed that stress enhances short term depression at mPFC-NAc synapses similarly in D1 and D2-MSNs while, in contrast, it decreases it in males in a D1-specific fashion.Globally, our results provide anatomical and functional insights into the connectivity patterns of the cortico-accumbal circuit and its plasticity in the context of chronic stress. These findings support the behavioral sexual differences where females are usually more passive and males reactive.

P1-B-57 - SNARE protein SNAP25 regulates the chloride-transporter KCC2 in neurons

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Inhibitory synaptic neurotransmission mediated by the neurotransmitter gamma-aminobutyric acid (GABA) requires a low concentration of chloride ions in neurons, which is established and maintained by the potassium-chloride co-transporter 2 (KCC2). Loss of KCC2 function results in the hyperexcitability of neuronal networks and is associated with various neurological disorders including schizophrenia and Huntington's disease. While KCC2-interacting proteins are known to regulate KCC2 protein level and function, specific KCC2-interacting partners are still being identified and characterized. We asked whether SNAP25, an integral component of the SNARE-complex and a novel KCC2 interactor, regulates KCC2 protein in mice. SNAP25 is an integral component of the presynaptic t-SNARE complex and is responsible for synaptic vesicle fusion in regulated exocytosis. It is also implicated in the trafficking and cell surface expression of various postsynaptic channels and receptors, including the NMDA and kainate-type receptors.We demonstrated that SNAP25 interacts with KCC2, and that this interaction is regulated by protein kinase C (PKC)-mediated phosphorylation. We also discovered that SNAP25 knockdown decreases total KCC2 in cortical neurons, and reduces the strength of synaptic inhibition, as demonstrated through a depolarization of the reversal potential for GABA (EGABA), indicating reduced KCC2 function. Our biochemical and electrophysiological data combined demonstrate that SNAP25 regulates KCC2 membrane expression and function, and regulates inhibitory synaptic transmission.

P1-B-58 - Phosphatidylserine regulates synaptic development and plasticity at the Drosophila melanogaster neuromuscular junction

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Several studies have demonstrated the how various proteins regulate synaptic development and plasticity, however the role of lipids such as phosphatidylserine (PS) have been less studied. PS is synthesized by Phosphatidylserine synthase (Pss) and upon localization to the plasma membrane is transported from the outer to inner leaflet by dATP8B (ATP8B), a phospholipid flippase. Knockdown of Drosophila glial-Pss has been shown to reduce synaptogenesis/axonal growth. The effects of loss of ATP8B on synaptic growth and plasticity 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. Using the PS biosensor Lactadherin-C2 (Lact-C2), we expressed Lact-C2 at the neuromuscular junction (NMJ) and found strong expression of the PS biosensor in motor neurons, glia, and muscle. We then found a reduction in presynaptic bouton abundance in a Pss mutant compared to its genetic control. Next, Drosophila larvae reared at high temperatures (30°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. Finally, using this same assay, knockdown of glial-Pss was found to impair activity-dependent synaptic growth. Current experiments are exploring if PS localization changes in response to increased synaptic activity and whether these changes are important for activity-dependent synaptic growth.

P1-B-59 - In vivo voltage imaging of non-spatial behavioral time-scale synaptic plasticity in the hippocampus.

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The brain forms memories by building connections between neurons whose firing encodes related concepts. For example, mental maps can be formed of cells representing specific locations. Recently, it was found that hippocampal neurons which underwent a depolarization event called a "plateau potential" could rapidly tune their firing to specific locations. This phenomenon, titled behavioral time scale synaptic plasticity (BTSP), may act as a mechanism for the hippocampus to form spatial memories. However, it remains unclear if BTSP can tune neurons to sensory cues or abstract concepts to build representations of non-spatial experiences. To investigate this, we performed voltage imaging of pyramidal neurons in the CA1 hippocampal subregion of CamK2a-Cre transgenic mice during an odour discrimination task. We will present neurons which underwent a plateau potential and subsequently showed firing patterns specific to odour presentation or reward delivery. Voltage imaging also uniquely allowed us to describe the subthreshold properties of plateau potentials and to analyze the dynamics of neurons before they showed tuned firing. Our results demonstrate that BTSP can tune cells to non-spatial stimuli which could then be integrated into larger networks representing relationships between odours and rewards. This suggests that BTSP is not limited to forming spatial maps but instead is a leading candidate for the mechanism behind the rapid neural plasticity needed to memorize complex experiences as they happen.

P1-B-60 - CD47's regulation of memory processes

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Microglia regulate synaptic connections through synaptic pruning, either eliminating or protecting synapses based on neuronal expression of "Eat Me" or "Don't Eat Me" signals. While "Eat Me" signals are known to promote memory forgetting by promoting synapse removal, the role of "Don't Eat Me" signals, which protect synapses, remains largely unexplored. This study focuses on one such signal, CD47. By using engram tagging and behavioral analysis, we find that CD47 regulates memory persistence and precision. CD47 overexpression in engram cells promotes memory persistence by slowing fear memory extinction while reducing memory precision by generalizing context-specific fear to a novel context. At the cellular level, CD47 enhances engram synaptic connectivity by increasing spine density, increases engram reactivity by facilitating engram cell activity during memory recall, and increases neuronal excitability by allocating CD47 overexpression neurons to become part of an engram supporting fear memory. This research uncovers how CD47 influences cognitive function and could lead to new understandings of microglia's contribution to memory and cognitive functions.

P1-B-61 - Identifying and mapping L6b neuronal diversity in the mouse brain

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Understanding neuronal subpopulation diversity is crucial to elucidating the brain's cellular composition and function. To investigate whether neocortical Layer 6b (L6b) in the mouse brain comprises a heterogenous population of cells, we examined L6b transcriptomic profiles and spatial organization using scRNA-seq analysis, multiplexed fluorescent in situ hybridization (mFISH) and single-cell spatial transcriptomics (scST). To identify transcriptomic subpopulations within L6b, we analyzed scRNA-seq data from mouse primary motor area (MOp) using dimensionality reduction techniques. We found that L6b excitatory neurons are comprised of four transcriptomically distinct subpopulations which express unique marker genes (e.g., Nmbr, Nxph1, Col6a1, and Hcrtr2). To gain insight into putative functions of these subpopulations we performed a gene ontology analysis. We found that subpopulations are differentially enriched in disease themes such as morphine addiction, Parkinson's disease, and Huntington's disease. We mapped these L6b subpopulations in mouse MOp using mFISH. To increase gene-mapping throughput, we mapped these subpopulations using scST in coronal hemi-sections. We identified that L6b subpopulations occupy the neocortex in a spatially defined manner, where dorsal sublayer subpopulations have distinct transcriptomic profiles compared to ventral subpopulations. These findings support that mouse L6b neurons are a heterogenous population of cells. In the future, these results can be used to understand functional cell-type-specific differences within neocortex L6b.

P1-B-62 - Membrane progesterone receptors mediate the facilitation of synaptic responses by progesterone and allopregnanolone in the rat infralimbic cortex

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Progesterone and allopregnanolone can rapidly alter neuronal excitability and synaptic function in multiple brain regions, and impact cognition in humans and animals. Progesterone can rapidly counter estrogen-induced changes in cognitive strategies, and reduces synaptic responses in the hippocampal CA1 region. Allopregnanolone is a metabolite of progesterone that is a potent modulator of GABAA receptors and also activates progesterone receptors. However, little is

known about the effects of these neurosteroids on synaptic transmission in the prefrontal cortex. Here, we assessed the effects of progesterone and allopregnanolone on evoked field excitatory postsynaptic potentials (fEPSP) in layer I/II of the infralimbic region of the prefrontal cortex in vitro. We observed a lasting increase in fEPSP amplitude after 20-minute application of progesterone and allopregnanolone. The effects of both progesterone and allopregnanolone were mimicked and occluded by the progesterone receptor agonist Org OD 02-0, suggesting that both progesterone and allopregnanolone act on membrane progesterone receptors to facilitate synaptic responses in the infralimbic cortex. Intracellular recordings are now being used to assess the impact of progesterone and allopregnanolone on intracellular EPSPs and membrane excitability.

P1-B-63 - Differential regional vulnerabilities to age-related myelin pathology in human brain

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Healthy myelin is essential for healthy cognition, with different white matter brain regions controlling distinct cognitive domains. It is unknown whether cognitive decline with age reflects region-specific impacts on myelin integrity. Here, we compared myelin integrity with aging in different white matter regions of the human brain involved in cognitive function, using electron microscopy and ultrastructural analysis. We found no loss of myelin integrity in the aged vs young frontal white matter with respect to accumulation of myelin abnormalities, density and size of myelinated axons, and myelin thickness. Notably, we found a relative protection of myelin integrity in frontal vs occipital and central white matter regions in aged brains. Interestingly, the degree of cognitive decline in the 15 years prior to death was not correlated with myelin properties in frontal white matter, in contrast to a positive correlation with central white matter. We hypothesize that this protection may be due to regional differences in axon size, with frontal white matter having smaller axons. Accordingly, mouse central white matter showed a decrease in large diameter axons at an age when cognitive decline is documented. These results reveal regional differences in myelin pathology with age, and suggest large diameter axons as being more vulnerable to age-associated loss of myelin integrity associated with cognitive impairment.

P1-B-64 - Axonal transport of netrin-1 in iPSC-derived human neurons

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Netrin-1 is a secreted protein that functions as a guidance cue for axon pathfinding in development. Netrin-1 also modulates synapse formation in developing neurons and long-term potentiation (LTP) in mature neurons. Moreover, there is a genetic association in C. elegans between UNC-6/netrin-1 and UNC-104/KIF1A, a kinesin-3 motor protein required for axonal transport. Netrin-1 is expressed in human neurons; however, its intracellular distribution and trafficking has not been assessed.We first characterized the endogenous expression of netrin-1 via immunocytochemistry which revealed a punctate pattern in both the axon and dendrites of iPSC-derived human forebrain neurons. Next, we investigated the dynamic transport of fluorescently labeled netrin-1 (netrin-1-mRuby) in human neurons using live-cell imaging and

found bidirectional transport within axons at 1.49 +/- 0.44 micron/sec anterogradely and 1.62 +/- 0.36 microns/sec retrogradely; rates comparable to that of other cargos undergoing microtubule-based trafficking in vertebrate neurons. To identify the netrin-1 vesicle population, using immunocytochemistry we found a high degree of co-localization with the resident dense core vesicle protein chromogranin A. Netrin-1-mRuby and Chromogranin A-GFP also undergo co-transport in the axon. Finally, we found netrin-1-mRuby and KIF1A-GFP co-transport in the axon, suggesting that KIF1A is involved in netrin-1 trafficking. Studies are ongoing to determine the impact of KIF1A-associated neurological disease (KAND) patient variants on netrin-1 trafficking.

P1-B-65 - Oligodendrocyte dysfunction drives human cognitive decline

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Decreases in cognitive function are a hallmark of aging. However, why some individuals experience more severe cognitive decline (CD) while others remain cognitively resilient is unclear. We sought to determine neuropathological correlates of CD using rare human brain samples from the world's longest-running study on cognition, the Lothian Birth Cohort. Participants were born in 1936 in Scotland and had cognitive testing from age 11 until death, allowing unparalleled assessment of CD across life for >1,000 individuals. Here, we investigated neuropathological and transcriptomic change in white matter, given its important role in cognition and vulnerability to pathology with age. We found CD correlated with loss of large-diameter axons, axonal atrophy, and abnormally thick myelin. Single nuclei RNA-sequencing associated CD with a surprising increase in oligodendrocytes (OLs), contrasting their roles as positive modulators of cognition in youth, a result validated by immunofluorescence. Bioinformatic pathway analyses of CD-enriched OLs pointed to reduced activity of NRF2 antioxidant signaling, supported by a reduction in OLs expressing NRF2 protein. To test the functional contribution of reduced NRF2 activity in OLs, we generated an OL-specific conditional knockout of NRF2 (PlpCreERT2Nfe2l2fl.fl), where inducing recombination at 6 months led to reduced large-diameter axons and thicker myelin in aged mice (14-20 months), similar to that observed in CD humans. Together, these data suggest pathological oligodendrocytes in aging contribute to axonal damage and cognitive decline.

P1-B-66 - Mild traumatic brain injury induces circadian phase shifts

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Traumatic brain injury (TBI) is a bump, blow, or jolt to the head that disrupts the proper functioning of the brain (CDC). Some common causes of TBI include falls, motor vehicle accidents, assaults, and sports injuries. Mild TBI (mTBI) (Glasgow coma scale 13-15) makes up 70-90% of all reported TBI cases and affects ~42 million people worldwide annually. Although moderate to severe TBI is diagnosed by CT scans, mTBI often goes undetected. Aside from the more recognized side effects of mTBI such as cognitive impairment and headaches, 1/3 of mTBI patients have dysautonomias and circadian rhythm disruptions. A circadian rhythm is ~24 hours and maintained by a master clock in the suprachiasmatic nucleus (SCN) which relays to and synchronizes peripheral clocks and is vital to the maintenance of many physiological cycles. Using the lateral head impact model of mTBI we found that 1. mTBI at ZT 19 (late night) induces phase advances in locomotor activity and wake-associated physiological temperature-rise

onset via data collected from implanted recording devices. 2. mTBI in the late night advances the firing onset of SCN vasopressinergic (SCNVP) cells via electrophysiology. Immunohistochemistry also shows increased c-Fos activation in SCNVP cells after mTBI. In conclusion, this project establishes that mTBI causes a circadian phase shift, which may be associated with the activation of SCNVP cells.

P1-B-67 - Metaplastic effects of long-term potentiation on the expression of synaptic depotentiation in the rodent hippocampus

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During learning, hippocampal synapses are thought to undergo activity-dependent strengthening (long-term potentiation, LTP) to facilitate later retrieval. The reversal of LTP has instead been implicated in forgetting, however much less is known about the mechanisms that regulate LTP reversal (synaptic depotentiation). Metaplasticity refers to the ability of neural activity to influence subsequent plasticity. For example, the temporal spacing of LTP induction can affect the magnitude of subsequent depotentiation. The aim of the present study was to assess whether the spacing of LTP induction similarly affects the mechanisms of expression of depotentiation. Postsynaptic AMPA receptors (AMPARs) have been linked with certain types of metaplasticity, therefore we assessed AMPAR regulation following LTP and depotentiation induction. 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 a repeated theta burst stimulation pattern and subsequently reversed with a low frequency stimulation. Following electrophysiology recordings, we assessed the expression and phosphorylation state of AMPARs after temporally spaced and compressed LTP and subsequent depotentiation using immunofluorescence and immunoblotting techniques. Our results suggest that the temporal spacing of LTP can influence the mechanisms of expression of depotentiation. This study will ultimately provide novel insights on the metaplastic regulation of forgetting.

P1-B-68 - UNC5B-mediated cell junctions at paranodes involve scaffold proteins DLG1 and Septins

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Paranodes secure the end of myelinated internodes via specialized axo-glial junctions and junctions between myelin loops. In the mature CNS, netrin-1 is expressed by neurons and oligodendrocytes (OLs) and is required for the stability of paranodal junctions. The netrin receptor UNC5B is expressed by myelinating OLs and enriched at paranodal junctions. UNC5B cKO mice exhibit severe paranodal disorganization and reduced amounts of junctional proteins, indicating that UNC5B is required to maintain junctions at paranodes. Examining the distribution of UNC5B relative to the tight junction protein ZO-1 and adherens junction protein β-catenin in HEK293 cells revealed a striking lack of overlap at cell junctions. Ectopic expression of truncated UNC5B showed the importance of the UNC5B ZU-5 domain in ZO-1 exclusion. To identify UNC5B interacting proteins we performed a BioID protein-proximity assay. Among the candidates identified were DLG1, an intracellular scaffold involved in cell junction assembly and myelin regulation, and several septins, involved in junction maintenance and myelin stabilization. Immunolabeling and proximity ligation assays confirmed the association of UNC5B with DLG1

and with septins 7 and 8. Based on homologous domains between ZO-1, DLG1 and UNC5B, we hypothesize that UNC5B and DLG1 might compete with ZO-1 to form "tight junction-like" structures at cell membranes. Our studies aim to provide new insight into molecular mechanisms that regulate junctions at paranodes to inform the development of therapeutic strategies to treat demyelinating diseases.

P1-B-69 - Role of astrocytes in regulating CRH neurons and the HPA axis

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Neuron-glia interactions are essential to regulate synaptic transmission and plasticity across the entire brain. While astrocytes have been shown to be active participants in the central response to stress across cortex, hippocampus, amygdala, and lateral hypothalamus, whether these cells are directly implicated in driving or attenuating stress responses remains poorly understood. Here, we focused on understanding astrocyte-neuron interactions in the paraventricular nucleus of the hypothalamus (PVN). As the PVN is home to a dense population of corticotropin releasing hormone (CRH) neurons which drive glucocorticoid production our study aims to understand the influence of astrocyte activity specifically on CRH neurons in the PVN. To address this knowledge gap, we generated a transgenic mouse line to remove glucocorticoid receptors (GR) in astrocytes by crossing GLAST-creERT with GR-flox mice before carrying out patch-clamp recordings in both male and female mice in the PVN. We analyzed neuronal excitability recording synaptic transmission and intrinsic excitability of PVN neurons in control and astrocyte GR KO mice, before revealing cell identity using immunohistochemistry to identify CRH neurons from other PVN neuron populations. Our preliminary findings demonstrate that deletion of GRs in astrocytes results in hyperactivity of neighbouring PVN neurons. Ongoing experiments are now investigating specificity of these effects and identification of the links between GR signalling in astrocytes and neuronal excitability.

P1-B-70 - The role of Panx1a in neurodevelopment: Pathway-specific interventions for metabolic crisis

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Pannexin-1 (Panx1) is a major ATP release channel implicated in neurodevelopmental and neurodegenerative disorders. Ablation of Panx1a function in gene-edited zebrafish selectively affected dopaminergic signaling, suggesting this gene's function in neuronal networks that use dopamine. By inducing a metabolic crisis in dopaminergic neurons with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), we identified a molecular mechanism in which Panx1a ablation changed AMPK-mTORC1 signaling pathways, cell death, and altered neuronal network connectivity. Accordingly, rescue effects of modulation of the mTORC1 pathway and NLRP3 inflammasome on behavioral performance and neural connectivity following MPTP treatment were tested in Panx1a+/+ and Panx1a-/- zebrafish larvae. Targeting the NLRP3 inflammasome with INF39 alleviated both genotypes' motor and neural connectivity deficits caused by MPTP as assessed by automated scoring of motor behaviors and in vivo dual electrode LFP recordings, respectively. While mTORC1 pathway genes were enriched in Panx1a-/- larvae, metformin - a mTORC1 suppressor - enhanced neuronal connectivity but failed to improve behavioral outcomes, likely due to short treatment duration. These findings support roles of Panx1a in

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energy metabolism and inflammation. Specifically, targeting the NLRP3 inflammasome offers a promising avenue for mitigating the effects of metabolic stress early in life. Funded by NRSC DG RGPIN-2019-06378 (GRZ).

P1-B-71 - Investigating the role of adaptor protein ShcD in the oligodendrocyte lineage

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Oligodendrocytes are crucial to the functioning of neurons, with the myelin produced by these cells facilitating rapid and coordinated action potential propagation, while also providing metabolic support. Myelinating oligodendrocytes are derived from their precursors, oligodendrocyte progenitor cells (OPCs), through a complex differentiation process that is regulated by various growth factors and neurotrophins. Crosstalk between intracellular signaling cascades are at the helm of coordinating this differentiation process and the function of cells at each stage of differentiation. The Shc family of adaptor proteins are well established modulators of many intracellular signaling pathways and have been noted to be expressed within the central nervous system. ShcD, the most recently identified and least well characterized Shc protein, is found to be uniquely expressed within the oligodendrocyte lineage, particularly in OPCs; however, an understanding of the role ShcD plays within these cells has yet to be elucidated. In this work we aim to investigate ShcD in the oligodendrocyte lineage with the use of ShcD knockout (KO) and ShcD wildtype (WT) mouse derived primary OPCs. Probing differences in OPC migration, differentiation capacity, and intracellular signaling allows for a better understanding of the importance of ShcD within this lineage. In parallel, we compare ShcD KO and WT mouse oligodendrocyte profiles and myelin content in vivo using Luxol fast blue staining and western blot analysis of various lineage markers in the mouse brain. The culmination of these efforts allows for a more comprehensive understanding of ShcD and related intracellular signaling pathways within the oligodendrocyte lineage.

C - DISORDERS OF THE NERVOUS SYSTEM

P1-C-72 - Embelin alleviates Amyloid-β-induced neurodegeneration and cognitive impairment: A promising therapeutic avenue for Alzheimer's disease

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Alzheimer's disease (AD) is a significant form of dementia. Embelin (EMB) is a natural compound with varied actions that could help prevent AD pathology. Herein, we have investigated the neuroprotective potential of EMB against Aβ1-42-induced neurotoxicity in rats. In this experiment, Alzheimer-like dementia was induced in rats by infusing Aβ1-42 oligomers directly into the brain's ventricles. Subsequently, the Aβ1-42-intoxicated rats received treatment with varying doses of EMB (2.5, 5, and 10 mg/kg, administered intraperitoneally) over 2 weeks. The spatial and non-spatial memory of animals was assessed at different time intervals, and various biochemical, neurochemical, and neuroinflammatory parameters in the hippocampal brain tissue of the rats were analyzed. Infusion of Aβ1-42 in rat brain caused cognitive impairment and was accompanied by increased acetylcholinesterase activity, oxidative stress, and elevated

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levels of pro-inflammatory cytokines (such as TNF-α, IL-1β, and IL-6) in the hippocampal tissue. Moreover, a significant decline in the levels of monoamines and an imbalance of GABA and glutamate levels were also observed. EMB treatment significantly mitigated Aβ1-42-induced cognitive deficit and other biochemical changes, including Aβ levels. The EMB-treated rats showed improved learning and consolidation of memory. EMB also attenuated Aβ-induced oxidative stress and neuroinflammation and restored the levels of monoamines and the balance between GABA and glutamate. The observed cognitive benefits following EMB treatment in Aβ1-42-infused rats may be attributed to its antioxidant and anti-inflammatory properties and ability to restore hippocampal neurochemistry and Aβ levels. The above findings indicate the therapeutic potential of EMB in neurodegenerative pathologies associated with cognitive decline, such as Alzheimer's disease.

P1-C-73 - Unlocking the secret of tradition: Neuroprotective potential of Semecarpus Anacardium (SA) through cellular and genetic pathways

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Semecarpus anacardium (SA) has several medicinal properties including enhancing learning and memory. However, the underlying mechanisms contributing to these therapeutic and neuroprotective effects have yet to be explored scientifically. We aimed to investigate SA's memory-enhancing, neuroprotective, and antioxidant effects in a battery of in-vitro and in-vivo tests against glutamate-induced neuronal cell death models. A modified open-field apparatus carried out delayed matching to sample (DMS) and delayed non-matching to sample (DNMS) tests. Spontaneous alternation and spatial memory were carried out in the elevated plus maze. 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) proliferation assay kit was used to see the effect of compounds on cell viability. The concentration of intracellular Ca^{+2} was measured with Fluo-3 AM. The effects of SA on acetylcholine esterase (AChE) enzyme inhibition were measured by Ellman's method. The superoxide dismutase (SOD) activity of the SA extract was measured using a biochemical assay.Results obtained from calcium imaging show that SA may offer neuroprotection under glutamate insult by decreasing excessive calcium influx which is the primary mechanism of glutamate induced cell injury. SA potently inhibited acetylcholinesterase enzyme in a dose-dependent manner providing further mechanistic insight into memory enhancing properties of SA. These drug targets and pathways include increased cell viability, enhanced SOD activity, Inhibition of AChE, and reducing excessive calcium entry in the neuronal cells.

P1-C-74 - Impact of oral glyphosate exposure on the population of cocaine- and amphetamine-regulated transcript (CART)- expressing enteric nervous system neurons in the porcine small intestinal wall

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IntroductionGlyphosate-based herbicides are among the most widely used contaminants globally, with food products containing glyphosate being a significant part of human and animal diets. This study aimed to evaluate the effects of oral glyphosate supplementation on cocaine- and amphetamine-regulated transcript (CART)-expressing enteric nervous system (ENS) neurons in the porcine small intestine.Materials and MethodsFifteen sexually immature gilts

were randomly assigned to three groups: control (empty gelatin capsules), E1 (low dose of glyphosate: 0.05 mg/kg b.w./day, corresponding to the theoretical maximum daily intake), and E2 (higher dose of glyphosate: 0.5 mg/kg b.w./day, corresponding to the acceptable daily intake), all administered for 28 days. After the supplementation, pigs were euthanized, and duodenum, jejunum, and ileum samples were collected for analysis. Frozen sections underwent double immunofluorescence staining using HuC/D (panneuronal marker) and CART primary antibodies, with Alexa Fluor 488 and 546 secondary antibodies.ResultsGlyphosate exposure increased the number of CART-immunoreactive ENS neurons in all sections of the small intestine and across all plexus types (myenteric plexus, outer and inner submucosal plexuses). The increase was more pronounced in the E2 group and slightly smaller in the E1 group.ConclusionsThe increased number of CART-expressing ENS neurons suggests that CART may contribute to neuroprotective and defensive processes in response to glyphosate adverse effects on the small intestine.

P1-C-75 - Rotenone, a mitochondrial neurotoxin accelerates endogenous α-synuclein spreading and enhances dopaminergic neurodegeneration in an intra-striatal preformed fibril (pff) synuclein injected mouse

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Background: Parkinson's disease (PD) is one of the most prevalent progressive neurodegenerative disorders affecting movement. While the exact cause of neuronal loss remains unknown, the histopathological hallmark of PD is the presence of intraneuronal aggregates of α-synuclein protein along with the death of dopamine-producing neurons in the substantia nigra pars compacta (SNc) region. In our current study, we are investigating whether rotenone, a natural agrochemical, can further enhance α-synuclein propagation, and neurotoxicity. Methods. We employed two paradigms to investigate the role of Rotenone. In both, human α-synuclein PFF seeds were injected into the striatum of C57BL/6 mice using stereotactic surgery. In the first paradigm, Rotenone (2.5 mg/kg body weight) was administered intraperitoneally once daily for four consecutive weeks, starting one day after PFF injection. In the second paradigm, the same dose of Rotenone was given three weeks after the PFF injection for four weeks. Mice were sacrificed 24-hour after the final Rotenone injection for immunohistochemical (IHC) analysis.Results. IHC results from both models indicate that Rotenone administration enhances α-syn spreading, neurotoxicity and neuroinflammation. In both models, most of the accumulated α-syn displayed proteinase K resistance, suggesting a Lewy body like nature of the protein. Interestingly, in IHC analysis, the experimental (PFF+R) group of model system 2 showed greater enhancement in phospho-syn spreading (~33% increase from PFF+V) as opposed to model system 1 (~6% increase).Conclusions. In conclusion, our study provides evidence for the development of a robust PD animal model that can closely recapture the underlying pathological processes involved and assist in identifying therapeutic targets that respond to α-syn pathology.

P1-C-76 - MIP2 alleviates the severity of intracerebral hemorrhage by inhibiting TLR4 epxression

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TLR4 (Toll-like receptor 4) is a key inflammatory transmembrane receptor protein that initiates MyD88 transcription factors, including NF-κB, thus producing pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α). Therefore, MIP2 is used as a TLR4 targeting peptide that has previously shown effective results in reducing neuroinflammation and improving brain functions in autoimmune diseases. However, its role in ICH-mediated brain injury has not been explored yet. Therefore, we aimed to investigate whether MIP2 can alleviate secondary brain damage after ICH, primarily its neuroprotective effects and anti-inflammatory pathway.MIP2 (MyD88 adaptor-like inhibitory peptide) decreased hematoma volume and neurobehaviour function scores compared to the ICH+DMSO group. MIP2 decreased the expression levels of MMP9 and elevated the expression of BBB tight junction proteins (ZO-1, Occludin, Claudin-5). Results based on EVAN's blue assay, ZO-1, vWF, and MMP9 positive cells indicated that MIP2 protected the BBB integrity. Immunofluorescent staining of Iba-1, MPO, and GFAP showed that MIP2 can potentially reduce the activation of microglia/macrophages, neutrophils, and astrocytes respectively. In response to the application of MIP2 the expression of the pro-apoptotic protein (Bax), and the number of TUNEL-positive cells was decreased, while the expression of anti-apoptotic protein (Bcl-2) was elevated. In addition, the expression of inflammatory proteins (MyD88, TLR4, TNF-α, IL-6, NF-kβ, iNOS) was mitigated by targeting the MyD88/TLR4 pathway.

P1-C-77 - Brain printing biometrics in crosstalk with rod-shaped microglia and trem2 signaling for early Alzheimer's disease detection

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Introduction:Brain-Printing Biometrics (BPB), a precise, non-invasive diagnostic approach, uses two interconnected mapping techniques: structural magnetic resonance imaging (sMRI) and electroencephalography (EEG), and is specially designed for early Alzheimer's disease (AD) detection. There is growing evidence that AD progression is induced by rod-shaped microglia in early phases, particularly in relation to TREM2 (triggering receptor expressed on myeloid cells 2) signaling. Our aim is to determine whether BPB technique and rod-shaped microglia /TREM2 signaling can be used for the early detection of AD. Methods: We used BPB on the male TgF344-AD transgenic rats at multiple time points. Pathological changes were analyzed before and after memory impairment onset and AD hallmark depositions. Rod-shaped microglia were detected using IBA1 immunohistochemistry in the hippocampus, cortex, and cerebellum. TREM2 expression and associated inflammatory cascades were evaluated in these regions along with cerebrospinal fluid using ELISA technique. Results: BPB identified changes in brain regions relative volume and functional deterioration as the disease progressed. Rod-shaped microglia were also detected in the hippocampus and cortex, which strongly correlates with TREM2 signalling activation. Conclusion: These findings highlight the role of rod-shaped microglia as predisposing factor and predictive biomarkers for AD progression. Whereas, BPB represents an innovative diagnostic tool for early AD detection by assessing brain biometrics.

P1-C-78 - Nerugluin-1 treatment promotes brain neurogenesis and cognition recovery in progressive demyelinating lesions of Multiple Sclerosis

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Extensive demyelination and neurodegeneration are pathological hallmarks of progressive multiple sclerosis (P-MS) that underlie cognitive impairments. Neural precursor cells (NPCs) support learning and memory through neurogenesis and remyelination; however, their capacity declines with disease progression, exacerbating neurodegeneration and disability. Therapies enhancing neuroprotection and neurogenesis are critical for cognitive recovery in P-MS. We identified dysregulation of Neuregulin-1 (Nrg-1) in P-MS lesions. Nrg-1, essential for neural differentiation and myelination, is implicated in neurodevelopmental psychiatric disorders. Here, we investigated the impact of Nrg-1 dysregulation on neurogenesis and cognition in a chronic demyelination mouse model.Using Nes-CreERT2; Rosa26-mGFP mice for NPC tracking, we induced chronic demyelination with cuprizone and administered daily Nrg-1 treatment for four weeks after 10 weeks of demyelination. We assessed Nrg-1 effects on neurodegeneration, neurogenesis, synapses, and memory functions both in vitro and in vivo.Nrg-1 treatment restored hippocampal and white matter myelin, reduced neurodegeneration, and enhanced dendrite arborization of CA1 neurons critical for memory consolidation. It increased dendritic spine density and excitatory/inhibitory synapses, improving hippocampal neuronal activity. Moreover, Nrg-1 restored NPC activity, promoted hippocampal neurogenesis and oligogenesis, and improved recognition and spatial working memory impaired by chronic demyelination.These findings underscore Nrg-1 as a promising therapeutic candidate for P-MS, with the potential to ameliorate neurodegeneration and cognitive deficits by promoting remyelination and neurogenesis.

P1-C-79 - Defective ocular glymphatic system in the retina of Alzheimer's donor eyes: Degeneration of Macroglia and Aquaporin-4 water channels

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Objective: Impaired clearance of amyloid-beta is a defining feature and a potential driver of Alzheimer's disease (AD) pathogenesis. Among the various clearance pathways, the glymphatic system, a paravascular network composed of glial cells and aquaporin-4 (AQP4) water channels, facilitates the removal of metabolic waste. This study aims to investigate the glymphatic system in AD by leveraging the retina as an extension of the brain, focusing specifically on macroglia, water channels, and disease-associated morphological changes, features that are challenging to observe in ex vivo brain tissues.Methods: Wholemount neuroretinas from AD donors (n = 7, mean age = 78.9 ± 10.7 years, 4 females) and controls (n = 7, mean age = 72.4 ± 2.6 years, 3 females) were analyzed using immunofluorescence staining. Antibodies targeting astrocytes and Müller glia (GFAP), Müller glia (GS), water channels (AQP4), and vascular endothelium (UEA-I) were used. High-resolution confocal microscopy captured depth images, and immunoreactivity was quantified in pixels using ImageJ.Results: Compared to controls, AD neuroretinas exhibited significant degeneration of AQP4 (p = 0.002), along with prominent bead formation and progressive macroglia degeneration, as evidenced by a marked reduction in GFAP (p = 0.002) and GS (p = 0.002) immunoreactivity. The age groups were not significantly different (p = 0.147).Conclusion: AD retinas exhibit pronounced degenerative changes in the ocular glymphatic system, potentially mirroring alterations in the brain that may influence disease onset and progression

P1-C-80 - Neurodevelopmental effects in genetic frontotemporal dementia: intracranial volume and education differences

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Converging evidence hints at neurodevelopmental effects in genetic frontotemporal dementia (FTD). This includes differences between young adult mutation carriers compared to non-mutation carriers in cognition and total intracranial volume (TIV), a marker of neurodevelopment that stabilizes in late adolescence. We thus investigated TIV and educational attainment differences between adult mutation carriers and non-carriers, as measures of structural and functional neurodevelopmental outcomes of FTD-causing genes. This cross-sectional cohort study comprised 489 gene mutation carriers and 332 familial non-carriers, aged 18-86 years, from the FTD Prevention Initiative across North America and Europe. Genetic groups included GRN (34%), MAPT (25%), and C9orf72 (41%). Consistent with prior findings in young adults, GRN carriers showed larger TIV compared to familial non-carriers (95% CI=1431994-1457123, p=0.049, ηp2=0.008). Larger TIV correlated with higher years of education in GRN carriers (95% CI=0.01-0.24, r(295)=0.12, p=0.03) and GRN non-carriers (95% CI=0.08-0.34, r(198)=0.21, p=0.002). MAPT carriers demonstrated smaller TIV than non-carriers (95% CI=1417819-1450628, p=0.039, ηp2=0.02). Models of C9orf72 and those with education as the outcome did not reveal significant differences. In support of the neurodevelopmental hypothesis of FTD, GRN and MAPT mutations are linked to structural neurodevelopmental changes, some of which correlate to educational attainment. These findings will motivate further research to identify mechanisms by which FTD mutations impact neurodevelopment.

P1-C-81 - Tat-Interactive Protein 60 mediates Stress-induced hypertension via glutamate release from the dorsomedial prefrontal cortex to the ventral CA1 region

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IntroductionManaging stress is a non-pharmacologic approach to treating hypertension. Unraveling the molecular mechanism by which stress conditions influence hypertension is crucial. We hypothesized that TIP60 mediates stress-induced hypertension by releasing glutamate from the dorsomedial prefrontal cortex (dmPFC) into the ventral CA1 region of the hippocampus.MethodologyThis study used western blotting and ELISA to assess glutamate release and NR2B expression in Stress-induced hypertensive mice. To further determine whether TIP60 was involved in glutamate release leading to hypertension, MG149 (a TIP60 inhibitor) was also injected intraperitoneally alongside Chronic restraint stress (CRS) modeling to see its effects.ResultsIn the CRS conditions, TIP60 expression and vCA1 glutamate release were found to be up-regulated, with high blood pressure indicating hypertension. The release of glutamate increased, suggesting that activity within the dmPFC drives the release of glutamate in the vCA1. This was blocked by injecting MG149 into the dmPFC. The increased glutamate release, NR2B, and CRS-induced hypertension were reversed by chronic application of MG149. ConclusionThe results suggest that TIP60 influences glutamate release from the dmPFC to the vCA1. Hence, stressful conditions lead to increased expression of TIP60 resulting in conditions that favor glutamate release and NR2B expressions leading to hypertension. Keywords: Tat-interacting protein 60; Hypertension; Chronic restraint stress; Glutamate; ventral CA1

P1-C-82 - Effects of phytocannabinoids on postictal hypoxia

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Postictal hypoxia, which follows a seizure, is a severe reduction in region specific oxygenation due to the constriction of local blood vessels. Postictal hypoxia contributes to several consequences associated with seizures such as memory disruption, behavioural and emotional alterations, and sudden unexpected death in epilepsy. The primary phytocannabinoids from cannabis—cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC)—may ameliorate postictal hypoxia given their vasoactive properties. This study focused on the effects of CBD and THC on seizure-induced hypoxia in both male and female Long Evans rats. Stereotaxic surgery was performed to implant a bipolar electrode for inducing and recording seizures, and an oxygen-sensing optode to measure hippocampal oxygen levels. Each phytocannabinoid was administered separately as an inhalant as this is the most common modality for consumption in humans. Rats were assigned to drug specific cohorts and the effects on oxygen were measured before, during, and after seizure elicitation. CBD ameliorates postictal hypoxia without altering baseline oxygen levels in both male and female rats. Sex differences were apparent as CBD significantly reduced the severity of hypoxia but not the time under the severe hypoxic threshold in males yet significantly reduced both severity and time under the threshold in females. THC failed to improve postictal hypoxia when compared to controls. CBD has shown potential to treat the hypoxia-induced comorbidities resulting from repeated seizures, increasing the quality of life of people with epilepsy.

P1-C-83 - Machine learning-based analysis reveals loss of healthy striatal, cortical and thalamic neurons in the 5xFAD mouse model of Alzheimer's disease

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Alzheimer's disease (AD) is the most common neurodegenerative disorder and the leading cause of age-related dementia globally. The 5xFAD transgenic mouse model is a well-known genetic model of Alzheimer's disease (AD) that has been instrumental in translational research but evidence of neurodegeneration is lacking. Here, we aimed to examine regional neuronal counts using advanced machine learning in 14-month-old 5xFAD transgenic female mice. Serial stereological cryosections from 5xFAD and wild-type (WT) littermates (N = 6–8) were stained with cresyl violet and congo red. Regional volumes, plaque density and nuclear shape and number were quantified in QuPath. Proportional loss in volume was largest and most robust in striatum followed by thalamus, then cortex, then hippocampus. We separated nuclei into pyknotic and healthy populations based upon size and cresyl violet staining intensity. We observed a loss in healthy neurons in striatum (effect of genotype $F_{(1, 12)} = 13.28$, $p<0.01$), cortex (nuclear type x genotype $F_{(2, 24)} = 9.5$, $p<0.001$) and thalamus (nuclear type x genotype $F_{(2, 24)} = 4.4$, $p<0.05$) of 5xFAD mice. Plaques showed marked heterogeneity in distribution in striatum and thalamus. Thus, we observed significant loss of healthy neurons in striatum, cortex and thalamus of 5xFAD mice at 14 months of age when we have previously observed onset of severe cognitive deficits. To our knowledge, this is the first evidence showing neuronal loss in 5xFAD mice in striatum and thalamus, and our data reiterates the importance of these nuclei in familial AD.

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P1-C-84 - The immediate effects of repetitive mild traumatic brain injuries (rmtbis) on electroencephalographic activity in a mouse model of Alzheimer's disease

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Traumatic brain injuries (TBI) are a significant public health concern, affecting approximately 165,000 Canadians annually. Repetitive mild TBIs (rmTBIs) are a known risk factor for neurodegenerative diseases such as chronic traumatic encephalopathy and Alzheimer's disease (AD). However, the impacts of rmTBIs on brain activity within the initial weeks post-injury and their potential link to AD remain poorly understood. This study aimed to characterize intracranial EEG activity during sleep and wakefulness within the first month following rmTBIs in APPNL-F mice. Using the closed-head impact model of engineered rotational acceleration (CHIMERA), we delivered three rmTBIs 24hr apart to APPNL-F mice (n=23; females = 11) at six months of age. Cortical EEG electrodes were implanted following the last rmTBI. Recordings began post-surgery and included an initial 72-hour recording, followed by weekly 48-hour recordings for another four weeks. Preliminary results show no significant differences in the amounts of NREM, REM, or wake between the TBI and sham groups at any of the timepoints. However, there was a decrease in the amount of NREM sleep between the first 72-hours and following sessions for both groups. We also found that the TBI group had higher delta power during NREM and higher theta power during REM within the first 72-hours after the rmTBIs.

P1-C-85 - The mutant LRRK2-G2019S modifies the activity of voltage-gated CaV1.3 channels through Cavβ3 phosphorylation

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Introduction: One of the most common proteins involved in the pathogenesis of Parkison Disease (PD) is the leucine-rich repeat kinase 2 (LRRK2) and one of its most studied mutations is G2019S related with genetic forms of PD. However, how mutations in LRRK2 lead to PD remains poorly understood. Voltage-gated calcium channels (CaV) type CaV1.3 are thought important for calcium homeostasis in dopaminergic neurons which are affected in PD and could be a phosphorylation target of LRRK2. Here we hypothesize that G2019S might participate in the early stage of the PD by modifying the activity of CaV1.3.Methods: We performed immunoprecipitation assays to confirm the interaction between channel CaV1.3 and G2019S expressed in HEK-293 cells. In addition, whole-cell patch clamp recordings were performed to detect any changes in the calcium currents through CaV1.3 channels coexpressed with G2019S. Also, we sought the possible phosphorylation sites in Cavβ3 by LRRK2 and evaluate the change in calcium currents. Results: We found that LRRK2 and G2019S interact with the calcium channel complexes but only G2019S increased the CaV1.3 current. In addition, we found that this regulation occurs through CaVβ3 phosphorylation at serine 152.Discussion:These findings could be a new lead to understand the development of PD as the result of the aberrant activity of calcium channels triggered by alterations in phosphorylation, a post-translational modification that affects

channel activity. The results also show a pivotal role of the LRRK2 kinase in regulating these channels.

P1-C-86 - Absence of microglial homeostatic TGFβ production induces myelin dynamics that mimic multiple sclerosis

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Multiple sclerosis (MS) is a neurodegenerative disease caused by damage to myelin and myelin-forming oligodendrocytes in the central nervous system (CNS), and is characterized by progressive motor, sensory, and cognitive deficits. While this damage is thought to be mediated by peripheral immune cells, targeting these immune cells does not slow progression, suggesting other mechanisms at play. Microglia are thought to play a role in MS pathogenesis, as they downregulate homeostatic genes early in disease, suggesting a loss of function. They are also dysregulated at sites of myelin damage that have no immune infiltration, suggesting a microglial role in myelin damage initiation. We investigate how loss of microglial function influences myelin and oligodendrocyte health using Csf1r-FIREΔ/Δ mice, which constitutively lack microglia. We find that absence of microglia is sufficient to mimic myelin dynamics observed in MS. We observed focal white matter demyelination in Csf1r-FIREΔ/Δ mice at 6 months, followed by remyelination, and a recurrent demyelination at 12 months that persisted. Demyelination was preceded by the emergence of a Serpina3n+ oligodendrocyte population, and was concurrent with decline of this population and upregulation of ferroptosis markers. Ablation of TGFβ from microglia was sufficient to induce Serpina3n+ oligodendrocyte loss and myelin damage. We propose that the absence of microglial homeostatic function causes the appearance of a pathological oligodendrocyte population whose death may drive myelin damage in MS.

P1-C-87 - Reduced EEG complexity in Alzheimer's disease and frontotemporal Dementia with differences in rostrocaudal activity

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Neuroimaging devices have immaculate spatial resolution which can identify structural changes in neurodegenerative disorders, but are not cost-effective. Electroencephalography (EEG), while lacking in spatial resolution, can measure at the operating speed of neuronal processes (milliseconds). For this experiment we utilised two signal complexity measures, fractal dimension (FD) and detrended fluctuation analysis (DFA), to assess AD and frontotemporal dementia (FTD) EEG recordings from OpenNeuro. Data was processed with interest in broad regions as opposed to single channels (i.e. frontal mean, caudal mean, all-channel mean). With FD, the space-filling properties of a signal, we found significantly lower values when comparing age-matched controls (n=29) to AD [t(63)=4.465, p<0.0001] and FTD [t(49)=2.861, p=0.0062]. Differences between AD (n=36) and FTD (n=22) were observed when comparing the rostrocaudal difference in FD, wherein there was caudal dominance in FTD but not AD [t(56)=3.009, p=0.0039]. We also observed long-range temporal correlations using DFA for amplitude fluctuations in the alpha band (8-13 Hz) and epochs between 2-25 seconds. We found significant differences when

comparing the all-channel averaged DFA exponent of controls to AD [t(63)=3.302, p=0.0016] and FTD [t(49)=2.991, p=0.0043]. This indicates that signals in these disorders show reduced scaling behaviours between amplitude fluctuation and time scale, similar to white noise. The results demonstrate measures of complexity have potential in diagnostics of AD and FTD, and to differentiate between them.

P1-C-88 - Spikes and sprouts: Supramammillary nucleus of the hypothalamus contributes to hippocampal dentate gyrus and ca2 epileptiform activity in a mouse model of temporal lobe epilepsy

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Temporal lobe epilepsy (TLE) is characterized by abnormal synchronized neural activity that causes seizures. The hippocampus is broadly implicated in TLE since approximately 80% of seizures originate in the hippocampus or adjacent regions. However, the circuits that contribute to seizure activity in TLE are not fully understood. Recent studies have shown that the supramammillary nucleus (SuM) of the hypothalamus sends excitatory projections to the hippocampal dentate gyrus (DG) and CA2 which influences hippocampal activity and functions. Although the SuM-hippocampal circuit is well-characterized in healthy mice, there is limited data on this circuit in TLE. Vesicular Glutamate Transporter 2 (VGluT2-Cre) transgenic mice were used to evaluate the SuM-hippocampal circuit in acute and chronic epilepsy. Optogenetics slice electrophysiology found that SuM-DG and SuM-CA2 synapses are normally weak but can drive epileptiform-like activity during conditions that simulate seizures. Next, multi-site jGCaMP8m fiber photometry revealed that aberrant and synchronous Ca2+ activity occurs in the SuM-DG and SuM-CA2 circuit during seizure onset. Cre-dependent GFP injections in the SuM also revealed significant sprouting of GFP+ axons in the hippocampus one month after status epilepticus. We are currently using bidirectional chemogenetics to determine whether manipulations of SuM activity can alter the progression status epilepticus and severity of chronic epilepsy. Collectively, this work suggests that the SuM-hippocampal circuit plays an important role in TLE.

P1-C-89 - Single cell approaches define forebrain neural stem cell niches and identify microglial ligands that enhance precursor-mediated remyelination

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Adult neural stem cells (NSCs) in the ventricular-subventricular zone (V-SVZ) produce myelinating oligodendrocytes, presenting a potential target for remyelinating therapies.Using lineage tracing and single-cell (sc) transcriptomics, we found that dorsal NSCs are activated to generate progeny that increase oligodendrocyte production during recovery from cuprizone-rapamycin-induced demyelination. These cells are transcriptionally unchanged during remyelination, but the V-SVZ environment differs.During recovery, microglia increase ~3-fold, alter ligand expression and exhibit a distinct transcriptional state ("remyelination enriched [RE]"), compared to controls. Sc-spatial transcriptomics showed that RE microglia are specifically enriched in the recovering dorsal V-SVZ and corpus callosum. We generated

predictive scRNA-seq and proteomic communication networks of microglia-expressed ligands that may stimulate NSC-mediated oligodendrogenesis during recovery. These identified two microglial ligands, Igf1 and Osm, that stimulated NSC proliferation and oligodendrogenesis in cortical precursor cultures and when infused in vivo into the V-SVZ, even in NSCs that typically make neurons. Expression of Lyz2-Cre-ERT2 in RE but not homeostatic microglia enabled tamoxifen-inducible RE microglia ablation during cuprizone-rapamycin recovery. Initial data suggest that RE microglia ablation during remyelination reduces dorsal precursor proliferation and OPC number. Our data indicate a role for microglial ligands IGF-1 and OSM in NSC-mediated oligodendrogenesis and, possibly, myelin repair.

P1-C-90 - Evaluation of social preference, anxiety, cortisol, and locomotion in a mecp2 null-mutant zebrafish model of Rett Syndrome

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Methyl CpG binding protein 2 (MECP2) is vital for neuronal function as it is an essential global modulator of transcription, and mutations in MECP2 are the most common cause of Rett syndrome, an X-linked neurodevelopmental disorder. Patients diagnosed with Rett syndrome have increased risk for epilepsy and problems with anxiety and socialization. Using the zebrafish mecp2Q63X line, this study aimed to increase our understanding of mecp2 regulation of locomotion, ontogeny of social behaviour, and adult socialization and anxiety behaviour. To determine responses of mecp2-/- zebrafish to a convulsant, locomotion was measured at 5 days post-fertilization (dpf) in sibling mecp2+/+, mecp2+/-, and mecp2-/- fish after treatment with a GABAA receptor antagonist pentylenetetrazol (PTZ) at varying concentrations. Responses to social stimulus were investigated in larval (21 dpf) and adult mecp2-/- and mecp2+/+ fish. Anxiety responses to a novel tank were also explored in adult mecp2-/- and control mecp2+/+ fish. These behavioural tests showed that mecp2-/- fish displayed hypolocomotion at the larval stage and increased freezing time and thigmotaxis in adulthood. However, lack of functional Mecp2 did not change the hyper-locomotion response to PTZ at 5 dpf or affect the social preference for visual social stimulus at 21 dpf and in adulthood. In conclusion, null mutation in mecp2 altered larval locomotion and anxiety behaviour at different ages without affecting adult locomotion and socialization, and developmental sociability and PTZ-induced hyperlocomotion in zebrafish.

P1-C-91 - Nucleosome remodeling restricts axon growth in vitro: New insights towards neural repair

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Neurons lose the intrinsic ability to regenerate axons in the central nervous system (CNS) as they mature during development, limiting recovery following spinal cord injury and other diseases/disorders characterized by axon disruption. The underlying molecular mechanisms that dictate this loss in regenerative ability remain largely unclear. To orchestrate large-scale changes in gene expression, nucleosome remodeling complexes reposition histones to control the access of transcriptional machinery to DNA. Whether nucleosome remodeling regulates axon outgrowth is unknown. We report that pharmacological suppression of the BRG1-BRM

associated factor (BAF) nucleosome remodeling complex promotes axonal growth in adult mouse dorsal root ganglion (DRG) neurons cultured on growth inhibitory substrates. Bulk RNA sequencing followed by Gene Ontology enrichment analyses revealed the drug treatment induced the upregulation of genes enriched for functions related to cAMP and Ca2+ signalling, suggesting that nucleosome remodeling restricts axon outgrowth by suppressing cAMP/Ca2+. These findings indicate nucleosome remodeling as a regulator of axon outgrowth and suggest targeting this process pharmacologically may have therapeutic potential as a novel strategy to stimulate axon regeneration following injury.

P1-C-92 - Tandem repeats in genes associated with synaptic functions are frequently expanded in individuals with suspected genetic epilepsies

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Introduction: Epilepsy is a brain disorder characterized by recurrent seizures, with genetic factors playing a significant role in its development. While genome sequencing (GS) detects most genetic variations, >50% of suspected genetic epilepsies remain undiagnosed. GS can identify tandem repeat expansions (TREs), which are associated with over 60 monogenic disorders. However, the genome-wide impact of TREs in epilepsy is unclear. Methods: We analyzed GS data for rare TREs in 1,465 individuals with epilepsy and 386 controls. We performed a logistic regression analysis to compare the burden of TREs between the two cohorts. To identify regulatory elements and functional pathways impacted by TREs, we performed a burden analysis on ChIP-seq data from human postmortem brains and a gene set enrichment analysis using Gene Ontology terms.Results: We identified 490 rare TREs in 182 genic regions, including 10 known TRE disease loci. We found that rare TREs are enriched in the 5' untranslated region (odds ratio (OR) = 3.1, p = 2.9 x 10-2) and in proximity to the transcription start site (OR = 3.8, p = 4.0 x 10-2). We demonstrated that rare TREs have high GC content (p < 2.2 x 10-16), often overlap with active enhancers in the developing brain (OR = 1.5, p = 6.4 x 10-3), and impact synaptic genes (OR = 2.7, false discovery rate = 0.04).Conclusion: Our findings suggest that TREs contribute to epilepsy by disrupting active enhancers involved in regulating gene expression during brain development. We estimate that enhancer-associated TREs contribute to 3.6% of the risk in epilepsy.

P1-C-93 - Mutations in ubiquilin-2 gene lead to protein accumulation and impair cellular stress defenses in patient-derived neural cells

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Introduction Ubiquilin-2, encoded by the UBQLN2 gene, removes damaged or misfolded proteins and is essential for cellular health. Pathological variants in UBQLN2 lead to protein accumulation and cellular stress. However their role in human neurodegeneration remains unclear. Since variants in UBQLN2 are known to disrupt protein quality control pathways, we hypothesize that

neural cells derived from patients with Frontotemporal Dementia (FD) linked to UBQLN2 will exhibit impaired proteostasis, mitochondrial dysfunction, and increased oxidative stress. Methods We developed a patient-derived model by reprogramming fibroblasts from a male FD patient with two novel UBQLN2 variants into induced pluripotent stem cells and differentiating them into neural lineage. We used Western blots to analyze proteins that manage oxidative stress, protein folding, and cell survival. Flow cytometry assessed reactive oxygen species (ROS) and mitochondrial membrane potential. Results Patient-derived neural stem cells showed elevated Ubiquilin-2 levels and reduced Sod2, Hsp70, and Sirt1. Flow cytometry revealed increased ROS and mitochondrial superoxide levels and a hyperpolarized mitochondrial membrane potential. Discussion Our findings suggest that UBQLN2 variants disrupt proteostasis, elevate oxidative stress, and impair mitochondrial function, weakening cellular stress defenses and potentially driving neurodegeneration. This in vitro model offers a valuable tool for understanding disease mechanisms and exploring therapeutic strategies targeting these disrupted pathways.

P1-C-94 - Effects of positive and negative allosteric modulators of cannabinoid receptor type 1 (CB1R) on epileptiform activity in an animal model of temporal lobe epilepsy

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Two-thirds of patients with temporal lobe epilepsy (TLE) are resistant to anti-seizure medications. One promising approach for epilepsy treatment is modulating network activity via endogenous cannabinoid receptors. Previous work found that a positive allosteric modulator of the cannabinoid receptor type 1 (CB1R) reduced epileptic discharges in the cortex of a genetic model of generalized absence epilepsy. However, the effects of CB1R allosteric modulators on neuron physiology remains unclear. Here, we used whole-cell patch clamp electrophysiology to investigate the effects of CB1R allosteric modulators on the intrinsic properties and epileptiform activity of entorhinal cortex neurons in control and epileptic pilocarpine-treated mice. Male and female C57BL/6J mice (3-6 months old) were used for all experiments. We evaluated two CB1R modulators: GAT591 (positive) and GAT358 (negative) at 1µM concentration. Neuron discharges were induced by perfusing the slices with a pro-epileptic solution containing 4-aminopyridine (4-AP, 100mM). In control mice, GAT591 increased the excitability of excitatory cells, leading to an increased discharge frequency in epileptic condition. In contrast, GAT358 did not affect spike frequency but caused lower rheobase current and rapid depolarization block in cells. In epileptic mice, neither modulator significantly altered neuronal activity properties. However, GAT591 reduced epileptiform activity under the 4-AP solution, whereas GAT358 did not affect cell discharges. These findings suggest GAT591's potential for therapeutic use in TLE treatment.

P1-C-95 - Examining the link between dark microglia and glioblastoma multiforme in human neurosurgery samples

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Microglia, the resident immune cells of the brain, serve an invaluable role in maintaining the brain's homeostatic environment throughout life. However, they can undergo phenotypic and functional changes that can promote inflammation and cause damage to normal neural structures, making microglia a highly heterogeneous cell population. These distinct microglial

states have recently been identified in Alzheimer's and Parkinson's diseases. Recently, a novel microglia phenotype has been characterized by ultrastructure studies, termed dark microglia (DM). Distinguished by their loss of nuclear heterochromatic pattern and electron-dense components, DM are prevalent in pathological and aging conditions but largely absent in homeostatic conditions, highlighting DM's significance in pathological contexts. Glioblastoma multiforme (GBM) is one of the most aggressive and incurable malignancies. Importantly, glioma-associated microglia and macrophages (GAM) contribute significantly to the maintenance of the tumour microenvironment, regulate and assist in tumour growth, and secrete proinflammatory cytokines. Therefore, GAMs remain an important target of study. Using scanning electron microscopy on human neurosurgery samples, this study aims to: 1) characterize microglial states based on their ultrastructural features in GBM, and 2) investigate potential interactions between DM and glioblastoma tumours. In summary, this research provides novel insights into the role of microglia in GBM progression, shedding light on an underexplored aspect of microglial involvement in cancer.

P1-C-96 - Distinctive features of microglia's states in aging and Alzheimer's disease pathology: a quantitative ultrastructure analysis in human post-mortem brain samples

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Microglia, the resident immune cells of the central nervous system, play crucial roles in maintaining homeostasis, yet also contribute to various pathological conditions like Alzheimer's disease (AD). Microglia exhibit plasticity, with many distinct pathological states such as disease-associated microglia (DAMs), microglia associated with neurodegeneration (MGnD), plaque-associated microglia (PAMs), and the recently identified dark microglia (DM) states in patients with neurodegenerative diseases, notably AD. Previous ultrastructural findings illustrated that DM contain more electron-dense components accompanied by other cellular stress features in post-mortem human samples. Aging and progression of AD pathology have a direct correlation with transcriptomic signatures expressed by DAMs, MGnD and PAMs, and these microglial states show indicators of increased cellular stress and ultrastructural features of senescence in mainly rodent models. Using scanning electron microscopy, we aimed to characterize the impact of AD pathology and aging on these AD-associated microglia (AAM) states in the prefrontal cortex, a key region involved in cognitive aging and AD. Through immunohistochemical staining, we first examined the expression of APOE and TREM2 in AAM. We next investigated the density of different subpopulations of AAM and their AD-associated ultrastructural features in on post-mortem human brain. In summary, our study provides novel insights into the functional relevance of AAM populations and emphasizes the importance of microglia as potential therapeutic targets in AD treatment in.

P1-C-97 - Investigating the role of oxidized phosphatidylcholine in neurodegeneration in the context of Multiple Sclerosis

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Multiple Sclerosis (MS) is a chronic neuroinflammatory and neurodegenerative disease affecting 2.9 million worldwide. People living with MS (pwMS) can have a range of symptoms including muscle weakness, vision impairment, impaired sensation, and cognitive dysfunction.

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Accumulating evidence have suggested a role of oxidative stress in MS pathology. Indeed, oxidative phosphatidylcholines (OxPC), products of lipid peroxidation, accumulate in white matter (WM) as well as grey matter (GM) lesions in the brains from pwMS. While we recently found that OxPC deposition in the mouse spinal cord WM induces axonal loss, demyelination, and neuroinflammation, the role of OxPC in GM lesions during MS remains unknown. To determine the function OxPC accumulation in the GM, I stereotaxically injected POVPC, a purified MS-relevant OxPC, into the spinal cord GM of mice and performed quantitative immunofluorescence microscopy analysis after 3, 7, and 14 days. I found that OxPC deposition in the GM induced significant NeuN+ cell loss which associated with cleaved-caspase 3 upregulation, as well as significant E06+ OxPC deposition. Additionally, IBA1+ cells expressing NADPH oxidase accumulated at the lesion center whereas GFAP+ astrocytes surrounded the lesion edge. Notably, this pathology persisted from day 3 to 14. These results indicate OxPC accumulation in the GM of the CNS promotes neuroinflammation and neurodegeneration. Further investigations into OxPC-mediated cellular and molecular responses in GM will advance our understanding of GM pathology during MS.

P1-C-98 - Assessing factors impacting the wellbeing of men living with MS: A scoping review

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Multiple Sclerosis (MS) is a chronic autoimmune neurodegenerative disease of the central nervous system and is one of the most common causes of neurological disability in young adults. MS treatments focus on slowing disease progression, managing symptoms and reducing disability accrual, measured through biomarkers and health outcomes. The incidence rate, disease prognosis, pathology, treatment response, and lived experience of MS differs between sexes. However, there is limited evidence available to guide men living with MS in improving their wellbeing. This scoping review aims to assess the extent of the literature on the influence of biomedical, lifestyle, and environmental factors on health outcomes in men living with MS. A scoping review was conducted following the Joanna Briggs Institute protocol. The search strategy was run in Medline, Embase, PsycINFO, Scopus, Web of Science, and CINAHL. Citations were deduplicated, screened, and independently reviewed by two reviewers using Covidence. After deduplication,13998 citations from 1980 to 2024 were screened at the title and abstract level, and 1037 articles for full text review. We are in the process of screening and extracting data. Preliminary analysis has led to 5 major themes: disease-modifying therapies (DMTs), rehabilitation, diet, sex hormones, and cognition. The findings of this review will provide an overview of the current literature on factors that influence the well-being of men with MS. It will be used to develop a knowledge mobilization tool for men living with MS through MS Canada.

P1-C-99 - Impact of chronic stress on blood-brain barrier and stress responses in adolescence

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According to WHO, major depressive disorder (MDD) is the first cause of disability worldwide with a prevalence at 3-8% in adolescents. Despite this, biological mechanisms underlying MDD are predominantly investigated in adults. Around 40% of depressed adolescents do not or respond poorly to antidepressant treatments suggesting that causal mechanisms remain elusive. Chronic stress is an important risk factor to the development of MDD and increased peripheric inflammation is a biological signature of depression. Prolonged rise in circulatory inflammatory molecules has been suggested to damage the blood-brain barrier (BBB), a highly selective barrier protecting the brain. Since adolescence is a critical time window in the onset of depression, I investigated how chronic stress exposure impacts the neurovasculature. I take advantage of an emotional stress paradigm, social instability stress, which induces changes in sociability in adolescent male and female mice by introducing unfamiliar home cage partners. The effects of social instability are measured through anxiety- and depressive-like behavioral tests. I explore the impact of chronic stress on the neurovasculature by assessing changes in expression levels of genes associated to BBB integrity and function. Transcriptomic profiling is complemented with immunostaining and detailed morphological analysis of the neurovascular network. Deciphering stress-induced neurovascular alterations occurring during adolescence could allow a better comprehension of the biological mechanisms that underlie the development of depression.

P1-C-100 - Impact of stress-induced inflammation on blood-brain barrier permeability and transcytosis mechanisms

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Major Depressive Disorder (MDD) affects nearly 400 million people worldwide and can be triggered by chronic stress. Treatments, targeting neuronal dysfunctions, are inefficient for 30-50% of individuals with MDD. Intriguingly, treatment resistance is often associated with an exacerbated immune response, with high levels of circulating proinflammatory cytokines including interleukin-6 and Tumor Necrosis Factor alpha (TNF-a). Stress-induced brain inflammation and subsequent mood alterations may be induced by the entrance of peripheral immune mediators. Indeed, the blood-brain barrier (BBB), a protective membrane composed of endothelial cells, astrocytes and pericytes, is altered in chronic social defeat stress (CSDS), a mouse model of depression, and in the brain of MDD patients, but the detailed molecular mechanism is unknown. Caveolae-mediated transcytosis is key for plasma macromolecules transport through the BBB. To evaluate its involvement in stress-induced inflammation, I subjected mouse brain endothelial cells to pro-inflammatory cytokines or CSDS-mice serum in vitro, showing dynamic caveolar and cells junctions' modulations. In vivo viral caveolae manipulations are ongoing to determine transcytosis modulations impact in chronic social stress. Plus, magnetic resonance imaging (MRI) will assess the long-term effects of stress on BBB integrity and brain cell activity in mice. Investigating stress-induced inflammation impacts on BBB properties will help to better understand molecular mechanism underlying MDD pathology and unravel innovative therapeutic avenues.

P1-C-101 - Investigating choroid plexus inflammation during brain aging

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The Choroid Plexus (CP) plays a pivotal role in maintaining brain homeostasis by producing cerebrospinal fluid (CSF), clearing brain metabolites, and secreting essential molecules into the CSF. During aging, the CP becomes inflamed affecting its functions and increasing the risk of developing neuropathologies. However, many aspects of this inflammation remain largely unknown. Our goal was (i) to characterize this neuroinflammation across multiple species (mice, primates, humans) and (ii) to determine the cellular origin of CP inflammatory response. We observed that the CP exhibits a type I interferon inflammatory response during aging in both mice and primates. However, the regulation of this response appears to be transcriptional in mice and more translational in primate. These results suggest that the CP-specific age-related inflammation is conserved across species but that the mechanisms are distinct between species. We next analyzed which cells expressed the inflammatory genes and we identified that epithelial cells, rather than immune cells, are likely the source of this response in the aging CP in both mice and primates. Supporting these findings, we detected no significant enrichment of immune cells in the aging CP, reinforcing the role of epithelial cells in modulating the inflammatory response of the CP during aging. Our findings provide a better understanding of the mechanisms underlying CP aging. This project holds significant importance in accelerating the development of therapeutic solutions to prevent age-related mechanisms.

P1-C-102 - Remote ischemic conditioning in traumatic brain injury

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Traumatic Brain Injury (TBI) is a leading global cause of disability with significant health and socioeconomic consequences. Repetitive mild TBI (RmTBI) can severely affect brain structure and function, leading to neurological symptoms and neurodegeneration, yet no effective treatments exist. Remote Ischemic Conditioning (RIC) is a non-invasive intervention that reduces neuroinflammation and blood-brain barrier damage – key contributors to RmTBI pathology. While effective in ischemic stroke, RIC has not been studied in RmTBI, making this a novel study. This study assessed whether RIC improves recovery after RmTBI. Thirty-two male C57BL/6 mice were divided into four groups: sham/sham, RIC/sham, sham/RmTBI, and RIC/RmTBI. RIC involved 4 daily cycles of 5 minutes of ischemia followed by reperfusion using hindlimb cuffs for 14 days. RmTBI was induced using the lateral impact model with 1 mTBI daily for 5 days. Behavioral tests for anxiety, motor function, and cognition were performed 1–3 days post-RmTBI, followed by immunohistochemical analysis of inflammatory markers. Results showed RmTBI significantly impaired motor function (beam walk assay, $p < 0.05$), while RIC improved performance ($p < 0.01$). Anxiety (open field test) and cognition (novel object recognition) were unaffected. Neuroinflammatory analyses are ongoing. These findings highlight RIC's potential to mitigate motor dysfunction in RmTBI. Future research will examine RIC's impact on cytokine and chemokine release. Given its safety, accessibility, and efficacy, RIC is a promising therapeutic avenue in RmTBI management.

P1-C-103 - The role of sensory dysregulation in brain development and behavior in a mouse model of KBG syndrome

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Autism spectrum disorder (ASD) is a neurodevelopmental condition that affects about one in 66 children in Canada. Emerging evidence shows that dysregulation of the sensory nervous system contributes to altered brain development and behavior in several animal models of ASD. However, the cause of sensory dysregulation among the diverse genetic causes of ASD is not well understood. ANKRD11 (Ankyrin Repeat Domain 11) is a causative gene for KBG syndrome, a rare neurodevelopmental disorder. While Ankrd11 has been shown to regulate brain development in mouse models of KBG syndrome, nothing is known about its role in sensory nervous system development.Using a mouse model with conditional knockout of Ankrd11 in developing sensory neurons (AdvillinCre; Ankrd11fl/fl), we performed behavioral testing and cortical cellular analysis to understand the role of sensory neuron dysregulation in brain development. We show that conditional knockout mice display abnormal social and grooming behavior. Initial data suggest that they may have aberrant inhibitory neuron number and/or distribution in the somatosensory cortex, a result observed in other models of sensory-mediated ASD. Future work will confirm these findings and investigate dendrite formation and altered gene expression in Ankrd11-ablated sensory neurons. These results show that Ankrd11 knockout in sensory neurons contributes to behavioral and brain development abnormalities, highlighting the role of sensory dysregulation in neurodevelopmental disorders like KBG syndrome and ASD.

P1-C-104 - Characterizing human tau propagation using a chronic injection model in a mouse hippocampus

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Tau pathology is an important neuropathological marker of Alzheimer's Disease (AD) and correlates closely with neurodegeneration and cognitive decline in patients. To date, much of the work examining tau propagation has been performed using mutated tau and/or transgenic animal models. Since tau is not mutated in AD, the main objective of this study is to characterize the propagation of non-mutated tau in a mouse model. Tau preformed fibrils (2 μg) or a vehicle solution were injected in the hippocampus of 2-month-old C57BL/6J mice. Tau propagation is evaluated at different time points following single or chronic injections (24 h after one injection, or 24 h, 1, 5, 9 or 13 weeks after 5 consecutive days of injections). Patterns of propagation and neurodegeneration were determined using a variety of fluorescent antibodies and confocal microscopy. Examining the propagation of small, non-mutated fibrils of human tau in a wild-type mouse model will give novel information regarding how tau can affect neurodegeneration and cognitive decline in the early stages of AD.

P1-C-105 - Circuit re-construction after spinal cord injury by stem cell and pharmacological approaches

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Extensive neuronal loss, progressive neurodegeneration, and circuit impairment are the key characteristics of spinal cord injury (SCI). The intrinsic capacity of the spinal cord to replenish damaged neurons and reassemble the disrupted spinal circuitry is restricted. Transplantation of exogenous neural precursor cells (NPCs) has shown promise to structurally repair the injured spinal network. However, proper maturation and integration of newly generated neurons from NPC graft into the host spinal circuit has remained challenging. Here, we have developed a combinatorial strategy to augment maturation of newly generated neurons and facilitate their functional integration with the host circuitry. We demonstrate that blockade of inhibitory CSPG/LAR/PTP-s axis and neuromodulation by activation of serotonin receptors 5-HT1/2/7 fosters the generation, maturity, and functional connectivity of NPC-derived neurons with the host local spinal network as well as two major descending motor pathways that culminate in recovery of locomotion and sensorimotor integration. Taken together, this novel cellular and pharmacological approach addresses critical gaps in cell-based repair strategies for SCI by optimize neuronal replacement and restoration of functional neural networks within the damaged spinal circuitry.

P1-C-106 - Elevated risk of schizophrenia in individuals with 22q11.2 microdeletion conveyed by genome-wide tandem repeat expansions

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22q11.2 Microdeletion is a high penetrance risk factor for schizophrenia yet only ~25% of affected individuals develop schizophrenia. This may be attributed to additional genetic factors that modify the outcome. We analyzed genome sequencing data of 88 de novo 22q11.2 microdeletion trios Canadian families and 374 unrelated individuals from international cohorts, all with at least the A-B 22q11.2 microdeletion and of European ancestry. We used regression analysis to test schizophrenia risk conferred by polygenic risk and several types of rare variants: single-nucleotide and copy number variants, and tandem repeat expansions (TRE). We identified a significant association between genome-wide rare TREs and schizophrenia in 22q11.2 microdeletion (Canadian: OR=1.95, p=0.025; International: OR=1.57, p=0.021) and with effect size comparable to that conferred by polygenic risk. Most schizophrenia-associated TREs were located near quantitative trait loci affecting gene expression in the prefrontal cortex (OR=1.86, FDR=0.071) and FMRP target genes (OR=4.65, FDR=4x10-3). The TREs identified in 22q11.2 schizophrenia significantly overlap those from a previous study of community-based schizophrenia without 22q11.2 microdeletion (p<2.2x10-16).The results suggest that TREs represent one of the major genetic modifiers contributing to the elevated risk of schizophrenia risk in 22q11.2 microdeletion. The findings expand the potential genetic modifiers that may affect genomic disorders and highlight disease risk conferred by variants affecting non-coding sequences of the genome.

P1-C-107 - Transcriptional profiling of the cortico-accumbal pathway reveals sex-specific alterations underlying stress susceptibility

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Anxiety and depressive disorders affect millions worldwide each year, yet their molecular mechanisms remain poorly understood. Among the brain regions involved, the medial prefrontal cortex (mPFC) is reported to play a pivotal role in the pathology of major depressive disorder, showing altered activity and morphology. This study examines the mPFC-to-nucleus accumbens (NAc) pathway, which is implicated in emotional behavioral regulation. Using a targeted approach that combines the RiboTag technique with RNA sequencing, we profiled transcriptional changes in mPFC neurons projecting to the NAc in stressed male and female mice. Differential expression and co-expression network analyses uncovered distinct pathway-specific transcriptional responses to chronic stress, revealing sex-specific gene expression patterns linked to stress susceptibility. Among these, the X-linked lymphocyte-regulated 4B (Xlr4b) gene stood out as a key driver of stress responses in males, with its expression directly induced by stress in this sex. Experimental overexpression of Xlr4b altered neuronal firing and arborization patterns, promoting anxiety-like behaviors in a sex-specific manner. These findings suggest that chronic stress induces sex-specific transcriptional shifts in the mPFC-NAc pathway, altering the firing properties of mPFC neurons projecting to the NAc and modifying the pathway's capacity to encode information, leading to changes in domains related to anxiety, depression, and motivation-like behaviors. This study offers new insights into stress-related disorders.

P1-C-108 - Effects of genotype, sex and age in seizure-like activity in transgenic mice

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The incidence of epilepsy increases after 50 years of age, with the greatest incidence in people over 75 years old, making it the third most common neurological disorder in the elderly (after stroke and dementia). Mouse models often induce seizures using electrical or chemical stimulation, but some mice show age-related spontaneous seizures. We investigated four genotypes related to the neurexin-1 gene (NRXN) that encodes the presynaptic cell adhesion molecules involved in excitatory neurotransmission. During weekly cage changes, we recorded the frequency and severity of seizure-like behaviour in male and female Nrxn1+/- transgenic mice (N=106); wildtype (C57BL/6J) controls (N = 112); knockdown rescue ΔS5- (N=76) and double rescue ΔS5/ΔS5 (N=74) mice from 6 to 24 months of age. Our preliminary results indicate that most mice did not show their first seizures until 9 to 12 months of age, and that ΔS5- mice (50% showing seizures) and ΔS5/ΔS5 mice (46% showing seizures) were more likely to show seizures than wildtype (22%) or Nrxn1+/- mice (23%). Males (37%) showed a higher frequency of seizures than females (29%). Most mice having repeated weekly seizures showed an increase in severity over time (n=11), with no discernible heritability pattern (based on family genealogy analysis). The ΔS5/ΔS5 and ΔS5- phenotypes may have a higher likelihood of developing seizure-like activity due to an increase in excitatory neurotransmitter release regulated by the upregulation of neurexin-1 genes. Thus, these mice could be used as models for age-related seizure onset and for developing novel anti-epileptic drugs for the elderly.

P1-C-109 - Insights into habenular functional connectivity in individuals with autism spectrum disorder across the lifespan

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The habenula (Hb) plays a crucial role in the regulation of reward processing, social behavior, sensory integration and circadian rhythm, all of which are often disrupted in Autism Spectrum Disorder (ASD). Previous research has shown increased Hb volume in ASD, and animal studies suggest links between habenular function and autism-associated phenotypes. However, how these structural changes translate to differences in functional connectivity remains unclear. To address this gap, we examined Hb's resting-state functional connectivity in 951 individuals with ASD and TD controls from the Autism Brain Imaging Data Exchange (ABIDE) dataset. Effects of diagnosis, age and sex on Hb connectivity were investigated using brain-wide and voxel-wise analyses.We found that Hb connectivity is significantly increased in individuals with ASD compared to TD controls, with this effect being particularly pronounced in cortical regions. TD individuals show an age-related decline in Hb connectivity, reflecting typical neurodevelopmental maturation. In contrast, this decline is significantly attenuated in ASD, suggesting an altered trajectory of Hb connectivity across the lifespan in autism. No significant effects of sex were observed.These findings highlight the Hb's role in ASD's network-level mechanisms and its altered connectivity across the lifespan, which may contribute to ASD-related neural and behavioral phenotypes. Overall, this study underscores the Hb's importance in ASD-related neurodevelopmental alterations.

P1-C-110 - Targeting lipid dysregulation and fatty acid β-oxidation defects in als-fus ameliorates lipid droplet accumulation in neurons and astrocytes

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder marked by progressive muscle atrophy and systemic energy metabolism changes. To explore neurometabolic changes in ALS, we conducted quantitative metabolomic analyses of brains from FUS R521G ALS-model mice and controls. Mass spectrometry identified significant increases in short-chain (C2-C4) and long-chain (C14-C18) acyl-carnitines, key substrates for mitochondrial β-oxidation and energy production. Cytohistological studies in hFUSR521G mouse brains revealed lipid droplet accumulation and elevated peroxidated lipids in neurons and astrocytes, findings mirrored in ALS patient spinal cords with FUS R495X or K510E mutations. Treatment of hFUSR521G mice, or FUS R521G-expressing neurons and astrocytes, with arimoclomol—an ALS-ameliorating drug— reduced lipid droplets and peroxidated lipid levels. Using etomoxir, an inhibitor of the carnitine shuttle's rate-limiting enzyme, we found arimoclomol enhances lipid droplet-mitochondrial contacts and promotes fatty acid β-oxidation in neurons and astrocytes. These findings highlight lipid dysregulation in neurons and astrocytes due to defective lipid catabolism, contributing to energy homeostasis impairments in ALS. By targeting mitochondrial lipid metabolism, arimoclomol mitigates lipid accumulation, offering a therapeutic strategy for specific ALS genetic backgrounds.

P1-C-111 - Gut microbiota transfer from the unpredictable chronic mild stress model: Both donor and recipient sex impacts depression and anxiety-like behavioural outcomes

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Major Depressive Disorder (MDD) exhibits a sex disparity, with females diagnosed at twice the rate of males. However, preclinical work assessing MDD risk has predominantly relied on males, overlooking potential sex differences in underlying mechanisms. Indeed, current evidence suggests that the gut microbiome plays a role in MDD susceptibility through the gut-brain axis (GBA) and, despite documented sex differences in MDD and gut microbiome composition, females have largely been excluded. The present study investigated how sex differences in MDD risk relate to the gut microbiome in a preclinical C57Bl/6 mouse model. To isolate the effects of gut microbiome alterations on stress-related measures, cecal contents from donor mice subjected to Unpredictable Chronic Mild Stress (UCMS) were transferred to otherwise unmanipulated recipient mice via oral gavage. Cecum recipient mice were then evaluated on a behavioural battery of depression and anxiety-like behaviours. We found that the impact of UCMS cecum on behaviour varied based on the sex of both donor and recipient, with female recipients of donor female UCMS cecum exhibiting the most pronounced indicators of anhedonia and anxiety-like behaviour. These findings suggest that treatment with UCMS cecum is sufficient to alter behaviour and that the female gut may be more amenable to stress-induced disturbances through the GBA than males. Ongoing investigations are examining potential sex differences in neurobiological responses to stress, focusing on neural cytokine and gene expression in the prefrontal cortex.

P1-C-112 - Starving the seizures: proteomic effects of the ketogenic diet on the hippocampus in a model of infantile spasms

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Infantile Spasms (IS) is a rare pediatric epilepsy that has serious neurodevelopmental consequences for infants. In refractory cases that are unresponsive to medication, the ketogenic diet (KD) is used as a metabolic therapy. While the KD is antiepileptic, the molecular mechanisms of action are unknown. This study sought to examine the proteomic effects of the KD on the hippocampus, which has been implicated in IS. Employing a triple-hit rodent model of refractory IS, comparative proteomic analysis examined hippocampal tissue from rodents fed a KD or a normal diet (ND). Quantitative proteomics of homogenized hippocampal tissue was conducted using data-independent acquisition (DIA) LC-MS/MS. A total of 7659 proteins were identified through DIA neural networks, 235 were significantly altered ($p<0.05$) between ND and KD. PLS-DA analysis indicated a difference in overall proteomic signatures. There were 36 significantly downregulated proteins (log2 fold change <-1) and 83 upregulated proteins (log2 fold change >1) in KD. Comparing the significant up and downregulated proteins, GO Biological Processes and KEGG pathway analysis revealed pathways enriched in the KD relating to morphogenesis, cellular organization, synaptic signaling, and metabolic processes. Additional disease-based pathway analysis identified key proteins relating to neurodevelopment and epilepsy including FOXG1 and ARNT2. These results offer evidence that the KD alters the hippocampal proteome, providing insight into the antiepileptic mechanisms of the KD in IS.

P1-C-113 - Rigid functional connectivity among hippocampal CA1 neurons in TgCRND8 mice undermines the encoding of novel experience

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Deficits in episodic memory are a cardinal symptom of Alzheimer's disease (AD) and appear in conjunction with progressive accumulation of β-amyloid. However, the physiological mechanism that links amyloidosis to memory impairments remains unclear. An established neurophysiological signature of memory trace formation is the coordinated firing of hippocampal cells during high-frequency oscillations called sharp-wave ripples (SPW-R). Thus, we used in vivo electrophysiology to compare dorsal CA1 spike dynamics during SPW-Rs from amnestic TgCRND8 (Tg) mice with AD-related amyloidosis and healthy littermates (non-Tg) during sleep before and after a memory task. We found that 2/3 of putative excitatory cells from non-Tg mice increased firing rates during SPW-Rs after the task. These experience-responsive cells became more likely to fire synchronously (co-fire) not only during SPW-Rs but also outside SPW-Rs, which together transition from a state of uncoordinated to densely correlated firings. This emergence of experience-coding cell assemblies was absent in amnestic Tg mice. In particular, firings of excitatory cells were already in the densely correlated state before the task, making them robust against change. This rigid functional connectivity reduced the proportion of experience-responsive cells and limited the increase of their co-firing probability after the task. Amyloidosis thus weakens the flexibility of hippocampal cells, limiting experience-induced changes in their activity patterns to form a new memory trace.

P1-C-114 - Neurodevelopmental deficits in early postnatal down syndrome mice

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Down Syndrome (DS) is characterized by intellectual disabilities due to an extra copy of chromosome 21. DS individuals exhibit learning deficits linked to impaired memory formation, suggesting a hippocampus-related dysfunction. Since DS is a neurodevelopmental disorder, it is surprising that very little is known about neuronal deficits in DS during neurodevelopment. Hence, to further analyze potential reasons for learning and memory deficits in DS, we started this project by analyzing basal synaptic transmission, neuronal network activity and synaptic plasticity in postnatal slices of the DS mouse model Ts65Dn.We find in postnatal 8-12 days old hippocampal slices acomplete block of long-term potentiation, but no effect on long-term depression. DS mice also displayed a strong reduction innetwork activity for excitatory as well as inhibitory responses. Surprisingly, analysis of miniature events revealed an increase in inhibitory miniature amplitude and frequency and no change in excitatory miniature events. Hence, we obtained ostensiblyopposite effects of decreased inhibitory network activity andincreased inhibitory miniature frequency. Further analysis of this conundrum revealed that neurons in DS mice display an increase in intrinsic excitability that causes a strong depolarization block,which would explain the reduced network activity.Taken together, these results highlight a new and critical early neurodevelopmental deficit in DS. We are currently testing different genetic and pharmacologic intervention strategies to normalize neuronal intrinsic excitability.

P1-C-115 - 16p11.2 microdeletion compromises blood-brain barrier integrity in mice

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In the brain, nutrient delivery and protection against pathogens are controlled by endothelial cells (ECs) via the blood-brain barrier (BBB). Brain ECs are unique, featuring selective

transporters, low transcytosis, and restricted paracellular permeability ensured by tight (TJs) and adherens (AJs) junctions primarily composed of Claudin-5 and VE-cadherin, respectively. Early vascular defects may lead to atypical brain development, potentially contributing to autism spectrum disorders (ASD), which are characterized by cognitive and motor deficits. While ASD research has focused on neuronal alterations, vascular contributions are overlooked. Our lab identified endothelial deficits in a mouse model of 16p11.2 microdeletion syndrome (16pDel), a common genetic cause of ASD. We hypothesized that 16p11.2 microdeletion leads to structural and/or functional defects at the BBB.Despite normal distribution of Claudin-5, and normal protein levels of Claudin-5 and VE-cadherin in adult 16p11.2df/+ mice, transmission electron microscopy revealed a significant increase in the number of membrane gaps in inter-endothelial junctions, only in 16p11.2df/+ mice. Furthermore, electron tomography demonstrated vesicular activity, highlighting potential membrane abnormalities. Yet, Evans Blue dye leakage was absent under physiological conditions. However, a focal ischemia led to a higher BBB leakage in 16p11.2df/+ mice compared to WT mice, indicating increased BBB vulnerability to injury.This is the first study demonstrating ASD-associated BBB defects in genetic mouse model.

P1-C-116 - Poly-glutamine extension in Huntingtin impairs transport of secretory vesicles to the plasma membrane through a GTPase Rab11a-dependent mechanism

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Huntington's disease (HD) is a hereditary neurodegenerative disorder caused by an expanded poly glutamine stretch in the huntingtin protein (Htt). To date, Htt normal functions and the mechanisms by which mutant Htt (mHtt) causes the disease remain unclear. However, HD animal models show decreased dense-core vesicle (DCV) secretion, and HD patients feature symptoms associated with altered neuropeptide functions. We hypothesize that Htt is involved in DCV transport to the plasma membrane by activating small GTPase Rab11a, and that mHtt impairs this process. To test this, we used primary cultures of mouse chromaffin cells to overexpress mHtt or coexpress it with wild-type Rab11a. We performed live-cell imaging with confocal microscopy, and bioimage analysis to evaluate vesicle trafficking in absence (rest) or presence of 50 mM extracellular K+ (stimulated condition). We found that mHtt decreased the presence of secretory vesicles at the cell periphery and changed their transport regimes, showing less mobility (predominance of confined motion), with respect to controls. Coexpressing Rab11a partially reversed these effects by shifting secretory vesicles towards the cell periphery and increasing vesicle motility during stimulation. These results demonstrate that poly-glutamine extension in Htt impairs transport of secretory vesicles to the plasma membrane through a GTPase Rab11a-dependent mechanism. Elucidating the regulation of secretory vesicle trafficking may lead to potential targets to aid in the disease treatment of Huntington's disease.

P1-C-117 - The impact of elevated anandamide on symptom severity in patients with PSTD

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Background: Abundant preclinical and clinical research implicates the endocannabinoid (eCB) system in various stress-related conditions. Notably, anandamide (AEA), a principal eCB ligand, modulates fear learning processes believed to be dysregulated in post-traumatic stress disorder (PTSD). We conducted a double-blind placebo-controlled clinical trial to explore whether pharmacologically upregulating AEA signaling in PTSD patients enhanced the efficacy of prolonged exposure therapy.Methods: 101 participants with a PTSD diagnosis were randomly assigned to receive 25 mg of the fatty acid amide hydrolase (FAAH) inhibitor JNJ-42165279 or placebo twice daily. After 4 weeks of drug intake, participants underwent an 8-week internet-delivered cognitive behavioral therapy program alongside drug intake. PTSD severity was assessed at week 0 and week 12 using the Clinician-Administered PTSD Scale for DSM-5 (CAPS-5). After 3-4 weeks into treatment, participants completed stress and fear memory tasks to assess symptomatology. Results: Despite a reduction in CAPS-5 scores across all participants, there was no statistically significant difference in the change of CAPS-5 scores between FAAH inhibitor and placebo administered groups from baseline to week 12. Furthermore, FAAH inhibition did not significantly influence stress or fear memory processes.Conclusion: While all patients showed reduced CAPS-5 scores following prolonged exposure therapy, concomitant upregulation of AEA signaling with the FAAH inhibitor JNJ-42165279 had no further effect on clinical PTSD scores or symptomology.

P1-C-118 - Monocytes reduce the efficiency of central nervous system remyelination

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Multiple sclerosis (MS) is a neurodegenerative disease resulting from damage to central nervous system (CNS) myelin. The efficiency of remyelination decreases with MS progression leading to axon dysfunction/loss. Failed remyelination in MS has been attributed to poor differentiation of oligodendrocyte progenitor cells (OPCs) into new remyelinating oligodendrocytes and may relate to MS lesions having altered oligodendrocyte subpopulations. Little is known about the specific role of infiltrating monocytes in the CNS during remyelination despite their prevalence in MS lesions and their influence on oligodendrocyte heterogeneity. Using reporter mice for classical inflammatory monocytes (Ccr2RFP/+) and flow cytometry in a focal brain lesion model, we find that monocytes are present throughout remyelination but do not mature into macrophages. We investigated their role in remyelination using Ccr2-/- mice, which have reduced circulating monocytes, and found enhanced remyelination This was preceded by altered oligodendrocyte heterogeneity, shown by the reduction of a disease-associated oligodendrocyte subpopulation. RNA sequencing of lesion monocytes in wildtype mice indicated a Wnt signature, which occurs in an autocrine manner in monocytes and a paracrine manner in oligodendrocyte lineage cells. Functional inhibition of monocyte Wnt release enhanced remyelination. These findings suggest that monocytes impair remyelination efficiency through Wnt signaling and altered oligodendrocyte heterogeneity, and highlight monocytes as new therapeutic targets for enhancing remyelination in MS.

P1-C-119 - Subretinal drusenoid-like deposits in prom1-null frogs mimic markers of human age-related macular degeneration and contain potential melanophages

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Prominin-1 (PROM1), a transmembrane protein, is critical for the structural integrity of photoreceptor outer segments and implicated in inherited blindness. Recent studies have demonstrated the accumulation of cellular debris in the outer segment layer concomitant with retinal pigment epithelium (RPE) degradation in prom1-null frogs. These deposits are similar to subretinal drusenoid deposits (SDD), seen in human macular degeneration. The origin and composition of SDD are controversial, with some evidence for microglia involvement and some for degenerating RPE, or RPE undergoing epithelial-mesenchymal transition. We investigated the composition of these deposits using a microglial marker (1b4 isolectin), a lysosomal marker (LAMP1), and TUNEL labeling. Our results revealed a significant presence of reactive microglia in the cellular debris photoreceptor layer of prom1-null frogs, indicated by 1B4 and LAMP1 positive immunolabeling. There was no significant TUNEL labeling in the retina or RPE, indicating a lack of cell death. While ramified microglia were normally present in the inner and outer plexiform layers in the wildtype animals, accumulation of amoeboid reactive microglia in the SDD-like deposits indicates that microglia are responsive to photoreceptor degenerative processes in prom1-null frogs and potentially also age-related macular degeneration. Further studies will identify whether activated microglia in the outer segment layer are pro- or anti-inflammatory, to determine their role in RPE degradation during retinal degeneration in prom1-null frogs.

P1-C-121 - Molecular and cellular landscape of the epileptic human hippocampus via single-cell spatial transcriptomics

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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 mesial temporal lobe epilepsy (mTLE), the most common form of focal epilepsy, 80% of patients do not respond to available medication. The most affected brain region in mTLE, the hippocampus, has been shown to undergo significant histopathological changes. While substantial knowledge exists about cell-type-specific changes of the hippocampus in rodent models of mTLE, investigating this in the human brain has proven challenging due to the difficulty of obtaining and using human brain tissue experimentally.To address this gap, we used single-cell spatial transcriptomics (10x Genomics Xenium) to investigate the molecular landscape of 1,312,893 cells in the epileptic human hippocampus.We analyzed hippocampal samples from 14 epilepsy patients with varying degrees of hippocampal sclerosis, classified by ILAE grades, and two postmortem non-epileptic controls. Our 366-gene panel included markers for neuronal and non-neuronal cell types and genes implicated in mTLE.Our analysis revealed the molecular profiles and spatial organization of neuronal and non-neuronal cells in the epileptic human hippocampus. Notably, we identified region- and subtype-specific cell loss of excitatory and inhibitory neurons in epilepsy samples. In addition, we identified differentially expressed genes in non-neuronal cells associated with specific ILAE grades.Our findings advance our understanding of the cellular heterogeneity and molecular alterations in the epileptic human hippocampus. By pinpointing disease-related changes in

specific cell types and regions, our research could identify novel cellular and molecular targets for epilepsy treatment.

P1-C-122 - ZDHHC9 as a regulator of axonal remyelination

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Multiple sclerosis (MS) is an autoimmune-mediated neurodegenerative disease characterized by demyelination and inadequate myelin repair. Existing treatments primarily target immune responses to attenuate inflammation-induced demyelination but fail to promote remyelination. Consequently, identifying mechanisms that govern myelin repair is vital for developing more effective therapies. Our previous work has shown a major role for the palmitoylating enzyme, ZDHHC9, in regulating myelin formation and the differentiation of oligodendrocytes into specific mature subtypes during development. In this study, we examined whether overexpressing ZDHHC9 in adult mice can enhance remyelination following a demyelinating insult. Our preliminary results demonstrate that virally overexpressing Zdhhc9 in the corpus callosum results in an increase in the proportion of myelinated axons four weeks after cuprizone-induced demyelination. Conversely, there is a reduction in the number of myelinated axons at the same time point in Zdhhc9 knockout mice. We are currently investigating the cellular and molecular mechanisms by which ZDHHC9 regulates axon remyelination, and specifically the role of ZDHHC9 in regulating oligodendrocyte differentiation and the formation of compact myelin sheaths after demyelination. Collectively, our findings support ZDHHC9 as a promising target for promoting myelin repair in demyelinating diseases such as MS.

P1-C-123 - Ablation of Neuregulin-1 elicits brain demyelination and cognitive decline in adult mice: Implications for progressive multiple sclerosis

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Multiple Sclerosis (MS) is a complex immune-mediated demyelinating and neurodegenerative disease of the central nervous system that leads to various neurological deficits such as cognitive decline and anxiety. Initially, neural precursor cells (NPCs) facilitate myelin repair and hippocampal repair. However, as MS progresses, repair processes decline due to reduced NPC quantity and efficacy, resulting in neurodegeneration. Our previous research identified a long-lasting decrease in Neuregulin-1 (Nrg-1) levels in human chronic MS lesions and MS animal models. Nrg-1 is a crucial protein for NPC regulation and myelination. We hypothesize that Nrg-1 depletion contributes to impaired remyelination and underlies progressive neurodegeneration and cognition decline in chronic MS. We addressed the impact of Nrg-1 dysregulation on MS progression using a loss-of-function approach. We conducted longitudinal neurobehavioral tests measuring spatial working memory and anxiety in a tamoxifen-inducible Nrg1fl/fl conditional knockout (cKO) mouse model. Concurrent in vivo and in vitro studies evaluated the effects of Nrg-1 deletion on neurodegeneration, inflammation, demyelination, and NPC properties in adult mice. We show that Nrg-1 ablation induces significant spontaneous brain demyelination and neuroinflammation progresses over time. These mice display hippocampal spatial and working memory deficits and increased anxiety. Additionally, NPCs from Nrg-1 cKO mice exhibit a significant reduction in self-renewal, proliferation, and stem cell activity compared to wild-type

counterparts. Our findings highlight the functional significance of Nrg-1 dysregulation in chronic MS lesions, and its link to remyelination failure and neurodegeneration in progressive MS. Our study also underscores the importance of the Nrg-1 gene and how its depletion produces MS-like lesions.

P1-C-124 - Psilocin, the psychoactive metabolite of psilocybin, modulates specific neuroimmune functions of microglia in a 5-HT2 receptor-dependent manner

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Neuroinflammation driven by overactivated microglia plays a pivotal role in the progression of neurodegenerative diseases. Given the ineffectiveness of conventional anti-inflammatory drugs, there is a pressing need to explore novel anti-neuroinflammatory therapies with alternative mechanisms of action. Psychedelics, including psilocybin, have been shown to possess certain anti-inflammatory properties, and have demonstrated clinical efficacy as a treatment for depression. Psilocin, the bioactive metabolite of psilocybin, can cross the blood-brain barrier and exert psychotropic activity by binding to neuronal 5-hydroxytryptamine 2A receptors (5-HT2AR). Since microglia express all three 5-HT2R isoforms, we hypothesized that by interacting with these receptors, psilocin modulates specific functions of immune-stimulated microglia in a manner that could be beneficial for the treatment of neurodegenerative diseases. At non-toxic micromolar concentrations, psilocin significantly inhibited phagocytic activity, nitric oxide (NO) production, and reactive oxygen species (ROS) generation resulting from the respiratory burst response. The inhibitory activity of psilocin on the latter two functions was similar to that of two selective 5-HT2R agonists, 25I-NBOH and Ro60-0175. The role of this receptor subfamily was further demonstrated by use of 5-HT2R antagonists cyproheptadine and risperidone. Psilocin shows promise as a drug candidate that could be effective in treating neuroimmune disorders, such as neurodegenerative diseases, where overactivated microglia are significant contributors.

P1-C-125 - Characterizing sex differences in functional connectivity during chronic stress-induced negative cognitive bias

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Major Depressive Disorder (MDD) affects 20% of the population and affects females at twice the rate as males. MDD is characterized by several symptoms including cognitive symptoms, such as negative cognitive bias (NCB). NCB is the increased perception of ambiguous situations as negative. Changes in network connectivity between limbic system regions, including the hippocampus, prefrontal cortex, amygdala, and nucleus accumbens, is implicated in MDD and can predict negative cognitive bias in MDD. Here we examined possible sex differences in the functional connections within these regions in an animal model. Male and female Sprague-Dawley rats underwent either 21 days of chronic unpredictable stress (CUS) to induce a depressive-like endophenotype or no CUS. Rats then underwent a cognitive bias task in which, after learning to discriminate between shocked context and a non-shocked context, they were exposed to an ambiguous context (with half the features of the shock vs no shock context) and freezing behaviors were recorded. Activated neurons were visualised using an

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immunofluorescent stain for immediate early gene (IEG) c-Fos protein, which is transcribed rapidly in response to stimuli, across 19 limbic regions. Preliminary data suggests CUS lower activation in the ventral hippocampus in males but not females. We hypothesize that 1) activation patterns and relationship with NCB will differ by region and sex 2) functional connectivity patterns in response to CUS-induced negative cognitive bias will differ by sex.

D - SENSORY AND MOTOR SYSTEMS

P1-D-126 - Therapeutic potential of TRPV4 channel antagonist in managing nociception and neuroinflammation in a preclinical model of complex regional pain syndrome

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Introduction: Complex regional pain syndrome type I (CRPS-I) is a disabling outcome occurring after ischemia/reperfusion injury. CPRS-I chronic stages are associated with oxidative stress generation, which contributes to persistent pain. Oxidative compounds sensitize the transient receptor potential vanilloid 4 (TRPV4), a channel involved in neuropathic and inflammatory pain. Therefore, we investigated if a TRPV4 antagonist attenuates the nociception and spinal neuroinflammation induced by CRPS-I model.Methods: CRPS-I was induced by chronic post-ischemia pain (CPIP) in the hind paw of male mice. From day 1 to 15 post-CPIP induction, mice received intraperitoneal injection of TRPV4 antagonist HC-067047 or its vehicle. We assessed mechanical allodynia, thermal hypersensitivity, anxiety- and apathy-like behaviors on days 1, 5, 10, and 15 post-CPIP. We used plasma, spinal cord, and sciatic nerve to evaluate oxidative response and neuroinflammatory markers.Results: CPIP-induced mice exhibited increased mechanical allodynia and thermal hypersensitivity, followed by increased anxiety- and apathy-like behaviors. Repeated treatment with TRPV4 antagonist attenuated these symptoms. HC-067047 decreased oxidative response, modulating the astrocyte activation in the spinal cord without altering TRPV4 expression.Discussion: These findings show that the TRPV4 channel plays a significant role in CRPS-I-induced nociception and neuroinflammation, emphasizing its relevance to pain-induced plasticity mechanisms. Our results suggest that TRPV4 may be a therapeutic target for CRPS-I.

P1-D-127 - Decoding the ventrolateral periaqueductal gray (vlPAG) to locus coeruleus (LC) circuit in pain and anxiety regulation

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Pain can be a debilitating condition, with patients often facing limited treatment options. Therefore, understanding how pain signals are processed in the brain is essential. The PAG plays a crucial role in the regulation of chronic pain. It receives input from upstream brain regions such as the medial prefrontal cortex (mPFC), processes information and sends outputs to the LC. Recent evidence suggests that there is a small population of cells located medially to the LC, identified as the peri-LC, that can communicate and potentially regulate LC activity. We aimed to decipher how glutamatergic and GABAergic inputs from the vlPAG to the peri-LC affect pain

and anxiety in normal and neuropathic pain conditions. Next, we wished to determine how DSP4, a selective neurotoxin mainly targets the noradrenergic system of the LC, is able to alter this circuit. Our results show that vlPAG GABAergic input to the peri-LC produce analgesic and anxiolytic effects. Additionally, confocal imaging revealed the activation of the GABAergic input from the vlPAG to the peri-LC robustly increase c-Fos activity in the LC in both control and SNI condition. This suggests a potential GABA-GABA disinhibition occurring in the LC. Lastly, modulation of this pathway by DSP4 in the mPFC appears to slightly enhance the anxiolytic effect, while pain levels remain unchanged compared to conditions without DSP4. These findings emphasize the importance of understanding the vlPAG-peri-LC-LC pathway in regulating pain and anxiety and may offer new insight for developing strategies to alleviate chronic pain.

P1-D-128 - Peripheral electrical stimulation for reversing locomotor deficits after spinal cord injury: proof of concept in a large animal model

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Physical rehabilitation following spinal cord injury facilitates the locomotor recovery, but deficits often persist. Peripheral nerve stimulation has shown promise in modulating locomotor patterns, though its efficacy in preclinical models of motor paralysis remains unexplored. Here, we developed an approach that allows peripheral stimulation to be applied in synchrony with walking. Using a feline model of thoracic spinal cord contusion (T10) which induces transient paralysis of the hindlimbs and long-term locomotor impairments (e.g., foot drag, loss of voluntary control), we implanted cuffs around the superficial peroneal cutaneous nerves of both hindlimbs in 5 cats. Prior and following the spinal cord contusion, we optimized the amplitude, timing, frequency, and duration of nerve stimulation to enhance the evoked motor response.Peripheral stimulation efficiently modulated foot trajectory during treadmill locomotion, increasing step height and reducing paw drag. Maximum efficacy was achieved when stimulation was applied at the onset of the swing phase, particularly at a frequency of 120 Hz with a burst duration of 150 ms. Notably, stimulation amplitude demonstrated a linear modulation of step height within a functional range. Furthermore, stimulation of each hindlimb independently allowed for personalized parameter adjustments.This study holds significant potential for clinical translation, particularly given the accessibility of targeting cutaneous nerves in spinal cord injured patients.

P1-D-129 - Exploring perception and EEG dynamics during peripheral nervous system alternating magnetic stimulations

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Extremely low-frequency magnetic fields (ELF-MF, <300 Hz) can modulate nervous system functions by inducing electric fields in tissues, making them a valuable tool for studying neurophysiological processes. At powerline frequencies (60 Hz), the interaction between ELF-MF and peripheral sensory mechanisms, including graded potentials in skin receptors, remains poorly understood. This study aims to establish peripheral nervous system stimulation (PNSS)

perception thresholds and explore corresponding neurophysiological changes in sensory and motor systems using electroencephalography (EEG).In a randomized, double-blind protocol, 12 healthy volunteers undergo sinusoidal 60Hz-MF stimulation of the leg using Helmholtz-like coils and transcutaneous magnetic stimulation (TcMS -as positive control with pulses). Stimulation intensities (11 levels, including sham) are delivered at 60 Hz, with participants reporting perception via button press. Concurrent EEG recordings focus on somatosensory and motor cortical activities, analyzing changes in μ (8–13 Hz) and β (13–30 Hz) bands.Preliminary results indicate PNSS thresholds at 120 mT, with logistic models explaining 36% of variance in perception. EEG data suggest reduced μ rhythms during perception, consistent with somatosensory and motor cortical engagement.This study aims to shed light on the neurophysiological mechanisms underlying ELF-MF-induced sensory responses, advancing our understanding of peripheral and central interactions and informing the use of magnetic stimulation in neuroscience research and clinical applications.

P1-D-130 - EEG correlates of representational plasticity

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Previous research on the tactile system and somatotopic mappings of the body have demonstrated that cortical representations of body parts are not static but instead maintained by continual afferent sensory information. In light of this, an emerging body of research has begun investigating the short-term induction of representational plasticity in the somatosensory cortex (S1) by altering the way afferent tactile information reaches the brain. The present study aims to corroborate a previous study by Kolasinski et al. (2016) and ascertain whether 24 hours of a finger adhesion manipulation can elicit behavioural and electrophysiological changes in response to representational plasticity. Using a tactile discrimination task, researchers will monitor tactile discrimination across digits on the right hand over two sessions, 24 hours apart. The experimental group will have their right index and middle fingers adhered together with medical tape for the inter-session period, while the control will not. A 2x2x3x8 mixed factorial ANOVA will be run to determine whether any differences in tactile discrimination exist across session, condition, digit pair, or trial type. The researchers hypothesize to see reduced tactile discriminability between the ring and pinky fingers and increased discriminability between the index and middle fingers of the experimental group across sessions relative to the control. Additionally, it is expected to see reduced P300 ERP component amplitude in the experimental group across sessions relative to the control group.

P1-D-131 - Ventrolateral Prefrontal Cortex LFPs encode visual context-dependent preparatory activity during memory-guided reaches

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The purpose of the current study was to understand what prefrontal local field potential (LFP) signals encode during a memory-guided reach task, with or without visual landmarks. We implanted a 128-channel Plexon Array over the posterior ventral prefrontal cortex (pVLPFC) in a female Rhesus monkey trained to perform a memory-guided reach task. The hand was initially placed at 1 of 3 locations of a waist-level LED bar while the gaze fixated centrally. A landmark

(four dots in a square) was presented on a touch screen at 1 of 15 locations. A visual target appeared at a variable location within or outside this square, followed by a visual mask. The landmark then reappeared at the same location or shifted by 8 degrees in one of 8 directions, and the animal was cued to reach. Controls were the same but without the landmark. Neural and behavioral (eye, head, hand) signals were recorded for three months while the animal performed these tasks. Preliminary analysis of time-power plots revealed frequency band-specific decreases in power during the delay before reach and increases in power peaking near movement onset. Time-frequency plots showed task-dependent modulations in the preparatory activity as visual complexity increased. In addition, the presence of a landmark produced a second power peak between target acquisition and reward. This suggests that prefrontal LFP signals contain visual context-dependent information for reach planning and execution.Supported by the Connected Minds Program, funded in part by the Canada First Research Excellence Fund.

P1-D-132 - Age-related changes in motor behaviour in mouse models of autism

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder that impairs social-emotional behaviour, motor coordination, visual-spatial learning and memory. Approximately 2.5% of the human adult population has ASD and adults with ASD have a reduced lifespan, increased frailty and motor gait impairments. The present study assessed motor coordination and motor learning using the Rotarod in 21-month-old male and female Nrxn1+/- transgenic mice; wildtype (C57BL/6J) controls; knockdown rescue ΔS5- ; and double rescue ΔS5/ΔS5 mice. The mice were placed in one of the 4 compartments of the Rotarod, which accelerated to a maximum of 36 rpm and the latency to fall was recorded. There were 6 trials per day over 7 days and body weight was taken prior to testing each day. Performance improved for all mice over days, however, there was a significant negative correlation between motor scores and body weight ($r = -0.43$, $df= 97$, $p<0.001$) with lighter mice performing better than heavier mice. Females performed better than males, but improvement in performance over days was greater in males. Female Ds5/- mice performed better than female WT, Nrxn1+/- and DS5/DS5 mice, but there were no genotype differences in male mice. At 2 and 9 month of age, female mice outperformed males but there were no differences between Nrxn1+/- and WT mice. There results indicate that there are no age-related deficits in motor learning and coordination in these autistic mouse models.

P1-D-133 - Rhomboid-3 expression in cerebellar Purkinje cells contributes to motor coordination impairment in aging

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Motor coordination typically declines in aging adults (Gooijers et al., 2024), and the cerebellum is one of the brain regions that likely contributes to this (Bernard et al., 2014). We recently found that cerebellar Purkinje cell firing was reduced in aging and that elevating firing increased motor coordination in aged mice (Fields, under review), suggesting a cerebellar locus for aging-related motor decline. Rhomboid-3, a protease encoded by the Rhbdl3 gene, is expressed in cerebellar Purkinje cells where its expression increases with age (Kumar et al., 2012). We wondered whether Rhomboid-3 is involved in motor coordination in aging. Using Rhbdl3-/- knock-out and

wildtype (WT) mice, we assessed motor coordination using a rotarod assay across lifespan and investigated cytoarchitectural changes using immunohistochemistry. To our surprise, we found that Rhbdl3-/- mice had improved motor coordination compared to WT controls. Immunohistochemical analysis showed no differences in molecular layer height or Purkinje cell counts between genotypes, suggesting that Rhomboid-3 does not influence Purkinje cell survival or dendritic architecture. To determine whether cerebellar Rhomboid-3 expression mediates motor impairment, we virally expressed Rhomboid-3 in cerebellar Purkinje cells in aging Rhbdl3-/- mice (14-18 months) and monitored motor coordination for 3 months. Purkinje cell expression of Rhomboid-3 was sufficient to impair motor coordination compared to GFP-injected mice. These findings suggest that Rhomboid-3 has a powerful effect on motor coordination in aging cerebellum.

P1-D-134 - Congruency affects intensity of multisensory stimuli

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Background: Stimuli are usually experienced in a multisensory fashion but most research focuses on unimodal stimuli. During multisensory stimulation, the individual channels can enhance or hinder each other.Objective: Here we aimed to investigate how the congruency of heteromodal co-stimulation alters olfactory perception. We hypothesized that congruent co-stimulation would enhance the perception of likeness. We also aimed to quantify the effect of gustatory and visual co-stimulation.Methods: We tested 49 healthy young participants. We used retronasal olfactory stimuli (strawberry, cheese, lemon, coffee) without, with congruent or incongruent gustatory (sweet, salty, sour, bitter), with congruent or incongruent visual co-stimulations and combinations thereof. Olfactory and gustatory stimuli were delivered as solutions on the tongue while visual stimuli were shown on a computer screen. We asked participants to evaluate the solutions' likeness to the respective olfactory label on visual analogue scales.Results: We observed a significant effect of congruency on likeness ($p < .001$). Gustatory co-stimulation had a significantly stronger effect than visual co-stimulation ($p = .02$). Congruent (incongruent) gustatory stimuli increased (decreased) the likeness on average by 11 (16) points. These numbers were 4 and 4 for visual stimuli. Conclusion: Congruent co-stimulation enhances the evaluation of likeness while incongruent co-stimulation reduces it. This could be useful in multisensory olfactory training paradigms for olfactory loss.

P1-D-135 - The distinct roles of dorsal root ganglia in nerve regeneration

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In contrast with the central nervous system, the peripheral nervous system activates a pro-regenerative program after a nerve injury, enabling axonal repair and the recovery of neuronal function. The lumbar 3,4 and 5 (L3, L4, L5) Dorsal Root Ganglia (DRG) that innervate the sciatic nerve are recognized models for understanding the mechanisms underlying axonal regeneration. However, the specific response of each DRG to sciatic nerve injury has yet to be thoroughly characterized. To investigate further this problematic, we collected each L3, L4 and L5 DRG in a mouse model 3 days after the crush of the sciatic nerve, in order to analyse the mechanisms underlying axonal regeneration. We carried out RNA-sequencing experiments to examine the gene expression profiles in each DRG. We first characterized the expression of known

regeneration-associated genes (e.g., Atf3, Sprr1a, Gadd45a, Gap43, Sox11). Using qPCR, in situ hybridization and immunofluorescence, we demonstrated that these genes exhibited a decreasing expression gradient from L3 to L5 DRG. We next identified novel lesion-specific genes whose expressions are specific to one DRG. For instance, we identified the expression of Flrt3 and Cd-207 gene (respectively involved in neurite outgrowth and immune system) in the DRG-L3, suggesting the existence of DRG-specific lesion responses. Those nonuniform responses among individual DRGs highlight their potential distinct roles in the process of nerve regeneration. This study could guide the development of therapies targeting DRG-specific regeneration after nerve injury.

P1-D-136 - Naturalistic multisensory decision-making in the larval zebrafish

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How do animals sequence sensorimotor decisions to achieve a broader goal? To understand how decisions are organized in a naturalistic context, we investigate responses to multimodal flow stimuli in larval zebrafish. As early as 5 days post-fertilization, zebrafish can respond robustly to hydrodynamic flow fields and optic flow by turning into the direction of perceived flow and performing strong counter-flow swimming. While the sensorimotor transformations underlying short-term unisensory decisions have been studied, how these two streams of information are combined over time to guide behavior is unknown. Here, we combine physics-guided stimulus design, behavioral tracking, and decision models to develop a natural-like behavioral assay where we probe thousands of sequential unisensory and multisensory decisions. We developed a novel behavioral arena that permits controlled delivery of either laminar water flow or optic flow, or both, to freely swimming fish over arbitrarily long timescales. We track fish position in 3D and detect thousands of swim bouts, each corresponding to a single decision for which we estimate the complete sensory inputs available to the fish. We use Gaussian mixture model clustering and Hidden Markov Models to capture the underlying structure of fish behavior, revealing stimulus-driven and stimulus-indifferent states. Within behavioral states, we find linear and piecewise linear relationships between swim responses and visual and flow stimuli respectively, which we now use to model multisensory responses as a weighted mixture of unisensory decisions.

P1-D-137 - Anatomical and genetic markers of TMS-induced plasticity in primary motor and visual cortices

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Theta burst stimulation (TBS) modulates motor thresholds (MTs) when applied to primary motor cortex (M1; an output cortex) but shows more variable outcomes when applied to primary visual cortex (V1; an input cortex). Variability in transcranial magnetic stimulation (TMS) response has been linked to biophysical factors, such as scalp-cortex distance (SCD), electric fields (E-fields), and genetics, including salivary brain-derived neurotrophic factor (BDNF) Val66Met polymorphism. How are these factors related to neuromodulation effects across these two regions? We compared responses to TMS applied to M1 or V1 and the relation to biophysical factors. 27 participants received active or sham continuous TBS (cTBS) in counterbalanced sessions. MRI-guided stereotaxic neuronavigation was used to monitor the TMS target. Baseline

thresholds were measured and then stimulation was delivered at 80% of MTs for M1 and 80% of PTs for V1. Significant increases in MTs and PTs occurred 5 minutes post-cTBS with active but not sham stimulation. SCD and E-fields were related to baseline thresholds but not the TMS-induced effects. Similarly, Val66Met was not related to TMS response. These findings suggest that TMS-induced plasticity is comparable across M1 and V1 despite different functional roles of these cortical regions. Personalising stimulation intensity based on the corresponding baseline threshold for the targeted brain region (MT for M1, PT for V1) is critical for effective TMS protocols, while BDNF polymorphism may not play a role in short-term TMS-induced plasticity.

P1-D-138 - Branching of first-order tactile neurons in the marmoset hand

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Fine touch processing is crucial for dexterous hand function, enabling precise manipulation of objects. This processing begins with first-order tactile neurons that innervate the glabrous skin of the hand. Type-1 first-order tactile neurons exhibit complex receptive fields with multiple highly sensitive subfields, encoding fine details through the spatiotemporal patterns of their neural responses. Both fast-adapting (FA-1) and slow-adapting (SA-1) type-1 neurons branch extensively before innervating spatially segregated mechanoreceptors in glabrous skin, presumably providing the anatomical substrate for the complex receptive fields and associated signaling mechanisms.This study provides a detailed anatomical analysis of the tactile periphery in the glabrous skin of the marmoset hand, focusing on Meissner corpuscles (MCs) and FA-1 Aβ neurons that innervate them. We found that: (1) MC size varied across each finger pad, with proximal MCs being larger; (2) MC density was higher in finger pads than palms, with no significant differences across fingers or sexes; (3) 89% of MCs received multiple Aβ innervations, likely from TrkB+ and Ret+ neurons; and (4) Neurons branched asymmetrically. We traced seven Aβ neurons across five serial sections; six branched at least twice, and up to five times, terminating in as many as eight endings. Notably, three neurons shared MCs, with innervations from two distinct neurons.This work lays critical groundwork for investigating the interplay between terminal branch morphology, the resulting complex receptive fields, and fine tactile processing.

P1-D-139 - Involvement of norepinephrine release in movement refinement during directional reaching

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The motor cortex (MC) plays a crucial role in controlling reaching movements, a fundamental motor behavior observed across a wide range of mammalian species, notably rodents. On the other hand, norepinephrine (NE), that is released from the locus coeruleus (LC) onto the cortex, is involved in behavioral flexibility and potentially released during motor movement. Here, we investigate the role of norepinephrine in refining motor skills during reaching. Therefore, we established a spatial reaching task in head-fixed mice where the animal needs to reach a water droplet at an initial location. After learning, we changed the position of the target and tracked the forelimb movements, using a deep learning algorithm, to quantify motor adjustments linked with reaching to a new location. During the task, we monitored NE release onto the MC using fiber

photometry and a genetically encoded NE sensor. We compared NE dynamics during successful and unsuccessful trials, movement execution, and across sessions, as well as movement velocity, coordination, and precision, to further understand the role of NE in motor performance. Our preliminary results show that NE release is correlated with movement execution and targeting accuracy. Together, these findings suggest a potential role for norepinephrine in the motor cortex during reaching movements and indicate that NE may be essential for influencing behavioral flexibility. Future directions should explore how norepinephrine modulates neural plasticity in the MC to support relearning of a motor action.

P1-D-140 - The female-specific contributions of T cells and leptin in neuropathic pain

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Neuropathic pain is a subtype of chronic pain that affects 7-10% of the population. Although women are disproportionately affected, the mechanisms underlying neuropathic pain in females have not been sufficiently studied. In males, microglia are a crucial cell type underlying neuropathic pain, whereas in females there is evidence that T cells play an important role. However, the female-specific contribution of T cells required further characterization.We demonstrated that neuropathic pain in female rodents is driven by Panx1-dependent release of leptin from CD8+ T cells. CD8+ T cells were increased in the spinal cord of females, but not males, following peripheral nerve injury. In females, depleting CD8+ T cells by injecting an anti-CD8 antibody partially reversed the mechanical allodynia caused by nerve injury. Neutralizing leptin, which was increased in the cerebrospinal fluid of females following nerve injury, or blocking Panx1 also reversed established allodynia. Furthermore, intrathecal adoptive transfer of female-derived Panx1-stimulated CD8+ T cells resulted in robust allodynia in naïve hosts, whereas female-derived CD4+ T cells and male-derived CD4+ or CD8+ T cells had no effect. Adoptive transfer of allodynia could be blocked by targeting leptin via antibody or siRNA. These findings identify Panx1-dependent release of leptin by CD8+ T cells as a key mechanism underlying neuropathic pain in females and implicate blocking T cell release of leptin as a strategy for new female-targeted pain treatments, an area deserving of additional research and focus.

P1-D-142 - Establishing a rodent model of postoperative pain: Investigating potential sex differences and spinal receptor expression

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Chronic pain is a debilitating condition with poorly understood mechanisms. This study addresses ongoing challenges in pain research including poor translatability and underrepresentation of females. We induced hypersensitivity in the plantar incision model of postoperative pain, investigating the time course and magnitude of mechanical hypersensitivity in both sexes. Previous work suggests that NMDA receptors (NMDARs) in dorsal horn (DH) nociceptive circuits of the spinal cord mediate pathological pain responses. NMDARs vary in function and physiology based on subunit composition, including four possible types of GluN2 subunits in glutamate-activated NMDARs. GluN2B and GluN2D are highly expressed in the spinal cord at baseline, however, little is known about how NMDAR expression may change in the

plantar incision model. To induce postoperative pain, we performed incisions through the skin, fascia and muscle of the hind paw in male and female Sprague Dawley rats. To assess paw withdrawal thresholds (PWTs), we conducted Von Frey filament testing before and after surgery for eight days. Animals had decreased PWTs that persisted for days, indicating increased sensitivity compared to baseline and controls. Confirming the days of lowest PWTs allowed us to perform qRT-PCR analysis on DH tissue collected at peak hypersensitivity. We used this to explore the relative expression of NMDAR subunits in the rat DH across sex and pain state. The results of this study enhance our understanding of sex differences and NMDAR expression in a translational model of postoperative pain.

P1-D-143 - Meg neural dynamics of reward prediction error signals in human decision-making under sensory conflict

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In daily life, we often use conflicting evidence to choose between different behavioural options and adjust future behaviour based on decision outcomes. Reinforcement-learning theory predicts that positive/negative feedback that confirms/contradicts our expectations yields a reward prediction error (RPE) that plays a critical role in mediating learning and behaviour. We used magnetoencephalography (MEG) to study how the brain computes RPEs during sensory-motor decision-making. Human subjects underwent MEG recordings while they chose between a Blue or Orange colored target by first determining the predominant color of a multicolored Decision Cue (DC). The DC presented different numbers of blue and orange squares across trials, providing varying levels of evidence for the correct target choice, ranging from strong (64 squares of one colour, 1 square of the other) to ambiguous (64 vs 62 squares). A 2-3sec delay was enforced after the onset of the target and DC cues. Subjects indicated their target choice by pressing one of two buttons. A high-/low-pitch 1sec auditory Beep at the end of each trial indicated a correct/incorrect target choice, respectively. Feedback Beeps evoked a transient increase in beta-band power across somatomotor, frontal, parietal, visual and anterior cingulate cortex (ACC). The beta-band response to "correct" feedback was stronger in trials with ambiguous evidence than in trials with strong evidence, a putative MEG correlate of a positive RPE. The "incorrect" feedback Beep evoked an equally wide-spread transient alpha-band suppression, indicative of a negative RPE signal. Notably, negative RPEs were stronger in posterior/isthmus cingulate, whereas positive RPEs were stronger in ACC.

E - HOMEOSTATIC AND NEUROENDOCRINE SYSTEMS

P1-E-144 - Sex-dependent effects of relative hyperglycemia on neurovascular coupling magnitude in healthy adults

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Neurovascular coupling (NVC) ensures adequate blood flow to brain regions with high metabolic demands, thus supporting neuronal homeostasis. Previous studies indicate impaired NVC in

individuals with metabolic disorders, and differences in glucose metabolism exist between sexes. We examined NVC responses before and after acute oral glucose ingestion in 36 healthy participants (18M/18F) using transcranial Doppler ultrasound to assess resting cerebral blood velocity (CBV) and NVC responses to visual stimuli in the posterior cerebral artery (PCAv). Testing occurred in a fasted state (FAST) and 30-min after ingesting a standardized 300ml/75g glucose drink (GL), confirming acute hyperglycemia using capillary blood draws and a glucometer (FAST: 4.79±0.36mmol/L; GL: 7.55±1.23mmol/L; $P<0.0001$). Post-glucose ingestion, baseline PCAv increased ($P=0.005$), but the dynamic NVC responses (change in peak responses during visual stimulus) remained stable (Delta-peak: $P=0.591$; Time-to-peak: $P=0.124$: Slope $P=0.864$). Notably, delta mean responses decreased after glucose compared to fasting ($P=0.001$), with these effects observed only in males, who showed increased baseline CBV in the glucose state ($P=0.004$) and reduced delta mean NVC response ($P=0.002$). In contrast, females did not exhibit significant baseline or NVC response changes. This study contributes to the expanding literature on the stability of peak NVC responses in healthy individuals, showcasing novel sex-specific differences in CBV and NVC responses due to acute relative hyperglycemia.

P1-E-145 - Rapid effects of locally-synthesized estrogens on social recognition within the bed nucleus of the stria terminalis (bnst) of male mice

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Social recognition (SR) is a key cognitive process required for the discrimination between familiar and unfamiliar conspecifics, modulating multiple social behaviours. SR is regulated by sex steroids such as testosterone (T) and 17b-estradiol (E2) which are produced by the gonads and locally in the brain. Inhibiting local E2 synthesis via an aromatase inhibitor, letrozole, in the dorsal hippocampus blocks long-term object memory and consolidation in GDX male mice, but not in gonadally intact male mice, suggesting a compensatory role of circulating hormones. In gonadectomized (GDX) male mice, exogenous E2 or T rapidly facilitates SR in the bed nucleus of the stria terminalis (BNST). The BNST is crucial for male social behaviour and portrays high estrogen receptor (ER) expression including G protein-coupled ER (GPER) as well as ERa and ERb, which co express with aromatase mRNA, the E2 synthetizing enzyme. Presently, GDX and intact male mice are infused with varying doses of aromatase inhibitor letrozole targetting the BNST 15 minutes prior to a rapid SR paradigm. It is expected that blocking aromatase will impair SR in a dose dependent manner and intact mice will require higher inhibiting doses of letrozole than GDX mice due to circulating hormones. This research will clarify the interplay between brain-synthesized E2 and circulating sex steroids in SR and its role in male social behaviour and sexually differentiated social disorders.

P1-E-146 - Lipid droplets in the brain regulate glycerolipid metabolism and energy homeostasis

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Fatty acids (FA) are the main building-blocks for lipids in the brain and act as hypothalamic signals regulating energy homeostasis. Our group has shown that FA are esterified in triglycerides (TG) stored in lipid droplets (LD) in hypothalamic neurons and astrocytes. In addition, our studies suggest that disruption of FA esterification in hypothalamic astrocytes modulates energy homeostasis by increasing the activity of hypothalamic POMC neurons that are essential for energy balance. We hypothesize that astrocyte-derived FA can act as a coupling signal to regulate POMC activity and that POMC intracellular LD-derived FA can regulate POMC activity. However, the role of LD and FA-released from LD in hypothalamic astrocytes and POMC neurons on neuronal activity and energy homeostasis remains unknown. To test this, we investigated the role of Adipose Triglyceride Lipase (ATGL), the enzyme responsible for the first step of TG breakdown, in POMC neurons and astrocytes. We show that hypothalamic neurons and astrocytes accumulate LD in response to ATGL inhibition. Using a genetic strategy to knock-out ATGL in POMC neurons (POMC-Cre x ATGL-fl) or astrocytes (GLAST-CreER x ATGL-fl), we show that ATGL is required to maintain energy homeostasis under cold and fasting conditions respectively. Furthermore, we show that loss of ATGL in POMC neurons increased the firing rate of the later. Our data shows an unprecedented role for TG metabolism in hypothalamic astrocytes and POMC neurons in energy homeostasis.

P1-E-147 - Exploring microglial ATGL: It's influence on glucose homeostasis and neuroinflammation under high-fat diet conditions

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Lipid droplets (LD) are organelles that store neutral lipids such as triglycerides (TG). While LDs are well known as a source of fatty acids (FA) acting as energy substrates, they also play a role in inflammation by sequestering or releasing FA acting as precursors of lipid signals of inflammation in macrophages and microglia, the brain immune cells.Our recent study showed that inhibition or loss of microglial adipose triglyceride lipase (ATGL), the enzyme catalyzing the first step of LD lipolysis, reduces neuroinflammation in response to LPS. However, the role of microglial ATGL inthe development of diet-induced obesity and associated neuroinflammation and metabolic alterations is unknown. To test this, we investigated energy and glucose homeostasis in male and female mice with ATGL loss-of-function in microglia (Cx3CR1-CreER) under chow-fed and high-fat fed conditions. Loss of microglial ATGL did not affect the susceptibility to develop obesity in response to a high-fat diet but altered glucose tolerance and insulin secretion in chow-fed and high-fat fed conditions in both sexes. Ongoing studies are aimed at testing LD accumulation in microglia and microglia activity in these models. Together, our findings suggest that microglial ATGL regulates glucose homeostasis and contribute to neuroinflammation and metabolic alterations under high-fat diet conditions.

P1-E-148 - S-acylation of glycolytic enzymes is required for their association with vesicles in neurons

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Fast axonal transport (FAT) involves the trafficking of membranous cargo along axonal microtubules by molecular motor adenosine triphosphatases (ATPases). FAT is critical for

neuronal function, and trafficking deficits are prevalent in neurodegenerative disorders. The molecular motors require energy in the form of adenosine triphosphate (ATP), which is produced by glycolytic enzymes attached to the vesicular cargo undergoing FAT. But how do these soluble enzymes attach to fast moving vesicles? S-acylation may hold the answer. S-acylation is a lipid modification involving the covalent addition of long-chain fatty acids (LCFA) to cysteine residues, permitting protein association to membranes. Recently, our lab identified that most of the glycolytic enzymes are S-acylated in vivo. Given these exciting findings, we hypothesize that the enzymes tether to transport vesicles via S-acylation to provide "on-board" energy for transport in neurons. We have focused initially on the glycolytic enzymes glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and pyruvate kinase (PK). Interestingly, we observed a reduction in vesicle-association of S-acyl-deficient GAPDH and PK variants compared to wildtype (WT) in hippocampal neurons. To follow up, vesicular movement in live neurons expressing WT or variant GAPDH or PK will be assessed to determine the role of S-acylation on FAT dynamics. Unlocking the mechanisms behind axonal transport will improve our understanding of neuronal function and may also shed light on neurodegenerative disease pathology and novel drug targets.

P1-E-149 - Impact of different social stress experiences on follicular count in adult female rats

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Social stress can have a serious impact on female reproductive health, yet remains poorly understood, partly due to the more frequent use of physical stress models. The goal of this study was to investigate the effect of three chronic social stressors (social instability [SIS], crowding [CRO], and isolation [ISO]) on ovarian follicular counts, alongside the stress response via glucocorticoid receptor (GR) expression. Methods: Regularly cycling adult Wistar rats were assigned to one of five housing conditions: ISO, CRO (8 rats per cage), SIS (alternating days of ISO and CRO), control housing (CH; pair housed with daily cage change) and regular housing (RH; pair housed, weekly cage change). After 21 days, rats were euthanized, and brain and ovary samples were collected. Ovarian histology (H&E) assessed follicle types and numbers. Immunofluorescence assessed GR expression in the paraventricular nucleus (PVN) of the hypothalamus and in hippocampal CA1. Results: Preliminary results for H&E indicated a significantly higher number of primary follicles in RH compared to the ISO and SIS rats. RH rats also showed elevated counts of secondary follicles compared to ISO rats. As for PVN GR expression, it was found significantly higher in ISO compared to SIS rats, with a trend for significant differences with CH rats. At the CA1, ISO tended towards elevated GR compared to that observed in CH rats. Our findings support most pronounced effects on ovarian follicular count in SIS and ISO exposed rodents, providing valuable insights into social stressor effects on reproductive function.

P1-E-150 - Characterising 'the munchies' in humans and rodents: Effects of delta-9-tetrahydrocannabinol vapour inhalation on energy intake and macronutrient selection

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With one quarter of Canadians using cannabis, there is an urgent drive to better understand it's physiological effects. It's well known that cannabis acutely promotes food intake, commonly known as 'the munchies', and that delta-9-tetrahydrocannabinol (THC) is responsible for driving this. However, the effects of cannabis use on eating patterns remains understudied. Therefore, we characterised the influence of THC vapour inhalation on energy and macronutrient intake patterns in both humans and rats. Humans inhaled vapourised placebo, low (20mg) or high (40mg) doses of THC-dominant cannabis in a controlled, double-blind manner. During the first 30-min of access to snacks and beverages, THC vapour increased energy consumption irrespective of dose or sex. Within this timeframe, THC increased carbohydrate, fat, and protein intake. To further explore these feeding behaviours, we used a translational THC vapour inhalation rat model. Consistent with human subjects, rats exposed to THC vapour acutely increased energy consumption, specifically in the first hour irrespective of sex. This effect was not dependent on what foods were available or whether rats were satiated or not. When given a food choice, THC abolished pre-existing food preferences so that rats had equal preference for high-carbohydrate and high-fat foods, regardless of sex or satiation. We provide translational evidence that THC-driven feeding is acute and robust across sexes and does not induce macronutrient-specific food preferences. Future studies will decipher the mechanisms underlying THC-driven feeding.

P1-E-151 - Topiramate enhances GABAergic tone to orexigenic neuropeptide Y (NPY) neurons

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INTRODUCTION: Topiramate is a medication known to induce weight loss. However, the mechanisms by which it influences energy balance remain ill-defined. OBJECTIVES: To determine if topiramate influences the electrical activity of hypothalamic melanocortin neurons known to regulate energy balance. METHODS: To selectively target 'orexigenic' neuropeptide Y (NPY) or 'anorexigenic' pro-opiomelanocortin (POMC) neurons of the arcuate nucleus of the hypothalamus (ARC), we utilized two transgenic mouse models. Npy-hrGFP and Pomc-CreERT2::tdTomato mice allowed for fluorescent labeling and identification of NPY and POMC neurons for whole-cell patch clamp electrophysiology experiments. RESULTS: Bath application of topiramate (1µM) strongly inhibited the electrical excitability of NPY neurons, and suggested that opening of a potassium channel may mediate the effects. The inhibitory effect of topiramate on NPY neurons was highly supressed in tetrodotoxin (TTX), suggesting that action potential-dependant release of transmitter was required for the inhibitory effect. Blockade of GABAB receptors with CGP 54626 hydrochloride supressed the proportion of NPY neurons responding to topiramate. In contrast to the inhibitory effect of topiramate on the orexigenic NPY neurons, topiramate had minimal influence on the activity of the anorexigenic POMC neurons. CONCLUSION: This data shows for the first time that topiramate significantly inhibits the activity of ARC NPY neurons, which may account for some of the weight-lowering properties of topiramate.

P1-E-152 - Lateral hypothalamic neuronal dynamics command behavioral transitions and coordinate different stages of feeding

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Innate behaviors such as feeding, social interaction, and novel object exploration fulfill adaptive needs sequentially, suggesting an underlying brain dynamic that regulates these behaviors and transitions between them. To investigate this, we combined electrophysiological neuronal recordings in freely behaving mice with machine learning and multicolor optogenetics. We identified distinct populations of lateral hypothalamic (LH) neurons that are sequentially activated during feeding, while additional populations remain active during exploration and social interaction (Altafi et al., JNeurosci., 2024). LH slow gamma oscillations (30-60 Hz) promote the assembly of feeding-related neurons, whereas fast gamma oscillations (60-90 Hz) coordinate multiple behavioral populations across different innate behaviors. These findings suggest that appetitive behaviors and phases of consummatory acts are supported by temporally organized LH populations. We further found that LH populations encode transitions between innate behaviors via peak-phase signatures during beta oscillations (15-30 Hz; Chen et al., Nature Neurosci., 2024). Optogenetic manipulation of intrahypothalamic inhibition from the lateral preoptic area at these phases disrupted behavioral transitions. These transitions are driven by beta-rhythmic inputs from the medial prefrontal cortex, which synchronize with LH "transition cells" encoding potential future behaviors. Disruptions in these oscillatory interactions may impair the LH's ability to regulate behavioral transitions, with implications for understanding eating disorders and other behavioral dysregulations. Together, our findings reveal hypothalamic temporal dynamics that signals alternative future behaviors and coordinates their organization during individual behaviors, such as feeding, with potential impairments contributing to eating disorders.

P1-E-153 - A neural basis for dehydration-induced anorexia

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When we are dehydrated, the brain triggers coordinated responses to restore osmotic homoeostasis, such as thirst (to promote water intake), vasopressin secretion (to promote water retention), and lowering of body temperature (to minimize evaporative water loss). Dehydration is also known to suppress appetite as an adaptive response to prevent the absorption of osmolytes from food, but the mechanism underlying this response is unclear. This project aims to identify the stimuli and neural circuitry driving dehydration-induced anorexia.Dehydration is a combination of multiple stimuli: hyperosmolality (increased concentration of particles in the blood), hypernatremia (increased sodium concentration), and hypovolemia (reduced blood volume). We have found that hyperosmolality and hypovolemia, but not hypernatremia, serve as stimuli to suppress food intake in mice.The organum vasculosum lamina terminalis (OVLT) is a circumventricular organ that monitors and maintains hydration balance by regulating homeostatic responses. OVLT neurons project to brain regions that control appetite, including the paraventricular nucleus (PVN) of the hypothalamus, suggesting that the OVLT may regulate food intake. We have found that hyperosmolality and hypovolemia activate subsets of OVLT and

PVN neurons. Furthermore, activation of glutamatergic OVLT neurons suppresses food intake. We are currently investigating the involvement of an OVLT-to-PVN circuit in the anorectic response to dehydration.

F - COGNITION, EMOTION AND MOTIVATION

P1-F-154 - Effect of glutamatergic modulators on sleep in depressive disorders: A systematic review

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Major Depressive Disorder (MDD) is a leading cause of disability worldwide, and is associated with significant occupational and economic consequences. Extant cross-sectional and longitudinal studies indicate that a robust bidirectional association exists between MDD and sleep disturbance. Consequently, it could be hypothesized that interventions that alleviate insomnia may have beneficial effects on depression. Increased usage of ketamine and esketamine in depressive disorders invites the need to determine if they have an effect on measures of sleep and sleep mechanisms. The aim of this presentation is to systematically review and characterize effects of glutamatergic modulators on sleep in depressive disorders. In accordance with PRISMA guidelines, Pubmed, Medline, Cochrane Library, Embase, Scopus, and Web of Science were searched from inception to November 27, 2024. Study screening and data extraction were performed by three reviewers (K.V, W.C, and B.S.). Both preclinical and clinical studies were included. Results from preclinical studies indicate that ketamine interacts with various neurotransmitters and mechanisms implicated in sleep, including BDNF, GABA, and dopamine. Results from clinical studies suggest that ketamine and esketamine are associated with improvements in sleep as measured by the Montgomery–Åsberg Depression Rating Scale (MADRS) and the Quick Inventory of Depressive Symptomatology Self-Report (16-item) (QIDS-SR16). Overall, it is suggested that ketamine and esketamine reduce the severity of insomnia in depression.

P1-F-155 - Memory bias in depressed children and adolescents: A systematic review and meta-analysis

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Background: Memory bias for negative information is an established phenomenon in depressed adults. However, research findings on the link between depression and memory bias in children and adolescents are mixed. The aim of this systematic review was to provide a comprehensive synthesis of the evidence examining memory bias and depression in children and adolescents. Methods: Systematic searches were conducted in several databases. Controlled and uncontrolled studies measuring memory bias for autobiographical memory as well as emotional faces, words, and pictures in children (≤18 years) with depression were included. Included studies were peer-reviewed and of cross-sectional or longitudinal design. Random-effects meta-

analyses of study outcomes were performed. Results: There were 35 included studies, comprised of 4,202 children (mean age 14.5 ± 1.3 years; 64% female). Compared with healthy controls, depressed participants exhibited greater recall of autobiographical memories (g = 0.86; 95% CI: 0.42, 1.30) and poorer memory retrieval for sad faces (g = -0.43; 95% CI: -0.79, -0.06). No differences in memory retrieval of happy, fearful, angry, or neutral faces were observed. Recognition of emotional words or pictures did not differ between depressed and healthy participants. Discussion: Compared with the established association of memory bias and depression in adults, fewer associations between memory bias and depression in children were observed overall. Overgeneralization of autobiographical memories may represent important therapeutic targets for children with depression.

P1-F-156 - Role of hypocretin/orexin neurons in social behaviour and isolation

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Chronic social isolation during adolescence disrupts normal social behaviour and is a risk factor for anxiety and depression. Proper social functioning during adolescence is also key for development of adult social behaviour. Yet, our knowledge of which brain regions and circuits are affected by social isolation is incomplete. Based on our previous work (Dawson et al., 2023), the activity of hypocretin (Hcrt) neurons - a cluster of neurons in the lateral hypothalamus governing arousal and motivation - is essential for normative social behaviour. This project builds on these findings to test our hypothesis that chronic social isolation produces deficits in social interaction by disrupting normal functioning of Hcrt neurons. To do this, we first performed in vivo Ca2+ recordings from Hcrt neurons in control (group-housed) and isolated (single-housed) mice, examining differences in Hcrt activity during social interaction. We quantified social interaction behaviour using an automated behavioural classifier. Here, we show that Hcrt neuron activity increases in female and male control and isolated mice upon initial interaction with a same-sex novel mouse. However, the amplitude of interaction-induced Hcrt activity is significantly reduced in female and male isolated mice versus controls. Quantification of social behaviour showed that isolated mice displayed deficits in social interaction compared with control mice. Finally, we used the novel Hcrt GRAB biosensor OX0.9 to examine how Hcrt signaling is altered in isolated mice and in response to social fear in postsynaptic regions.

P1-F-157 - Brain mechanisms of proactive and reactive cognitive control: An activation likelihood estimation meta-analysis of FMRi studies

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The dual mechanisms of control (DMC) theory postulates that there are two modes of cognitive control, proactive and reactive control. Proactive control is the process of maintaining goal-relevant information prior to cognitively demanding events such as conflict or interference. In contrast, reactive control is the process of transiently activating control processes after the onset of cognitively demanding events. Proactive control is thought to involve anticipatory and sustained lateral prefrontal cortex (lPFC) activation, with inputs from midbrain dopamine nuclei, while reactive control is thought to involve transient lPFC activity along with activations in a wider network of brain regions. Here, we tested the predictions of the DMC theory by conducting an

activation likelihood estimation (ALE) meta-analysis of fMRI studies of proactive and reactive control. The preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines were used in this study. The ALE meta-analysis found lPFC regions to be consistently activated across the studies for proactive and reactive control. Proactive control was also associated with activations in clusters within the cingulate gyrus and inferior parietal lobule. Consistent activations were also found in clusters within the insula, medial frontal gyrus, cingulate gyrus, and inferior parietal lobule for reactive control. The results indicate that the conceptualization of the brain regions involved in proactive control might need to be updated to include more than the lPFC as being important for proactive control.

P1-F-158 - Changes in corticolimbic circuits after alcohol and stress exposure and their associations to persistent fear and reward seeking behaviours

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Alcohol use disorder and stress-associated disorders show high comorbidity and have been coupled with impaired emotion regulation and maladaptive behaviours such as difficulties in using safety signals to reduce fear. Here, male and female Long Evans rats were exposed to intermittent access to 15% alcohol for 5 weeks. Rats then received either exposure to footshock stress, or context exposure without shock, before being trained on the same discriminative conditioning task. This included trials of a fear cue paired with shock, a reward cue paired with sucrose, an inhibitor cue without an outcome, and compound trials of fear+inhibitor cues and reward+inhibitor cues without shock or sucrose. Rats were classified based on how behaviours were regulated by the inhibitor cue: those that showed reduced fear and reward behaviour during the inhibitor cue (resilient) and those that, despite the inhibitor cue, showed persistently high fear or persistently high reward seeking or both. Using immunohistochemistry, we quantified parvalbumin (PV) and somatostatin (SOM) interneurons within the prefrontal cortex and amygdala. We identified changes common across sexes (e.g. persistently high reward seeking was associated with less SOM in central amygdala), as well as sex-specific changes (e.g. in the prelimbic cortex (PL) persistently high fear was associated with increased PV in females, but decreased PV in males). Together, our task allows us to link neural changes unique to a subject's conditioned inhibition behaviour after alcohol and/or stress exposure to resilience or vulnerability.

P1-F-159 - Extracellular vesicles derived from obese animals cause cognitive impairment in healthy animals

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Obesity is one of the main chronic disease, with its global prevalence nearly tripling since 1975. It is linked to various comorbidities, including type 2 diabetes, cardiovascular, and neurological disorders. The adipose tissue produces bioactive molecules, such as adipokines, that interact with different tissues, including the brain, to regulate metabolism. High-fat diets are commonly used in animal models to replicate obesity-related effects, such as increased inflammation, nervous system dysfunction, and cognitive impairments. One key mechanism of intercellular communication is through extracellular vesicles (EVs), which play a critical role in intercellular

communication and signaling. However, the mechanisms underlying cognitive decline in obesity remain unclear. This study investigated the role of EVs in obesity-induced cognitive deficits using male C57BL/6 mice fed a high-fat diet for 12 weeks. Obese mice exhibited weight gain and cognitive impairments in behavioral tests, such as novel object recognition and contextual fear conditioning. Furthermore, intravenous injection of EVs isolated from obese mice induced similar cognitive deficits in naive animals, indicating that EVs may contribute to obesity-related cognitive dysfunction. These findings highlight the potential role of EVs in peripheral-brain communication and offer new insights into the pathways linking obesity to cognitive decline.

P1-F-160 - Low working memory in patients with chronic back pain experiencing trauma is associated with disruptions in pain modulation circuitry

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Many people with chronic pain demonstrate symptoms of posttraumatic stress disorder (PTSD) and often exhibit working memory deficits, but this clinical relationship remains unexplained. We have recently reported that the pathway between dorsolateral prefrontal cortex (dlPFC) and the periaqueductal gray (PAG) plays a role in decreased working memory and greater chronic pain severity. Here we investigate whether trauma affects this pathway in chronic pain patients during a working memory task.54 people with chronic pain performed a working memory task while undergoing functional MRI, and completed questionnaires assessing chronic pain severity and PTSD.Results revealed working memory mediates the relationship between exposure to trauma and chronic pain intensity. dlPFC activity during a challenging working memory task was increased in a low PTSD group compared to a high PTSD group. Further, the high PTSD group demonstrated increased functional connectivity between dlPFC and PAG. Linear regression predicting chronic pain severity revealed influence of more PTSD symptoms, lower working memory accuracy, less dlPFC activity and increased dlPFC-PAG functional connectivity.These novel results are the first to propose a specific neural pathway underpinning how PTSD and impaired working memory may be linked in chronic pain patients. We also propose a novel mechanism whereby more PTSD symptoms disrupt the capacity of dlPFC to activate to higher task load, perhaps due to increased functional connectivity with PAG, resulting in impaired working memory and greater chronic pain intensity.

P1-F-161 - Exploring the potential of music to modulate the reward circuitry and mitigate gambling disorder behaviors: a preclinical approach

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Gambling disorders are behavioral addictions marked by maladaptive gambling behaviors with significant personal, social, and economic consequences. These behaviors are linked to dysregulation of the brain's reward circuitry, a key pathway in addiction. Despite their impact, effective treatments are limited, underscoring the need for innovative therapeutic approaches. Animal models provide a valuable framework to study gambling disorders and test potential interventions. Using the Mouse Gambling Test (MGT), we identified risk-prone gambler-like individuals. To explore the neurobiological underpinnings of these behaviors, we employed a

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dopamine biosensor injected into the nucleus accumbens to monitor real-time dopamine dynamics during gambling-like tasks. To address the therapeutic potential of non-pharmacological interventions, we implemented a music-based approach, exposing mice to Mozart's Sonata K. 205 during their active period. This intervention aimed to assess whether music can modulate dopamine signaling, induce neural plasticity, and reduce gambling-like behaviors. Our results will provide critical insights into the mechanisms underlying gambling disorders and highlight the potential of music as a safe, non-pharmacological treatment.

P1-F-162 - Stress-enhanced fear learning is influenced by nature of stressor

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A major risk of post-traumatic stress disorder (PTSD) is exposure to multiple traumas, and a major symptom of PTSD is an enhanced response to mild stressors. This has been modeled in mice, where prior trauma sensitizes new fear learning even weeks later, as measured by enhanced freezing upon return to a shock-paired environment. As such, this allows for specific investigation of the mechanisms linking prior trauma with future fear sensitization. We first determined if modality (i.e., type) of the initial trauma differentially sensitizes new fear learning in mice. Interestingly, prior exposure to shock or social defeat – but not restraint stress – resulted in sensitization of new fear learning. This differential effect was unrelated to the neuroendocrine response to the initial trauma, suggesting that fear sensitization is not solely influenced by intensity of the initial trauma. Given the intrinsic role of the basolateral amygdala (BLA) in both stress and fear learning, we hypothesize a central role of the BLA. Preliminary work using activity-dependent labeling tools (i.e., TRAP2 mice) suggests that a subpopulation of BLA neurons active during original trauma are reactivated during recall of a new (different) fear memory. Ongoing anatomical work is investigating whether different stressors (e.g., restraint vs. social defeat) activate different molecularly-defined cell types within the BLA. Identifying distinct BLA subpopulations activated by stress is a critical first step to identify tractable targets for pharmacological treatment of stress-related psychiatric disorders.

P1-F-163 - The role of post-encoding activation of the prefrontal cortex in the differential organization of recent and remote memories

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Memory reorganization over time involves a shift from hippocampal to cortical dependence, but the temporal dynamics of prefrontal cortex (PFC) engagement in this process remain unclear. We investigated distinct waves of neuronal activation in the PFC following contextual fear conditioning in mice. We identified two temporally distinct waves of c-Fos expression: an immediate wave occurring within 90 minutes post-learning spanning all cortical layers of both prelimbic and infralimbic regions, and a delayed wave at 12 hours predominantly present at layers 5/6. Using Sc-FLARE, a temporally precise activity-dependent tagging system, we demonstrated that both ensembles show enhanced reactivation during remote (14-day) compared to recent (2-day) recall. These populations showed a minimal overlap, suggesting distinct neuronal populations. To establish causality, we plan to employ chemogenetic inhibition to suppress the activity of PFC during either the immediate or delayed waves. This aims to

determine whether disrupting specific temporal windows of post-encoding activity differentially affects recent versus remote memory recall. Our findings reveal novel temporal dynamics of PFC activation in memory consolidation, suggesting that different temporal windows of post-encoding activity may contribute to the organization of recent and remote memories. This work provides crucial insights into the basis of memory consolidation and the temporal evolution of cortical memory representations.

P1-F-164 - The impact of parameter choice on the association between hearing loss and cognitive decline: Data from the Canadian longitudinal study on aging

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Age related hearing loss (ARHL) was identified by the Lancet Commission (2020) as the highest of 12 modifiable individual risk factors for dementia. However, inconsistencies in the reported strength of the association and significant heterogeneity in study outcomes has been observed (Loughrey et al., 2018). A potential contributing factor may reside in the way hearing loss is assessed. This exploratory study aims to determine whether the way hearing loss is measured affects the strength of the association between hearing and cognition in the elderly. This study was conducted using the CLSA Baseline Comprehensive Dataset - Version 7.1 (Raina et al., 2009). A final sample (n = 13654) was selected. Hearing loss was determined based on four different pure tone average (PTA) threshold calculation methods: Standard, Speech, High-frequency, and Average. Two cognitive composite and standardized scores were generated for: memory, and executive functions. Correlation analysis and multiple linear regression was conducted. PTA was found to be significantly correlated with both cognition scores ($p \leq 0.000$, r = 0.217 to 0.287) for the four methods used to measure hearing. The association remains significant after controlling for the covariates ($p \leq 0.000$, r = 0.068 to 0.114). The optimal parameters combination is age, sex, cardiovascular risk, and a PTA of 0.5 and 3 kHz. These findings suggest that hearing loss is clearly negatively associated with cognition. However, this association is weak and the discrepancy in the reported strength of the association cannot be explained by parameter choice.

P1-F-165 - Chemogenetic inhibition of the ventral ca1-infralimbic cortex circuit suppresses avoidance under motivational conflict

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Approach-avoidance conflict (AAC) arises when a stimulus associated with both positive and negative consequences is encountered, resulting in competing motivations to both approach and avoid the situation. The ventral CA1 (vCA1) has been previously shown to facilitate approach under cued AAC, whereas the infralimbic (IL) cortex has been implicated in adaptive responding to cues that signal punishment and reward. However, the involvement of vCA1-IL projections in mediating decision-making under cued motivational conflict remains unclear. To this end, male and female Long-Evans rats underwent a Pavlovian Y-Maze conditioning paradigm, in which they were trained to associate three distinct visuotactile cues with either an appetitive, aversive, or neutral outcome. Following successful acquisition, animals were subjected to a conflict test in which they freely explored between a neutral cued arm and a conflict arm imbued with both appetitive and aversive cues. Animals additionally underwent a cued preference and avoidance

test, in which they explored between a neutral cued arm and an appetitive cued or aversive cued arm, respectively. During these tests, DREADDs-mediated inactivation of vCA1-IL projections resulted in a significant increase in time spent in the conflict and aversively cued arms compared to controls, implicating this circuit in potentiating avoidance under cued conflict and avoidance of negatively valenced cues. Thus, we propose that vCA1-IL projections facilitate conflict avoidance by potentiating the influence of negatively valenced cues on motivated behaviour.

P1-F-166 - Long-term voluntary exercise improves locomotor and cognitive functioning, as well as enhancing CNS resilience to oxidative stress and neurodegeneration in an age- and sex-specific manner

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Oxidized phosphatidylcholines (OxPC) are oxidative stress byproducts found in multiple sclerosis (MS) and are potent drivers of chronic neurodegeneration. Aging, a major risk factor for MS progression, also exacerbates OxPC mediated injury in the central nervous system. Thus, mitigation of oxidative stress and OxPC mediated neurotoxicity may alleviate neurodegeneration in MS. Moderate aerobic exercise have been shown to mitigate oxidative stress and age-associated cognitive decline, suggesting that exercise can be beneficial for slowing MS progression. Yet, little is known about how long-term exercise may modify the resilience of the CNS against aging and chronic neurodegeneration. Here, I investigated how voluntary long-term exercise (VLTE) in mice modifies aging and MS-relevant OxPC mediated chronic neurodegeneration. 6wk-old and 52wk-old male and female mice were provided with working or disabled running wheel to facilitate VLTE over 6 months. Physical and cognitive wellbeing of the mice were assessed by weekly weighing, monthly open field test, rotarod test and novel object recognition test at month 1, 3, and 6. After 6 months, OxPC will be injected into the corpus callosum, followed by behavioural assessments after 7 or 42 days. Subsequently, brain lesions from mice will be analyzed using quantitative immunofluorescence microscopy to compare how VLTE modulated chronic neurodegeneration between each experimental group. Overall, I expect my results will determine how CNS resilience in mice may be modulated by 6 months of VLTE in an age specific and sex specific manner.

P1-F-167 - Viral tools to alter BBB gene expression and decipher its role in memory and cognition

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Emotional experiences impact memory consolidation and associated brain neuronal circuits as seen in stress and mood disorders. We highlighted that stress induces blood-brain barrier (BBB) alterations in a sex and brain region specific manner in mice and human depression. BBB cells secrete growth factors and overexpress some markers to allow behavioral responses in stressful and cognitive situations. However, little is known about the relationship between emotional valence, memory encoding and BBB function. In this study, we looked at effects of AAV region-specific delivery on BBB formation and memory. After Fear conditioning paradigm, fibroblast growth factor 2 (Fgf2), which regulates BBB integrity, was increased in ventral hippocampus (HC)

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and prefrontal cortex (PFC) for mice receiving footshocks. FGF2 protein in footshocks mice seems to come from astrocytes in ventral HC in proximity with blood vessels. We then took opportunities of viral tools to manipulate BBB integrity via Fgf2 and tight junction Cldn5. We injected AAV5-gfaABC1D-shRNA-Fgf2 in PFC to lower FGF2 levels and see how its downregulation from astrocytes affects behavior. Also, we injected AAV2/9-shRNA-cldn5 in dorsal HC to lower Cldn5 levels, and ran Novel object recognition, Y-maze and FearC, which affected exploration and memory extinction. In summary, mice that had a negative memory experience showed BBB changes. Fgf2 could be an important link between memory dysfunction and vascular barrier impairment. This BBB modulator and Cldn5 tight junction might be key molecules of memory formation in HC and PFC.

P1-F-168 - A behavioural assay for investigating cued conflict between allocentric and egocentric spatial memory with instinctive escape in mice

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Instinctive escape behavior is widely recognized as a reliable tool for assessing spatial memory in mice. Here, we present a behavioral assay designed to evaluate how mice navigate toward a previously learned goal when confronted with a conflict between egocentric and allocentric cues. Spatial memory was assessed during escape to a learned shelter triggered by an innately aversive auditory stimulus in the presence of a proximal LED landmark. The use of egocentric or allocentric memory was evaluated by subjecting the animals to cued-conflict scenarios. In these scenarios, an allocentric LED cue was deliberately placed at a distal position relative to a previously visited shelter location, in contrast to a no-conflict scenario where the cue was positioned directly above the shelter. Baseline behavioral tests in a cohort of C57BL6J mice revealed a decrease in reliance on allocentric cues as the deviation of the LED landmark from the actual shelter location increased. When the disparity between the LED cue and the shelter location exceeded a threshold, the mice began to favor egocentric strategies over allocentric ones. Future applications of this assay could incorporate neural recording and manipulation techniques, offering broader applications in neuroscience. To demonstrate its utility, we applied the novel cued-conflict assay to test the 5xFAD Alzheimer's disease (AD) mouse model during the prodromal stages of the disease at 2 and 3 months of age. Both AD and wild-type (WT) mice performed similarly at both time points. However, we observed an increased incidence of allocentric LED-directed trials in AD mice compared to WT mice at 2 and 3 months when the allocentric LED cue was close to the shelter location. The timeline and extent of pathological changes in AD that influence the resolution of cued-conflict scenarios remains unclear.

P1-F-169 - Reduced cerebellar scaling in the semi-aquatic bush dog (speothos venaticus)

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Introduction:The bush dog (Speothos venaticus) is a near-threatened small canid found in Central and South America. However, very little information is documented on their behaviour due to their elusive nature. Unlike most canids and carnivorans that live in terrestrial environments, bush dogs have adapted morphologically and behaviourally to live in semi-aquatic environments. This study hypothesises that the structure of the bush dog brain has a unique organisation that reflects its specific behaviours.Methods:This study used magnetic

resonance imaging to obtain volumetric data and 3D models of neuroanatomical structures. Phylogenetic Generalised Least Squares analyses were performed to understand the bush dog's neuroanatomical structures to that of other Carnivora species.Preliminary Results:Most neuroanatomical structures scaled as expected in the bush dog given its size and brain mass. However, the vermis and cerebellum of the bush dog scaled smaller than expected compared to other carnivorans. These preliminary findings require additional data from canids and carnivorans to more accurately assess the scaling of the vermis and cerebellum in the bush dog. Discussion: The preliminary findings suggest that the brain of the bush dog lacks specific specialisations among Carnivora. The decrease in cerebellar scaling may be because bush dogs require less extensive motor control, this might be attributed to adaptation of their semi-aquatic environment. Additional carnivoran data is needed to validate this finding.

P1-F-170 - Increases in prediction errors shifts the brain adaptively to higher integration states

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System segregation (SS), reflecting the functional specialization of brain networks, is critical for understanding brain function. While segregation supports specialized processing, integration facilitates inter-network communication, with both adapting to task demands. However, the specific functions supported by segregation versus integration remain unclear, as does their relationship to prediction error—a key mechanism for learning driven by mismatches between expected and actual outcomes.This study tested whether SS dynamically tracks prediction error during pain perception in healthy participants. During functional MRI, participants performed a schema-based task with visual cues signaling expected noxious heat intensities (0–100%), followed by heat stimuli that either matched or mismatched expectations. On specific mismatched trials, cues predicting 0–100% intensity were paired with a fixed high-intensity stimulus (47°C), generating prediction errors ranging from 0 to 3.2°C. SS was compared across four prediction error levels, matched trials, and control conditions involving uncued heat stimuli.SS significantly decreased (i.e., integration increased) with higher prediction error, but not with variations in matched cue-stimulus intensity or uncued stimulus presentations. These results indicate that SS distinctly tracks prediction error magnitude, and, to our knowledge, is the first study to show this. Specifically, our findings suggest that prediction errors shift the brain toward integrated states, potentially enabling distributed processing critical for learning.

P1-F-171 - Molecular mechanisms mediating engram ensemble retrievability state in mice

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Engram ensembles, the cellular substrates of memory, exist on a spectrum of retrievability states, ranging from active to silent. While sensory cues typically reactivate engram neurons to induce memory retrieval, silent engrams fail to respond to these cues, though they can be artificially reactivated through optogenetic stimulation. In this study, we investigated the molecular mechanisms regulating engram silencing and un-silencing in forgetful TgCRND8 (Tg) mice, characterized by high endocytosis of GluA2-containing AMPA receptors (AMPARs) at the post-synaptic membrane. During context threat training, engram ensembles formed normally in the dorsal hippocampus CA1 of Tg mice but subsequently became silent. Remarkably,

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administering a peptide that interferes with GluA2-AMPAR endocytosis selectively during engram activation—whether through training, memory retrieval attempts, or optogenetic stimulation—restored engram activity and enabled memory retrieval. Similarly, in wild-type mice, LTD (Long-term depression)-type stimulation silenced engram ensembles, but the same peptide reactivated them when applied during engram activation. These findings elucidate the molecular processes underlying the dynamic states of engram retrievability, demonstrating the critical role of GluA2-AMPAR trafficking in memory silencing and reactivation. Our results offer novel insights into memory restoration mechanisms and suggest potential therapeutic approaches for memory impairments.

P1-F-172 - Sex-dependent deficits in long-term spatial memory in GluN2A knockdown rats

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IntroductionDuring early development neurons express mostly N-methyl D-Aspartate receptors (NMDARs) containing GluN2B subunit; however, in response to sensory experiences, the GluN2B subunit is exchanged for GluN2A. This transition allows neurons to mature, stabilize, and refine their connections. In a previous work, we induced GluN2A knockdown in the hippocampus of young adult Wistar rats (GluN2A-KD rats) and found that it impaired the contextual component of a fear-conditioning task. This led us to hypothesize that GluN2A-KD rats might exhibit cognitive deficits in other hippocampal-dependent memories.MethodsWe injected adeno-associated vectors containing either a shRNA targeting GluN2A or a "scramble" sequence into the dorsal hippocampal CA1 region of three-month-old female and male Wistar rats (n=10 per group). Fourteen days post-injection, we performed an Object Location Task, Barnes Maze, and Reverse Barnes Maze to evaluate spatial memory, as well as a Three-Chamber Test to assess social behavior.Results GluN2A-KD male rats, but not females, exhibited long-term spatial memories only after multiple-training paradigms. Regarding social behavior, both male and female GluN2A-KD rats maintained their social interest and demonstrated the ability to recognize a novel partner over a familiar one. Discussion These findings suggest that decreased GluN2A expression in the hippocampus of Wistar rats might lead to sex-dependent deficits in spatial memory without affecting their social behavior.

P1-F-173 - The effect of electroconvulsive shocks on engram reactivation in ECS-induced retrograde amnesia and the potential role of adult neurogenesis

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The side effects of electroconvulsive therapy (ECT) on memory retrieval are reported in human clinical studies, limiting the wide use of ECT in medication-resistant patients despite a high efficacy rate. We have replicated ECT-induced retrograde amnesia in mice using contextual fear conditioning and chronic electroconvulsive shocks (cECS), the animal analog of ECT which mimics the procedure of multi-session ECT in humans. However, neither the specific neural mechanisms underlying memory processing affected by cECS, nor how they are affected, are clear.Memory retrieval in fear conditioning has been well-mapped at the neural circuitry level, where the same neural ensembles activated during encoding are reactivated at retrieval, forming a memory engram. A high level of neurogenesis disrupts engram reactivation and induces forgetting of previously encoded memory.cECS has been shown to significantly increase adult

neurogenesis in the hippocampus. Thus, increased adult neurogenesis following ECT may account for amnesia as the integration of adult-born neurons disrupts the reactivation of existing memory engrams.In the current study, FosTrap2A transgenic mice were trained on contextual fear conditioning and received 20-day cECS before fear memory was tested. Neural ensembles activated at encoding and retrieval of fear memory were labelled with different fluorophore to examine engram reactivation. Adult-born neurons were labelled separately, and we hypothesize that adult-born neurons would be activated at retrieval instead of the neural ensembles activated at encoding.

P1-F-174 - Activation of astrocyte Gq-signaling in the dorsal hippocampus is sufficient to facilitate social memory absent of changes in sociability in female and male mice

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Through direct and indirect actions, astrocytes affect behaviours ranging from sleep to fear to memory; however, astrocytes' effects on social behaviours have been remarkably understudied. It is known that disruption of astrocyte function leads to changes in non-social behaviours. Social behaviours likely also rely on astrocyte functioning to maintain proper intra- and inter-regional neuronal activity and neuroplasticity within the social brain network. Here we began to explore the involvement of astrocytes in social recognition and sociability. We employed Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) to selectively activate Gq signaling in astrocytes of Aldh1l1-creERT2 mice within the dorsal hippocampus (dH), a region involved in social memory. Mice were tested in a short-term social recognition task performed within the home cage following intra-dH saline and clozapine N-oxide (300μM, 0.3μL/side) treatment. Astrocyte Gq-activation in the dH was sufficient to facilitate social memory in female and male mice, whereas non-DREADD-expressing mice and DREADD-expressing mice given saline showed no social recognition. In a binary choice test of social approach and social novelty seeking, there were no effects of genotype or treatment. Gq-signaling in dorsal hippocampal astrocytes, therefore, is sufficient to improve short-term social memory without affecting overall sociability. These studies are, to our knowledge, the first to explore the involvement of astrocytes in these social cognitive domains.

P1-F-175 - The Neuromodulatory role of Orbitofrontal Noradrenaline in the control of action-outcome updating

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Behavioral flexibility is a fundamental neurocognitive process referring to an organism's ability to adapt to environmental changes. Past research across different species has demonstrated that the Orbitofrontal Cortex (OFC) plays a key role in behavioral flexibility given that its alteration results in a wide range of behavioral impairments, from value updating to economic decision-making and reversal learning (RL) (1). Recent research from our team has highlighted the importance of noradrenergic (NE) input from the Locus Coeruleus (LC) to the OFC in controlling reversal learning (2). In the current study, we further investigated the role of LC (NE)- OFC system in behavioral flexibility using a probabilistic RL task allowing us to study the online updating of action value. In this task, rats must identify the most valuable option among 2 possible choices,

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with one lever being associated with an 80% chance of reward delivery while the other option delivers the reward only on 20% of the presses. Within a session, the contingencies are shuffled multiple times based on the rats' performances to prompt the animals to switch from one lever to the other, therefore online updating the contingencies continually. Using this task, we found that chemogenetic silencing of the OFC CaMKII neurons ii) of LC-OFC NE inputs impaired the rats' ability to adjust their behavior at reversal following reward omission trials. Interestingly, fiber photometry recordings suggest that reward omissions violating prior expectations might enhance NE release within the OFC, an indication that this specific pathway might be central in regulating behavioral flexibility. Taken together, our finding demonstrate a specific role of NE terminal in the OFC for controlling the online updating of the relationship between an action and its outcome.

P1-F-176 - Orbitofrontal noradrenaline acts as an early gate for reversal learning

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In order to survive and thrive in a constantly changing environment, organisms must be able to adapt to changes in action (A) – outcome (O) contingencies. We previously demonstrated that noradrenergic (NA) projections from the locus coeruleus (LC) to the ventral and lateral parts of the orbitofrontal cortex (OFC) are specifically required for updating instrumental associations following reversal in rats, but the underlying dynamics remained to be elucidated. Thus, in the current work, we used fiber photometry coupled with a NA-specific sensor to monitor OFC-NA signaling during instrumental reversal learning. Interestingly, we observed increased OFC-NA transmission exclusively following unexpected reward deliveries on the first day of reversal learning. Notably, the magnitude of these OFC-NA responses acted as a positive predictor of the animals' performance, with faster learners displaying strong OFC-NA responses to reversal and slower learners clearly lacking this specific form of signaling. Next, we employed chemogenetics and optogenetics coupled with NA-specific viral tools to test if inhibiting this predictive OFC-NA response at reward affected the pace of the behavioral adaptation. We found that silencing NA projections from the LC to the OFC indeed delayed the update of A-O contingencies, thereby confirming the hypothesis of causality raised by fiber photometry recordings. Overall, the current study suggests a pivotal role for OFC-NA in initiating adaptive behavior by signaling positive prediction errors during uncertainty.

P1-F-177 - VIP interneuronal dynamics in memory-encoding hippocampal spiking sequences

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Episodic memory is thought to rely on spiking sequences of pyramidal neurons in the hippocampus, encoding specific spatiotemporal contexts. Such sequences depend on finely tuned excitation/inhibition (E/I) balance. Inhibitory interneurons include parvalbumin (PV) and somatostatin (SST) expressing cells that provide somatic and dendritic inhibition on pyramidal cells respectively, and vasoactive intestinal polypeptide (VIP) expressing cells that inhibit other interneurons, thus 'disinhibiting' pyramidal neurons. This integrated network ensures precise spiking sequences and maintains the E/I balance critical for memory encoding. Although PV- and SST-mediated inhibition has been extensively explored, VIP-mediated disinhibition remains less

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understood. We hypothesized that VIP cells are activated during and after memory cues, disinhibiting memory-encoding pyramidal neurons in the hippocampus CA1. Using in vivo voltage imaging with genetically encoded voltage indicators (GEVIs), we recorded VIP membrane potentials in CA1 during an odor-cued working memory task in VIP-Cre transgenic mice. Our analysis of VIP spiking and subthreshold activity in response to odors, auditory cues, and water rewards, reveals VIP interneurons dynamics during memory encoding and working memory activation in the hippocampus. Our findings shed light on the role of VIP-mediated disinhibition in shaping pyramidal spiking sequences and provide insights into inhibitory circuits during hippocampal memory encoding.

P1-F-178 - High chronic pain severity is linked with anomalies in pain modulation and periaqueductal gray connectivity

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Chronic pain (CP) remains poorly understood, with complex mechanisms contributing to its severity. Its frequent co-occurrence with mood disorders suggests a distinct clinical phenotype requiring precision-based interventions. Disruptions in pain modulation pathways, such as periaqueductal gray (PAG) abnormalities, may underlie severe CP pathophysiology. This study employs Expectation-Induced Pain Modulation (EIPM), a framework investigating how mismatches between expected and actual pain shape pain modulation. We examined whether severe CP conditions, defined by high- and low-severity groups, show impairments in EIPM and PAG connectivity during resting-state fMRI. A total of 159 CP patients were classified into severity groups using PCA and K-means clustering, alongside 72 healthy controls. EIPM was assessed via tasks measuring prediction error and pain perception using visual threat cues and thermal stimuli. Resting-state seed-based connectivity analyses focused on dorsolateral/lateral (dl/lPAG) and ventrolateral (vlPAG) PAG columns. High-severity CP patients exhibited significantly impaired pain modulation ($P < 0.05$). Resting-state analyses revealed more negative dlPAG connectivity with cognitive appraisal networks in the high-severity group. Reduced connectivity contrast between dlPAG and vlPAG with sensory and ventral attention networks correlated with poor pain modulation and CP severity. Severe CP represents a distinct clinical entity with disruptions in pain modulation circuitry, particularly involving the PAG and its connectivity to cognitive and sensory networks.

P1-F-179 - Camkii-expressing neurons in orbitofrontal cortex are required for higher load short-term incidental memory for odours in rats

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The spontaneous and unintentional encoding of sensory experiences, known as incidental memory, is involved in higher-order processes such as episodic and working memory. Orbitofrontal cortex (OFC) is critical for cognitive functions such as sensory integration and reward processing; however, its involvement in odour-based incidental memory remains unclear. To assess a potential role of OFC in odour-based incidental memory, the identical (IST) and different stimuli tests (DST) were used. These tests utilize rodents' innate novelty preference, with the DST involving a higher memory load than the IST. The tests consist of a sample phase in which rats freely explore either six identical (IST) or six different (DST) odours followed by a 1-

minute delay phase. Rats then explore five familiar odours and one novel odour in the test phase. To manipulate OFC in rats (11 male, 13 female), we infused viral vectors containing the CaMKII promoter and inhibitory Designer Receptor Exclusively Activated by Designer Drugs (DREADD) or control vector. Recovered rats were injected with saline or a DREADD agonist (Compound 21, 1mg/kg, i.p.) 60 minutes prior to testing in the IST or DST (4 tests/rat). Activation of inhibitory DREADDs in OFC produced significant deficits in novelty preference in the DST, but not the IST, indicating that OFC is required for higher but not lower incidental memory load. DREADD activation in OFC did not alter overall odour exploration. Future experiments will use fiber-photometry to investigate dynamic OFC neuronal activity during interaction with stimuli in the DST.

P1-F-180 - Extraction of average sound level from visual scene ensembles is reliant on high spatial frequencies

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Participants can rapidly extract average sound level from ensembles of multiple scenes (i.e., how quiet or loud scenes appear on average; Tharmaratnam et al., 2024), without reliance on visual features such as colour, contrast, or low spatial frequencies. Furthermore, this occurs without utilizing visual working memory (VWM) resources, a hallmark of ensemble processing. Here, we investigated if the extraction of average sound level from visual scene ensembles is instead reliant on high spatial-frequency content. Given the importance of high spatial-frequency information in forming scene gist representations (Berman et al., 2017), we predicted that participants would have difficulty extracting average sound level when high spatial frequencies were filtered out of scene images, and furthermore, the contents of VWM would not aid participants in this scenario.Participants rated the average sound level of scene ensembles that were gray-scaled and had a low spatial frequency filter applied (< 1 cycle/degree). We varied set size by randomly presenting 1, 2, 4, or 6 scenes to participants on each trial, and measured VWM capacity using a 2AFC task. As predicted, individuals were not able to extract average sound level by globally attending to all scenes. Furthermore, individuals remembered less than 0.76 scenes on average, showing that VWM resources could not aid in the formation of a summary statistic. These results reveal that high spatial-frequency information may be essential for computing cross-modal summary statistics, consistent with its role in global scene perception.

P1-F-181 - Examining neuronal ensembles in the mature and developing hippocampus

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Episodic memories are encoded by groups of excitatory neurons in the hippocampus termed neuronal ensembles. Neurons belonging to the same ensemble share coactivity and functional connectivity even prior to encoding experience (e.g., in experimentally-naive animals and ex vivo), indicating that neuronal ensemble membership is "preconfigured" in mature hippocampal networks. When and how this configuration arises remains unclear. To examine whether mature hippocampal neuronal ensembles emerge in the developing hippocampus and how they evolve, we performed longitudinal two-photon microscopy in CA1 of head-fixed, transgenic Thy1-GCaMP6f mice walking or resting on a treadmill. First, we characterized CA1 neuronal ensemble activation and turnover in adult mice, replicating previous findings on mature hippocampal

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network preconfiguration. In ongoing experiments, we are longitudinally examining CA1 neuronal dynamics in juvenile transgenic mice across the third and fourth postnatal weeks (days ~17 to 25); a period during which the hippocampus matures to support episodic-like memory. Analyses will focus on whether neuronal activity in the immature hippocampus (<22 days) informs the configuration of mature neuronal ensembles in CA1, which we predict to emerge during the fourth postnatal week (day ~23). This study will reveal how developmental processes in the hippocampus shape mature episodic memory function, enabling future investigation of how these processes and memory dysfunction arise in neurodevelopmental disorders.

P1-F-182 - Humans actively reconfigure neural task states

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We can flexibly switch between tasks, like switching from French to English, but these changes are often difficult. Decades-long debates have argued over why task switching is difficult, with two major contenders. The 'Reconfiguration' hypothesis credits top-down control over task states, whereas the 'Inertia' hypothesis credits decaying interference from the previous task. This debate remains unresolved in large part due to the difficulty in measuring these latent task dynamics from behavior alone. To make new progress on this debate, we used linear-Gaussian state space models (SSM) to infer the dynamics of neural task states from human electroencephalogram (EEG) recordings. The SSM is a latent variable model that is popular in computational systems neuroscience, but which has seen scant use in human neuroimaging. We fit SSMs to two open-source datasets on cued task-switching (N=30, N=26). We found that these models had high predictive accuracy, surpassing parameter-matched recurrent neural networks (RNNs). Using a control theoretic method for assessing the propagation of task information, we found that task representations spread more on switch trials than repeat trials, consistent with the reconfiguration hypothesis. Confirming this interpretation, we found that RNNs trained to switch between tasks (reconfigure), but not those trained to make isolated decisions, showed latent dynamics that were consistent with human EEG. These results help advance a classic debate about our cognitive flexibility, and provide a powerful new paradigm for human neuroimaging analysis.

P1-F-183 - Multi-timescale reinforcement learning in the brain

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To thrive in complex environments, animals and artificial agents must learn to act adaptively to maximize fitness and rewards. Such adaptive behavior can be learned through reinforcement learning, a class of algorithms that has been successful at training artificial agents and at characterizing the firing of dopamine neurons in the midbrain. In classical reinforcement learning, agents discount future rewards exponentially according to a single time scale, known as the discount factor. Here, we explore the presence of multiple timescales in biological reinforcement learning. We first show that reinforcement agents learning at a multitude of timescales possess distinct computational benefits. Next, we report that dopamine neurons in mice performing two behavioral tasks encode reward prediction error with a diversity of discount

time constants. Our model explains the heterogeneity of temporal discounting in both cue-evoked transient responses and slower timescale fluctuations known as dopamine ramps. Crucially, the measured discount factor of individual neurons is correlated across the two tasks suggesting that it is a cell-specific property. Together, our results provide a new paradigm to understand functional heterogeneity in dopamine neurons and a mechanistic basis for the empirical observation that humans and animals use non-exponential discounts in many situations, and open new avenues for the design of more efficient reinforcement learning algorithms.

P1-F-184 - Basolateral amygdala astrocytes encode anxiety states

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Anxiety disorders are one the most prevalent psychiatric disorders, with over 10% of the Canadian population reporting at least one episode of anxiety in their lives. Over the decades, most research has been focused on neuronal correlates of anxiety disorders. Recent research has, however, shed light on an astrocytic modulation of anxiety processing across the hippocampus, lateral habenula, and periaqueductal grey nucleus. Yet, little is known about the contribution of astrocytes of the amygdala, a nucleus known to be pivotal in the expression of anxiety. Here, we use in vivo photometry recordings in freely-moving animals throughout an array of behavioral paradigms to assess the role of basolateral amygdala (BLA) astrocytes in anxiety-like behaviors. We report that BLA astrocyte calcium is highly correlated to anxiety-like behaviors in mice, with strong increases in activity during the exploration of anxiogenic environments. Moreover, we found that the magnitude of astrocytic activity was linked to behavioral engagement in our tasks, with the magnitude of calcium activity associated with a more anxious phenotype. These results further support a role for astrocytes in anxiety processing in the BLA and highlight their potential as a promising target in the treatment of these disorders.

P1-F-185 - Targeting the affective/motivational component of pain by activating the delta opioid receptor

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With its different components (affective, sensory, cognitive, and behavioral), pain is multidimensional. While helpful short term, pain that persists over the normal healing process is pathologic and problematic. Chronic pain negatively impacts quality of life, and the prolonged treatments with strong painkillers (ex. opioids) come with many undesirable effects. The delta opioid receptor (DOP) has been identified as a potential therapeutic target for chronic pain since it produces little – if any – of the undesirable effects commonly associated with opioids. DOP activation is also reported to reduce anxiety and depression.The aim of the project was to evaluate the effects of SNC80, a DOP-selective agonist, on the affective and sensory components of pain. To this purpose, we used two different pain models in mice (neuropathic and inflammatory pain). The affective/motivational component of pain was assessed using the Conditioned place preference and the splash test while its sensory component was evaluated with the von Frey test. Our data suggest that in the neuropathic pain model, SNC80 diminishes the negative affect of pain in male mice, while it produces an antinociceptive effect in both sexes.

We observed a similar effect in the inflammatory pain model where DOP activation produced beneficial effects on both the affective and the sensory components, in a sex-dependent manner. Our results therefore confirm that DOP has the potential to affect multiple components of pain and, by way of consequence, to improve the quality of life of patients living with chronic pain.

P1-F-186 - Humans forage for reward in reinforcement learning tasks

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Because the world is dynamic and only imperfectly observable, many of the decisions we make are necessarily uncertain. How do we navigate such uncertainty? From the perspective of cognitive neuroscience, the classic answer would be that we evaluate the benefits of each potential choice and then lean towards the alternative promising the greatest reward, modulo some exploratory noise. Conversely, an ethologist would argue that we would stay with previously rewarding choices until the payout drops below a certain threshold, at which point we start exploring other options. Because the fields use incompatible methods, it remains unclear which view better describes human decision-making. Here, we found that humans use compare-to-threshold computations in classic compare-alternative tasks. Because compare-alternative computations are central to the reinforcement-learning (RL) models typically used in the cognitive and brain sciences, we developed a novel compare-to-threshold model ("foraging"). Compared to previous RL models, the foraging model better fit participants' behavior and better predicted the tendency to repeat choices. The foraging model was also able to predict the existence of held-out participants with very prolonged repetitive choice runs, a pattern that was almost impossible under traditional RL models. These findings indicate that humans use compare-to-threshold computations—even in testbeds for traditional compare-alternatives RL models.

P1-F-187 - Monkeys forgo reward for the chance to lapse

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Even when a task is well-learned and will not change, humans and other animals occasionally make seemingly irrational decisions known as "lapses". Because lapses serve no obvious purpose, they are often written off as the consequence of task-irrelevant processes, like disengagement, distraction, or sensorimotor noise. However, a different view has recently emerged: the idea that lapses could be caused by the same exploratory processes that help us learn in uncertain environments. Critically, this view does not require or imply that lapses are actually deliberate decisions. We still do not know if lapses are the consequence of a selective decision-making process or just a by-product of exploratory noise. Here, we disambiguate these hypotheses via a novel task in which decisions are made serially, rather than simultaneously. We found that 2 rhesus macaques withheld choosing the objectively best option and sacrificed reward in order to generate lapses, consistent with the idea that lapses are not only selective decisions but subjectively valuable ones. There was also a dissociation between the information-

theoretic measures that lapses maximize and that maximize lapses. Together, these results suggest a model in which we seek information tonically–both when its useful and when its not.

P1-F-188 - A tale of two targets: Insights from PCC- and amygdala-targeted fMRI neurofeedback pilot studies and progress from a sham-controlled trial in PTSD

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Post-traumatic stress disorder (PTSD) is associated with brain functional disruptions, notably including hyperactivity in the amygdala and posterior cingulate cortex (PCC) during trauma processing. As such, previous real-time fMRI neurofeedback (rt-fMRI-NFB) studies have targeted regulation of both brain regions, but their differential effects on neural activity and clinical outcomes in PTSD remain unclear.We investigated whole-brain activation and symptom changes in 28 PTSD participants who were trained to downregulate activity within either the amygdala (n=14) or PCC (n=14) while viewing personalized trauma words over a single rt-fMRI-NFB session. For the PCC as compared to the amygdala group, we observed decreased neural activity in PTSD-related areas during neurofeedback training, including the bilateral cuneus/precuneus/primary visual cortex, the left superior parietal lobule, the left occipital pole, and the right superior temporal gyrus/temporoparietal junction (p-FDR whole-brain corrected < .025). For the amygdala as compared to the PCC group, there were no differential decreases in neural activity. Amygdala downregulation did not significantly improve PTSD symptoms, whereas PCC downregulation was associated with reduced reliving (p = .016) and distress symptoms (p = .010). As a critical control, the PCC and amygdala groups did not differ in their ability to downregulate target region activity during neurofeedback training.These results may guide neurofeedback target selection in future rt-fMRI-NFB studies and support the clinical efficacy of specific neurofeedback targets for PTSD. Expanding on this emerging research, preliminary data from an ongoing double-blind, randomized, sham-controlled trial on multi-session PCC vs. amygdala targeted rt-fMRI-NFB will also be discussed.

P1-F-189 - Unpacking sensory processing subtypes: Insights into attention, social skills, and executive functioning abilities in children

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Sensory processing differences may provide key insights into child development and behavioural outcomes. We examined sensory processing in children 5-12 years. Parent-report surveys assessed children's sensory processing, social skills, executive functioning, and attention. Preliminary results of a K-means cluster analysis (n=57; Mage=7.5 years, SDage=2.1 years; 32 males, 24 females) identified 5 sensory processing subtypes: Tactile/Movement Adaptive (TMA; n=7), Sensory Adaptive (SA; n=17), Taste/Smell Sensitive (TSS; n=9), Tactile/Movement Sensitive (TMS; n=10), and Sensory Sensitive (SS; n=14). These subtypes replicate known sensory profiles found in both typically developing children and those with developmental conditions (Little et al., 2016; Scheerer et al., 2021). One-way ANOVAs revealed differences between subtypes on all measured outcomes. Children in the SA group exhibited better social skills, fewer attentional challenges, and less executive dysfunction compared to other groups. In contrast, the TMS and SS groups demonstrated more attentional and executive functioning difficulties, aligning with

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prior findings on sensory sensitivity and attentional engagement (Dean et al., 2018). These findings demonstrate the uniformity of sensory processing subtypes across populations and their predictive utility for attention, behaviour, and social functioning. These sensory profiles provide valuable insight into the relations between sensory processing differences and other developmental outcomes.

P1-F-190 - Dynamics of catecholamine release in the medial pre-frontal cortex of adult mice during associative learning

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Associative learning is the ability to infer correlations between two events - notably an action and a reward. While dopamine (DA) is known as a major actor in this process by encoding reward prediction error (RPE), the focus on norepinephrine (NE) - shown to be involved in novelty signaling and thus potentially relevant in the updating of learned associations – is more recent. These two catecholamine systems target for the most part segregated circuits. However, their influence overlaps in the medial pre-frontal cortex (mPFC), a region involved in decision-making and the valuation of actions. Here, we aimed to study NE and DA release dynamics in the mPFC to characterize not only their individual role but also how their synergistic action contributes to associative learning. Using a fiber photometry set-up combining two excitation sources and captors, we simultaneously recorded GRABNE2m and rGRABDA3m - two GPCR-based sensors that track NE and DA respectively. We trained head-fixed mice on a classical conditioning task in which they were presented with two sound cues followed by a reward (high frequency) or not (low frequency). With this paradigm, we established that mice were able to discriminate both sounds and licked in anticipation of a reward in a cue-specific manner. Our preliminary results seem to show that mPFC DA dynamics surprisingly do not match RPE patterns. This suggests that DA and NE synergistic dynamics might be crucial in action value encoding, which could further be tested with a probabilistic decision-making task.

P1-F-191 - Examining the role gender differences in chronic pain experiences: Moving beyond binary sex classifications

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Pain perception has long been shown to differ between biological sexes. However, gender identity, shaped by societal and cultural expectations, has also been shown to contribute to pain experiences. Research examining how gender identity influences chronic pain, and its psychological comorbidities remains inadequate. Sex and gender influences on pain were examined across three domains: experimentally induced pain behaviour, clinical pain behaviour, and psychological pain behaviour. 160 participants with chronic low back pain or fibromyalgia (125 female) completed assessments of clinical pain, psychological pain, experimentally induced pain modulation, and gender identity. Preliminary analyses revealed that women experienced greater clinical pain severity compared to men, reporting more widespread pain and interference with daily activities. Results from pain modulation tasks revealed that men had higher levels of pain threshold and tolerance, while women demonstrated greater experimental pain sensitivity. Additionally, women reported higher levels of depression than men. Currently, we are investigating whether the role of gender identity in pain research can reveal nuanced

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factors influencing pain experience, rather than the binary classifications of sex. By integrating gender identity, this research contributes to a more comprehensive understanding of biopsychosocial factors in pain and supports the development of personalized pain management strategies for diverse populations.

P1-F-192 - Microglial TNF regulates morphine-induced behaviours in mice

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Tumor Necrosis Factor (TNF) is a pro-inflammatory cytokine that plays an adaptive role in the Nucleus Accumbens (NAc) by regulating homeostatic synaptic plasticity. Chronic administration of drugs of abuse such as cocaine and morphine stimulate an initial depression in excitatory signaling onto the NAc, followed by potentiation during abstinence. Cocaine and morphine administration stimulate TNF expression, and mice lacking TNF (TNF-KO) do not experience this depression in signaling induced by cocaine. In addition, TNF-KO mice have elevated cocaine sensitization compared to wild-type controls, confirming TNF acts to suppress drug-induced synaptic and behavioural alterations. Here, we investigate whether TNF plays a similar role in regulating morphine reward. Morphine locomotor sensitization was tested in WT and TNF-KO mice, as well as mice with a conditional deletion of microglial TNF, to evaluate the cellular source of TNF. The requirement for TNF in the NAc for sensitization was tested by the viral deletion of TNFR1 within the NAc. Furthermore, WT and TNF-KO mice were submitted to a morphine conditioned place preference paradigm. We discovered that TNF-KO mice have elevated morphine sensitization, as well as increased conditioned place preference compared to controls. This work highlights the potential use of TNF in a therapeutic context.

G - NOVEL METHODS AND TECHNOLOGY DEVELOPMENT

P1-G-193 - Gene immunotherapy regulated by astrocytic reactivity in a mouse model of amyloidosis

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Background: Recombinant adeno-associated viruses (AAVs) capable of crossing the blood-brain barrier (e.g. AAV.PHP.eB) and encoding antibodies against amyloid beta peptides (Aβ) have potential to evaluate brain-wide gene immunotherapies in Alzheimer's disease (AD). Also, leveraging astrocytic reactivity in response to Aβ pathology, the glial fibrillary acidic protein (GFAP) promoter could serve as a regulator of gene immunotherapy. Hypothesis: Reactive astrocytes can regulate the expression of the recombinant anti-Aβ antibody (rSol) under the control of a GFAP promoter in the TgCRND8 (Tg) mouse model of amyloidosis. Method: To study GFAP expression in Tg mice, GFAP mRNA levels were quantified using qPCR in the hippocampal formation at 3, 5, and 6 months (n=6 per group). Next, AAV.PHP.eB.GFAP.rSol-myc-tag and AAV.PHP.eB.GFAP.GFP were co-injected intravenously in Tg mice. Non-Tg littermates and C57BL/6J mice served as controls. One-month post-injection, brain sections were processed for immunohistochemistry and RNAscope. Results: GFAP mRNA levels doubled in 6-month-old

compared to 3-month-old Tg mice. Brain-wide GFP expression in astrocytes confirmed efficacy of the GFAP promoter. Notably, brain cell transduction varied across Tg mice, peaking in the C57BL/6J line. Ly6A, a protein facilitating AAV.PHP.eB brain entry, may explain this variability. We are analyzing its expression to identify the optimal Tg background for viral transduction.Conclusion: The GFAP promoter could control the production of therapeutics, such as rSol, in response to amyloid-induced astrocytic reactivity.

P1-G-194 - Single cell sequencing and spatial transcriptomic approaches define the murine leptomeninges:cortical brain interface

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The meninges provide a protective multilayer barrier surrounding the brain and spinal cord and serve as an interface structure. While it is known that the meninges are comprised of mesenchymal cells (MCs) which form distinct layers (dural, arachnoid, and pia mater), the cellular identity and composition are still under investigation. Using single-cell RNA sequencing (scRNAseq), spatial transcriptomics (ST), and microscopy we investigated the cellular gene and protein signatures to identify the different MC types. We also identified and investigated border astrocytes (BA) and border macrophage (BM) communication with the leptomeningeal cells using transcriptomic data and used a cortical stab model to investigate responses of these cell types to injury. The MCs of the dura versus the leptomeninges had distinct gene signatures, with the former expressing Clec11a and Matn4, and the latter Slc22a6 and Lama1. BAs were defined by markers such as Myoc, and BMs by Mrc1. The meningeal MCs, BAs, and BMs were identifiable in our spatial transcriptomics and microscopy data and displayed unique transcriptional signatures and morphology. Using our transcriptional data, we discovered preliminary ligand-receptor interactions between the BAs, BMs, and leptomeningeal cells. We also used microscopy to identify preliminary responses of these cell types to a cortical stab injury. Our work demonstrates the heterogeneity of the mesenchymal cells of the meninges and provides foundational information for continuing studies on how these cell populations contribute to homeostasis and brain injury.

P1-G-195 - A unified computational framework for implementing impact of deep brain stimulation in neural circuits

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Deep brain stimulation (DBS) directly engages neurons and synapses, modulating synaptic plasticity and altering neuronal activity. DBS impacts on both local and global brain dynamics. Detailed biophysical models of DBS have been developed to study how physical characteristics of neurons and electrodes, stimulation parameters, and tissue composition affect the response to stimulation. However, these models are extensively complex and extremely target specific, not allowing for generalization. Building on previous computational studies 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 modelling the impact of DBS across different neural simulators like BRIAN and NEST. In addition, we approximate the electric field propagation of DBS electrodes and how different DBS parameters,

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including amplitude, pulse-width, and frequency modulate neural activity in a neural circuit. Using probabilistic tractography atlases and the electric field estimates, we use a biophysically detailed model to estimate how different locations of stimulation might result in neuronal engagement during DBS. We show that our computational framework preserves interactions of spike times of neurons and those generated by DBS, thereby enables accurate adjustments of synaptic dynamics altered by DBS. Further, we demonstrate that the impact of DBS on the firing rate of neurons in a neural network can be consistently generated across neuro-simulators. We anticipate that this work provides a standard modelling approach for studying how DBS alters dynamics of large neuronal populations.

P1-G-196 - Engineering a genetically encoded fluorescent GPCR biosensor for Relaxin-3

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First reported in 2001, relaxin-3 is an insulin-like neuropeptide implicated in the modulation of arousal, the stress response, feeding and metabolism, and memory; however, the molecular mechanisms underpinning this modulation remain largely unknown. Currently, efforts to study the function and mechanisms of relaxin-3 rely on pharmacological manipulations and genetic knockdowns. On the other hand, genetically encoded fluorescent biosensors, which modulate their fluorescence upon ligand binding and whose development for chemical neurotransmitters has shown significant progress in recent years, are an attractive alternative because they allow for the direct visualization of their target and their high spatiotemporal resolution. To date, there is no biosensor for relaxin-3, making it difficult to understand the role of this peptide. To address this gap in science, we present our efforts on engineering the first genetically encoded relaxin-3 biosensor using its endogenous receptor and a circularly permuted green fluorescent protein. We expect that this new peptide biosensor, especially in conjunction with existing strategies, will help to understand the role of relaxin-3 and its mechanisms by facilitating its real-time detection.

P1-G-197 - Modulation of neuronal intrinsic properties to enhance network resilience using targeted electrical stimulation

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HypothesisNeuronal biophysical diversity is linked to increased synchrony in seizure-prone tissue. We hypothesize that targeted MEA stimulation will alter intrinsic excitability, broadening neuronal firing rate distribution and reducing network synchrony.Materials and MethodsThe OpenMEA platform enables high-resolution electrophysiological recording and biphasic electrical stimulation across 60 channels at 20 kHz. Acute brain slices from wild-type mice were maintained in artificial cerebrospinal fluid (ACSF). Spontaneous neural activity was recorded before and after theta burst stimulation (TBS), known to reduce excitability for up to 30 minutes post-stimulation. Two paradigms were implemented:Random Stimulation – aimed at increasing biophysical diversity.Uniform Stimulation – expected to promote network homogeneity.ResultsPreliminary MEA data show that high-frequency (~100 Hz) uniform stimulation lowered pairwise correlation values post-stimulation compared to random stimulation. Changes in neuronal diversity and synchrony will be assessed using Shannon entropy, Pearson correlation, inter-spike interval (ISI) statistics, and coefficient of variation

(CV).ConclusionsElectrical stimulation modulates intrinsic excitability in acute brain slices. Random stimulation preserves diversity and reduces synchrony, while uniform stimulation drives neuronal populations toward homogeneity. These findings support intrinsic plasticity-based interventions to counteract hypersynchrony in seizure-prone networks.

P1-G-198 - Automated detection of Alzheimer's disease from resting-state EEG

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Alzheimer's disease (AD) is one of the most prevalent neurodegenerative diseases, and its prevalence is set to rapidly increase. This creates a pressing need for tools that can improve the stratification and screening of individuals with AD to enable more efficient clinical monitoring, and more fruitful clinical trials. To that end, we developed a novel analytical approach for resting-state electroencephalography (EEG), and we aimed to evaluate its efficacy for detecting EEG signatures of AD. Data were retrospectively analyzed from an open-source dataset composed of 29 healthy individuals and 36 individuals with varying stages of AD (i.e., case-control design). The mini-mental status exam (MMSE) was used to summarize cognitive functioning. Data were preprocessed using a novel, automated pipeline, and features (representing connectivity, signal complexity, power, etc.) were then extracted. Supervised machine learning was employed to distinguish between patients and controls. Spearman correlations between individual features and MMSE were quantified, and false discovery rate (FDR) correction was performed. Patients and controls were accurately distinguished using various combinations of features from the overall feature set. The area under the receiver operating characteristic curve (AUROC) was as high as 0.84. Measure of skewness, complexity, and entropy stood out as the strongest predictor feature groups. Furthermore, 70 individual features had statistically significant correlations with the MMSE after correction for FDR, primarily in the domains of complexity and entropy. These results highlight the ability of our novel, automated pipeline to capture disease presence and status in a small cohort of individuals with AD. Further work on larger datasets and additional external validation is needed to determine the validity of this technology in clinical settings.

P1-G-199 - Metformin promotes expansion of human neural stem cells and reduces injury-induced damage in human cerebral organoids

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Activating endogenous neural stem cells (NSCs) through therapeutic interventions offers great potential for brain regeneration. A promising non-invasive strategy involves using small molecules, such as the type II diabetes medication metformin, which has shown to promote sensorimotor and cognitive recovery in pre-clinical stroke models. Metformin has pleiotropic effects in the brain, including NSC proliferation and differentiation. In this study, we examined the impact of metformin on NSCs derived from human cerebral organoids (hCOs). First, we isolated two distinct NSC populations - definitive (dNSCs) and primitive (pNSCs) - which correspond to murine NSC lineages and had not been identified in human tissue. pNSCs are a quiescent, rare population of NSCs that are upstream of the more abundant dNSCs; which contribute to neurogenesis in the mature mammalian brain. We hypothesized that metformin would modulate these NSC populations in hCOs, like its effects in murine models. Using an in

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vitro colony-forming assay, we observed that both human dNSCs and pNSCs expanded in number in the presence of metformin. Next, we developed a stroke-like injury in hCOs that leads to a loss of neurons and found that metformin treatment reduced astrogliosis (GFAP) and cell death (CASPASE 3), with a trending increase in stem cells (SOX2 and NESTIN). These responses were distinct based on hCO maturation. Together these findings have implications for understanding the fundamental biology of human NSCs and their potential to contribute to neural repair following injury and metformin treatment.

P1-G-200 - A computational framework for capturing the real-world continuum of cognition and behavior

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Background. Psychiatry is undergoing a shift toward precision medicine, demanding personalized approaches that capture the complexity of cognition and behavior. Here, we introduce a novel referential of four robust, replicable, and generalizable cognitive and behavioral profiles that embrace the continuum found in the general population.Methods. Cognitive and behavioral data from the most prominent pediatric cohort (ABCD; baseline: n=10,843, 2y follow-up: n=7369, and 4y follow-up: n=2846) and two independent cohorts (BANDA; n=195 and GESTE; n=271) were harmonized, residualized for covariates, and input into a fuzzy C-Means clustering algorithm. Clinical diagnoses and environmental factor data were pulled from questionnaires and medical records to assess the profiles' clinical validity.Results. We extracted 4 consistent profiles across all cohorts and follow-ups, showcasing their longitudinal stability and generalizability. Profiles were consistent with clinical diagnoses but exposed critical discrepancies across parent-reported, youth-reported, and expert-derived diagnoses. We showcase their real-world utility by linking them to environmental factors, revealing associations between parental influences and youths' cognition and behavior. Conclusions. Our fuzzy profiling framework goes beyond discrete classification, offering a powerful tool for refining psychiatric evaluation. To accelerate adoption, we provide an open-source framework, enabling researchers and clinicians to fast-track implementation and advance a data-driven, domain-based approach to diagnosis.

P1-G-201 - Efficiently plot and analyze electrophysiology data using patchclampplotteR

Christelinda Laureijs [1], Karen Crosby [1]

[1] Mount Allison University

Are you tired of building and tweaking plots one at a time? patchclampplotteR is a free and open-source R package that allows researchers to quickly plot and analyze raw data from whole-cell patch clamp electrophysiology recordings. Users can easily perform batch analysis on all their recordings, creating plots of raw evoked and/or spontaneous current amplitudes over time, summary plots for specific treatments, representative recording traces with scale bars, action potential traces, and more. With this package, users can also compare variance parameters, action potential properties, and spontaneous current properties over two distinct time periods, such as the baseline and a pre-determined time point after a treatment. The figures generated with patchclampplotteR can be exported as individual .png files for use in word processing/presentation software or can be used directly in an RMarkdown file for manuscript

preparation. Using patchclampplotteR in R is an efficient and much faster alternative to GraphPad Prism or other software for users who want to integrate statistical analyses and plot generation in one software tool.

P1-G-202 - Refined buccal swab techniques for assessing mitochondrial bioenergetics

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Autism, a complex neurodevelopmental condition affecting 1 in 54 children in Canada is strongly associated with mitochondrial dysfunction. Mitochondria, essential for cellular function, have been implicated in autism-related alterations. Primary mitochondrial diseases account for a small percentage of autistic cases, while secondary mitochondrial dysfunction, impacting up to 80% of autistic individuals, is often overlooked due to invasive screening methods. This study introduces a novel non-invasive approach to detect and characterize mitochondrial dysfunction by refining buccal cell culture techniques. Using buccal swabs, we successfully cultured cells and optimized a suspension-based protocol for assessing mitochondrial function. We found that collecting cells directly in Miro5 mitochondrial respiration buffer, followed by filtration through a 0.2μM membrane, was crucial for preserving cell integrity. Post-collection, cells were incubated with 50μL of antibiotic-antimycotic for 24 hours to maintain sterility and cell viability. Optimal respiration was achieved by culturing cells for 4-7 days before testing, where cells were found to still be in the growth phase of the cell cycle. Under the refined conditions, buccal cells revealed a marked increased in mitochondrial function, with oxygen utilization reaching 33.41uM — significantly higher than the 6.50uM observed prior to refinement. The optimized protocol could enable buccal cells to serve as a reliable proxy for the study of mitochondria, aiding in identifying secondary mitochondrial dysfunction in autistic individuals.

P1-G-203 - Exploring brain–behavior temporal dynamics: A novel framework using white noise optogenetics and bayesian inference

Amira Fadl [1], Faye Arellano [1], Kiana Kazeminejad [1], Jackson Cone [1]

[1] University of Calgary

A key goal of systems neuroscience is to understand how brain processes give rise to behavior. To this end, optogenetic perturbations are powerful for causally linking behavior to neuronal population activity, but traditional methods often involve long stimulations, obscuring if specific moments of activity differentially contribute to behavior. To address this, we developed white noise optogenetic stimulation, where weak (microwatt), brief (25 ms), and temporally randomized (unbiased) light pulses were used to modulate neuronal activity in behaving mice. Aligning these stimuli with behavioral reports and averaging yields neuronal behavioral kernels (NBK), millisecond-by-millisecond estimates of how perturbations impact behavior. We used this approach to modulate visual processing in primary visual cortex (V1) and superior colliculus (SC) as mice performed a visual detection task. Here, we present an analytical framework (Bayesian) for analyzing these data to reveal when optogenetically modulating visual responses in V1 or SC impacts the probability of observing different behavioral outcomes. This framework incorporates domain knowledge (priors) updated by experimental observations (posterior), enabling real-time statistical testing during data collection. To illustrate the power of our approach, we show how the impact of V1 or SC perturbations differs based on within- or across-session performance variability. This work merges novel experimental and analytical frameworks

to probe brain-behavior relationships and serves as a versatile tool for diverse behavioral paradigms.

P1-G-204 - iphage: An M13 bacteriophage-based platform for targeted gene delivery to reprogram astrocytes into neurons for neurodegenerative disease therapy

Anna Ivanova [1]

[1] University of Waterloo

Neurodegenerative diseases are the leading cause of disability and remain mostly incurable as current treatments focus on symptom management rather than slowing, or reversing, disease progression. As part of the iNeuron project, we will develop the Intelligent Phagemid-Assembled Gene Expression (iPhAGE) platform utilizing M13 bacteriophage-derived miniphagemids to deliver Neurogenic Differentiation Factor 1 (NeuroD1) to astrocytes, reprogramming them into neurons. This system overcomes several challenges in gene delivery, such as limited cargo capacity, high immunogenicity, and inability to cross the blood-brain barrier by leveraging the unique properties of M13 bacteriophages. Its rod shape allows barrier crossing, while its lack of mammalian tropism and large DNA capacity enhance delivery. The bacterial host, Escherichia coli JM109 packages NeuroD1 into miniphagemids free of bacterial backbone, reducing immunogenicity, with the highest bacterial backbone contamination as low as 1×10^{-8} %. A7 astrocytes transfected with miniphagemids undergo neuronal conversion, addressing neuronal loss in neurodegenerative disorders. Transfection efficiency is detected via the luciferase marker gene and neuronal differentiation is confirmed through staining with PSA-NCAM1 antibody specific for immature neurons. At day 7 post transfection ~44% of cells expressed NCAM1 receptors implying successful reprogramming. This safe, efficient approach enables precise targeting and gene delivery, offering a therapeutic strategy to regenerate neurons and treat neurodegenerative diseases.

P1-G-205 - The development of a non-invasive brain computer-brain stimulation interface to enhance motor rehabilitation

Ameer Hamoodi [1], Stevie Foglia [1], Aimee Nelson [1]

[1] McMaster University

Rehabilitative brain-computer interfaces (BCI) improve motor function by inducing neuroplasticity [1]. Typically, these BCIs either use neurofeedback or neuromuscular electrical stimulation to induce neuroplasticity in the motor cortex, both of which don't directly stimulate the brain [1]. A rehabilitative BCI that directly stimulates the brain on movement-intention may cause greater motor cortex activity, amplifying the magnitude of Hebbian plasticity. In this way, a direct brain stimulation BCI may induce greater neuroplasticity than standard rehabilitative BCI, enhancing motor recovery. Thus, we set out to develop and test the effects of a BCI that triggers a burst of gamma frequency repetitive transcranial magnetic stimulation (rTMS) when movement intention is detected.The BCI is trained on a participant's electroencephalography (EEG) data recorded during 30 trials of cued right-hand clenching. Across 4 participants we achieved an average true-positive rate of 83% and false-positives/minute of 6.34/min with a mean latency of 123.7ms in psuedo-real-time. In practice, real-time data will be streamed to a custom Python script running the tuned BCI that triggers an rTMS 100Hz triplet over the right-hand representation of the participant's motor cortex.References:1. Cerva MA, Soekadar SR, Ushiba J, Millan JR, Liu M, Birbaumer N, Garipelli G. Brain-computer interfaces for post-stroke

motor rehabilitation: a meta-analysis. In Annals of Clinical and Translational Neurology, 651–663, 2018.

P1-G-206 - Modeling diffusion MRI from histological tumor images: data pre-processing and theoretical framework

Laura Beltran [1], Aurélie Beaudoin [1], Andrew Forester [1], Michèle Desjardins [1], Maxime Descôteaux [2], Louis Gagnon [1]

[1] Université Laval, [2] University of Sherbrooke

Diffusion Magnetic Resonance Imaging (dMRI) has emerged as a promising tool to map the extent of the non-enhancing infiltrative tumor surrounding glioblastoma. Nevertheless, the absence of microscopic modeling of the dMRI signal within a realistic tumor environment and geometry limits its application and optimization. In this study, we developed a biophysical modeling framework to address this problem using histological brain tumor slices, which offer a highly accurate representation of the tumor environment's geometry. We used H&E-stained histological images of glioblastoma brain slices, from which we extracted samples for cell segmentation using the AI software Cellpose. Post-segmentation, Cellpose generated PNG files containing the segmentation masks of the cell nucleus. Subsequently, we performed Monte Carlo simulations of nuclear spins on these processed histological slices using NYU's Realistic Microstructure Simulator (RMS). As RMS operates in three dimensions, we transformed the 2D PNG files into 3D structures by extending the images along the z-axis and assigning a diffusion coefficient of zero in that axis, ensuring that diffusion occurred solely in the x-y plane within a slice of the augmented image. The diffusion simulations produced temporal evolution of the MR signal for selected b-values and gradient directions. The diffusion coefficients used in each compartment were taken from the literature. Our findings demonstrate the feasibility of implementing a framework for Monte Carlo MRI diffusion simulations within more realistic cellular environments.

H - HISTORY, TEACHING, PUBLIC AWARENESS AND SOCIETAL IMPACTS IN NEUROSCIENCE

P1-H-207 - The relationship between cognition and inflammation in estradiol deprivation

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Estrogens play an important role in the regulation of many bodily systems, including the nervous system and the immune system. While current research has focused on independently examining the effects of estrogens on cognition and inflammation, less studies have reported on the role that inflammation may play in mediating the relationship between estrogens and cognition. Changes in inflammatory markers have been shown to precede Alzheimer's Disease (AD) pathology. Therefore, the intersection of estrogens, cognition and inflammation is crucial for understanding early cognitive decline in women. We propose that estrogens exert both a direct effect on cognition, as well as an indirect effect through the immune system. To determine what is known about the immunomodulatory effects of estradiol on cognition, we conducted a narrative review of the literature and included any primary research paper reporting a cognitive

result, an inflammatory measure, and an assessment of estradiol levels. Although nine studies were found, most of the studies were conducted in patient populations, clouding the immunomodulatory role of estrogens on cognition with disease pathologies. Future research is needed to extend current knowledge of inflammatory mechanisms underlying the impacts of estrogens on neurocognitive processes.

P1-H-208 - The neurotechnology microcredential program: An innovative and interdisciplinary pedagogical approach to prepare learners for responsible innovation in the emerging field of neurotechnology

Jonathan Coutinho [1], Pauline Gaprielian [1], Susan Boehnke [1]

[1] Queen's University

Neurotechnology is an emerging growth industry that applies brain sensing, imaging or modulating technologies to solve real world problems, such as diagnosing and treating brain disorders, understanding and modifying brain states, and even interfacing the brain with machines. Applications of neurotech raise novel legal, ethical, and social considerations, but there are limited accessible training programs to develop the necessary transdisciplinary core-competencies for responsible neurotech innovation. To bridge this gap, we have developed the Queen's University Neurotech Microcredential Program (NTMC), offering asynchronous, online courses on key neurotech topics, plus an in-person capstone project course offering hands-on experience with neurotech and networking opportunities with academic and industry experts. Our enrollment demographics (113 survey respondents) reveal diverse learners with near gender-parity (45% female), only 47% university students, 26% >35 yrs old, and 48% based outside Canada (US:21% Europe:14%, Australia:9%). This highlights the widespread demand for neurotech training across the globe and at various career stages. In our upcoming Neurotech Ethics course, learners explore the basics of neuroscience, neurotech, and applied ethics before investigating case studies of applications of neurotech devices across different sectors (medical, consumer, organizational). To facilitate learners' transdisciplinary evaluation of benefits, concerns, and risks, we developed a unique neurotech impact assessment tool (TALES - Tech, Analysis, Legal, Ethics, Social).

C - DISORDERS OF THE NERVOUS SYSTEM

P1-C-209 - A comprehensive analysis of the effects of tranexamic acid in hemorrhagic stroke patients

Pinky Pinky [1]

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Abstract Background: Hemorrhagic stroke, characterized by the rupture of blood vessels within the brain, leads to significant morbidity and mortality. Tranexamic acid (TXA), an antifibrinolytic agent, has been explored for its potential to stabilize clots and reduce hemorrhage expansion. This review aims to critically assess the impact of TXA on clinical outcomes in patients with hemorrhagic stroke. Methods: A systematic literature search was conducted across major databases including PubMed, Cochrane Library, and Google Scholar, focusing on studies published in the past two decades. Keywords included "tranexamic acid," "hemorrhagic stroke,"

"intracerebral hemorrhage," and "clinical outcomes." Both randomized controlled trials (RCTs) and observational studies were included. The primary outcomes assessed were mortality, functional recovery, and adverse events. Results: The review identified and analyzed 15 RCTs and 10 observational studies. Overall, the administration of TXA was associated with a modest reduction in hematoma expansion and early mortality. However, the evidence on long-term functional outcomes remained inconclusive. Adverse events, particularly thromboembolic complications, were reported in a minority of cases but were not significantly higher than in control groups. Conclusions: TXA shows the potential to improve early survival rates in patients with hemorrhagic stroke by limiting hematoma growth. However, the benefits of long-term functional recovery and quality of life are less clear, necessitating further largescale, high-quality studies. Careful patient selection and monitoring for adverse events are crucial for optimizing outcomes with TXA therapy in hemorrhagic stroke.

P1-C-210 - High-Resolution Spatial Transcriptomics of the Maternal Brain in a Rodent Model of Postpartum Depression

Maria Eleni Fafouti [1], Shahrzad Ghazisaeidi [2], Hugo Hudson [2], Liisa Galea [2], Brian Kalish [3], Bharti Kukreja [4], Romina Garcia De Leon [1]

[1] University of Toronto, [2] Centre for Addiction and Mental Health, [3] Harvard Medical School, [4] The Hospital for Sick Children

Pregnancy and the postpartum period are characterized by remarkable neuroplasticity in the maternal brain, yet they also represent times of heightened vulnerability to mood disorders such as postpartum depression (PPD). To investigate the molecular and cellular underpinnings of this vulnerability, we employed two complementary spatial transcriptomics platforms—Slide-seq and Slide-tags—to chart gene expression changes in maternal rat brains. Our focus is exclusively on maternal tissues (without offspring data), enabling a detailed examination of how the peripartum brain remodels in PPD.Using a validated rodent model of PPD based on high levels of postpartum corticosterone administration (PD2), we have generated comprehensive spatial maps of limbic regions implicated in mood and cognitive regulation (e.g., hippocampus, prefrontal cortex) across multiple timepoints (nulliparous, gestational day 20, postpartum day 8 and 23). Both Slide-seq and Slide-tags provide genome-wide expression profiles with varying spatial resolutions, facilitating the identification of cell type-specific transcriptomic shifts alongside region-specific patterns of immune signaling and neuroplasticity. By integrating these cutting-edge technologies, our study offers a uniquely detailed perspective on the spatial organization of gene expression and protein changes in the maternal brain. These findings lay the groundwork for uncovering novel molecular targets for PPD treatment and illustrate the power of advanced spatial transcriptomics to illuminate mechanisms of peripartum neuroplasticity in mothers.

E - HOMEOSTATIC AND NEUROENDOCRINE SYSTEMS

P1-E-211 - Psilocybin induces sex- and context-specific recruitment of the stress axis

Sarah Gibson Cook [1], Mijail Rojas-Carvajal [1], Tamás Füzesi [2], Jaideep Bains [3]

[1] University of Calgary, [2] Hotchkiss Brain Institute, [3] University Health Network

Following decades of prohibition, psychedelic drugs have reemerged as promising therapeutics for stress-related disorders, including depression and post-traumatic stress disorder. Still, their impact on stress-related brain regions, such as the hypothalamic-pituitary-adrenal (HPA) axis, remains unclear. This work explores the acute effects of psilocybin on the primary regulators of the HPA axis: corticotropin-releasing hormone neurons in the paraventricular nucleus of the hypothalamus (CRHPVN). Here, using blood plasma measurements and single-fiber photometry, we demonstrate that psilocybin induces robust activation of the HPA axis via CRHPVN neurons, with more pronounced responses observed in female mice. Our findings emphasize the interplay between the serotonergic and stress systems and support the considerable influence of contextual factors on psychedelic experiences. This study provides some of the first real-time, in vivo evidence of neural activation following psilocybin administration and has significant implications for optimizing the therapeutic potential of psychedelic-assisted therapy.

P1-E-212 - Sex-specific modulation of microglia activation by clodronate and minocycline in response to inflammatory stress

Rachael Rowe [1], Mansi Purohit [2], Phuc Tran [2], Julia Schneider Krimberg [2], Quintin Whitman [2], Patrick McGowan [1]

[1] University of Toronto, Scarborough, [2] University of Toronto

Microglia are crucial mediators of neuroinflammation, yet sex differences in their responsiveness remain poorly understood. Here, we studied how chronic minocycline treatment, an anti-inflammatory, alters microglia reactivity to lipopolysaccharide (LPS) in both male and female Long Evans rats. Animals (n=12/condition) were given minocycline (45mg/kg/day, 30 days) prior to LPS administration (0.05mg/kg, 2 x 0.5 mg/kg, or 1mg/kg). After a 0.05 mg/kg dose of LPS, males showed significantly lower baseline microglia density compared to females (p = 0.005) and a substantial reduction in IBA1/CD68 colocalization, a marker of microglia activation (p < 0.0001). Minocycline significantly reduced IBA1/CD68 colocalization in males (p = 0.007) following a single 0.5 mg/kg dose of LPS. Although a reduction in colocalization was observed in females, it was not statistically significant and only occurred after the second 0.5 mg/kg dose (p = 0.372). In contrast, females required a 1 mg/kg dose of LPS to exhibit a similar male responsiveness (p < 0.05). To further explore sex differences in microglia expression, we utilized clodronate as a second mode of microglia knockdown in a maternal high fat diet (mHFD) model. Preliminary data showed a significant reduction in microglia density, activation and arborization (Sholl decay) of male mHFD animals compared to females, following clodronate administration. These findings highlight strong fundamental sex difference neuroimmune reactivity, with male rats exhibiting greater sensitivity to lower LPS doses, while females require higher dosage or alternative suppression methods. This research emphasizes distinct neuroimmune regulation and the necessity of sex-specific approaches to neuroinflammatory research.

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POSTER SESSION 2

A-DEVELOPMENT

P2-A-01 - To be or not to be: Understand how neurons choose their identity and what disrupts it in autism

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Abstract Nerve cells (or neurons) are the basic elements of the mammalian brain. To build complex networks for high-order functions, the brain needs a vast variety of neurons that differ in their morphology, biochemical composition, activity property, molecular features, etc. Having these unique and correct structural and functional identity is critical for individual neurons to know what they need to be and do to support brain functions. Despite the long recognition of the diversity of neuron types in the brain, we still know little about what mechanisms ensure the correct identities of different types of neurons. This is an important question because incorrect neuron identity can result in human diseases like autism spectrum disorder (ASD). My study is to tackle this fundamental question in neuroscience, focusing on a gene called Csde1 (cold shock domain containing E1) that, when mutated in humans, causes ASD. CSDE1 encodes an RNA binding protein that controls gene expression by dictating the life cycle and function of messenger RNA. Therefore, my research on Csde1 will give me a unique opportunity to not only explore the little-studied post-transcriptional mechanisms in neuron identity specification during brain development but also dissect the disease mechanisms of ASD. To this end, I employed transgenic mouse models in which the CSDE1 gene was deleted in a temporospatial-specific way to study the role of Csde1 in neurons as well as to mimic the disease condition in human ASD. I examined the effects of CSDE1 loss on neuron identity specification using immunohistochemistry and confocal microscopy. The results of my study will provide critical knowledge to advance our understanding of how different types of neurons adopt their correct identities and how it causes functional defects when this process goes wrong in ASD.

P2-A-02 - P2 purinergic receptor activation rectifies autism-associated brain endothelial dysfunction

Julie Ouellette [1], Sareen Warsi [2], Purva Khare [3], Shama Naz [4], Leya Aubert-Tandon [4], Chantal Pileggi [4], Sozerko Yandiev [2], Moises Freitas-Andrade [1], Cesar H. Comin [5], Mary-Ellen Harper [4], Devika S. Manickam [3], Armen Saghatelyan [4], Baptiste Lacoste [1]

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Brain development and function are highly reliant on adequate development and maintenance of vascular networks. As such, early cerebrovascular dysfunction can affect brain maturation by impacting trophic support and energy supply. Our recent published evidence in a 16p11.2 deletion mouse model of autism spectrum disorder (ASD) revealed neurovascular abnormalities associated with brain endothelial dysfunction postnatally, as well as a compensatory shift in adult brain metabolism. Yet, the endothelial alterations eliciting these changes remain unknown. To address this knowledge gap, we first isolated brain endothelial cells (ECs) from 14-day old

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16p11.2-deficient male mice and wild-type littermates to assess endothelial parameters in vitro. We discovered that 16p11.2 deletion-induced endothelial dysfunction is linked to a bioenergetic failure with reduced intracellular ATP in ECs. Intra- or extra-cellular ATP supplementation rescued the function of 16p11.2-deficient ECs in vitro via P2 purinergic receptor activation, specifically P2Y2 receptors. Moreover, we find that 16p11.2-deficient ECs display distinct Ca2+ responses following administration of extracellular ATP. Finally, engaging P2Y2 receptors with a selective agonist in vivo restored 16p11.2 deletion-associated mouse behaviors. Taken together, this study demonstrates that metabolic reprogramming of brain ECs via P2Y2 receptor activation may represent a new therapeutic avenue for ASD-associated cerebrovascular deficits.

P2-A-03 - Mechanisms regulating axon diameter and their impact on neuronal function in health and disease

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Axon diameter is a fundamental characteristic of neurons that varies over a 100-fold between neurons and can be dynamically regulated throughout life. This critical parameter influences conduction speeds, myelination, and axonal transport, directly impacting the precise timing of signal transmission and neuronal function. Yet despite its importance, what determines axon diameter and how it is regulated over time remain poorly understood. We aim to uncover the mechanisms regulating axon diameter and explore how changes in axon diameter impact neuronal function. To achieve this, we have established zebrafish as a model to study axons diameter, leveraging their transparency to employ super-resolution live-imaging techniques to follow individual neurons and their axons over time. We demonstrate that axon diameter can be regulated independently of axon outgrowth and cell body size and is not dependent on myelination in the central nervous system. Furthermore, through genetic screens we identify roles for ipo13, dync1h1, eif2b5 in axon diameter growth, with both dync1h1 and eif2b5 being genes linked to neurological disorders. This work establishes new molecular players in axon diameter regulation and provides a foundational framework for understanding how axon diameter influences neuronal function and disease pathology.

P2-A-04 - Comparison of the projection density to the lateral entorhinal cortex among prefrontal cortical subregions in mice

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The prefrontal cortex (PFC) is involved in a diverse array of cognitive and affective functions, with each function potentially being mediated by distinct subregions. In particular, the role of the PFC in learning and memory relies on close interactions with the hippocampus and the lateral entorhinal cortex (LEC). However, the anatomical organization supporting this interaction is incompletely understood. We compared the density of projections to the LEC originating from four subregions of the PFC: the orbitofrontal cortex (OFC), prelimbic cortex (PL), infralimbic cortex (IL), and anterior cingulate cortex (ACC) in mice. To visualize PFC cells projecting to the LEC, retrograde viral vectors carrying fluorophores were infused into the LEC. Between the examined PFC subregions, the highest density of labeled cells was detected in the OFC. Furthermore, LEC-projecting cells were more prevalent in superficial than deep layers in all PFC

subregions. In parallel, variations in viral spread within the LEC, ranging from the superficial layers only to both superficial and deep layers, had a greater impact on labeling density in the PL and ACC compared to the OFC and IL. These findings reveal that the PFC subregions exhibit distinct patterns in the intensity and targeting of outputs to the LEC. These anatomical differences may provide a foundation for the varying involvement of PFC subregions across different stages of memory processes (encoding, consolidation, retrieval) and the content of memory (aversive, appetitive).

P2-A-05 - From cloud nine to cognitive decline: Sex- and concentration-dependent cognitive and neural effects of adolescent nicotine e-cigarette exposure in rats

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The shift from tobacco smoking to vaping raises concerns about its effects on the developing adolescent brain. Preclinical and clinical studies show a strong correlation between nicotine dependence and cognitive deficits in attention, working memory, and impulsivity, linked to changes in the hippocampus (Hipp) and prefrontal cortex (PFC) leading to long-term cognitive deficits even after adolescent smoking cessation. However, causal mechanisms remain unclear due to the lack of pharmacologically clinically representative models. This study uses a reverse-translational approach to assess the cognitive effects of adolescent nicotine vapor exposure (ANVE) at behavioral, and electrophysiological levels in the medial prefrontal cortex (mPFC), dorsal hippocampus (DH), and ventral hippocampus (VH).Methods: Adolescent male and female Sprague-Dawley rats (PND 35-44) were exposed to 0, 20, or 40mg/mL nicotine e-cigarette vapor three times daily for 10 days using the OpenVape system. In adulthood (PND>75), rats underwent spontaneous alternation (SA) to assess spatial memory, novel object recognition (NOR) for short-term memory, and prepulse inhibition (PPI) to assess sensorimotor gating. In vivo electrophysiology measured local field potentials (LFPs) in the mPFC, DH, and VH.Results: Rats exposed to 20 or 40mg/mL nicotine showed hyperlocomotion during adolescence, but not adulthood. Adult rats exposed to 40mg/mL nicotine exhibited decreased SA scores, with males primarily showing deficits in NOR while females exhibited deficits in PPI and startle habituation. Electrophysiological data revealed altered LFPs in the ventral subiculum of male rats exposed to 20mg/mL nicotine. 40mg/mL nicotine electrophysiological analyses are ongoing. These divergent findings highlight the long-term sex- and nicotine-concentration-specific risks of adolescent nicotine vapor exposure.

P2-A-06 - Region-dependent inputs differentially mediate early life stress induced changes in prefrontal glutamate release in pre-adolescent male rats

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Exposure to early life stress (ELS) can exert long-lasting impacts on emotional regulation. The corticolimbic system including projections from the basolateral amygdala (BLA) and ventral hippocampus (vHIP) to the medial prefrontal cortex (mPFC) plays a key role in fear learning. Previously, we found that ELS suppressed fear-induced glutamate release in the prelimbic (PL)

mPFC of pre-adolescent males but not females. Here, we determined whether reduced glutamatergic inputs and/or elevated inhibitory tone might contribute to this diminished mPFC glutamate response. We used a limited bedding paradigm (LB) between postnatal days (PND)1-10 to induce ELS in the offspring. We assessed presynaptic glutamate transmission in the layer II/III and layer V of PL mPFC slices, targeting the projections from BLA and vHIP, respectively. ELS increased presynaptic glutamate release probability in layer II/III but decreased it in layer V of the PL mPFC. Retrograde tracing (CTb) revealed that the bilaminar distributions of the BLA-PL mPFC and vHIP-PL mPFC projections were also disrupted by ELS. Furthermore, immunostaining of Fos, parvalbumin (PV), and somatostatin (SST) in the PL mPFC after fear conditioning showed that the activation of PV, but not SST interneurons, was elevated by ELS in fear-exposed pre-adolescent males. Our results suggest that ELS modifies the excitation/inhibition balance in the PL mPFC of pre-adolescent males, inducing layer-specific changes in glutamatergic transmission, probably mediated by altered long-range afferents and local interneuron activity.

P2-A-07 - Establishing primary fibroblast cell cultures from Ursus maritimus to model neural development in vitro

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Polar bears (PB), known for their high intelligence and large brain-to-body ratios, represent a captivating subject in evolutionary biology research. Furthermore, PBs are particularly susceptible to the effects of climate change and environmental pollution in their rapidly warming sea ice habitats. This research project focuses on bridging the understanding of neural development in PBs to the broader effects of environmental toxins on these processes. To investigate these effects, we have successfully generated primary fibroblast cell cultures from PBs. We confirmed their genetic and structural integrity via DNA barcoding and karyotypic analysis, and they have been sub-cultured for over 20 passages.With our cell cultures, we aim to develop a novel PB-specific induced pluripotent stem cell (iPSC) model. These cells have the potential to differentiate into all three germ layers, allowing for the recapitulation of critical developmental steps and the study of cellular and molecular changes in culture. Additionally, iPSCs enable the replication of organ development, such as in cerebral organoids. These organoids will provide a platform for studying the impacts of environmental pollutants on PB growth and health, contributing to advancing conservation efforts for these animals. This study is invaluable for phylogenetics, Arctic conservation, and evolutionary biology. Importantly, by pursuing a One Health approach, we aim to foster collaboration across disciplines to identify, prevent, and manage health risks through the perspective of humans, animals, and their environments.

P2-A-08 - Astroglial VEGF regulates postnatal cerebrovascular development in mice

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Astrocytes interact structurally and functionally with the brain vasculature and are critical players in brain angiogenesis during development. While it is known that astrocytes produce and

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release vascular endothelial growth factor (VEGF) during postnatal development, whether astrocyte-derived VEGFA controls the growth and maturation of the brain vasculature remains to be elucidated. To identify mechanisms underlying gliovascular growth during postnatal brain development, we assessed gene expression changes in astrocytes between P0 and P14. We found that Vegfa expression by astrocytes increases after birth and peaks at P7. This was confirmed by fluorescent in situ hybridization for Vegfa mRNA in Aldh1L1-eGFP mice. We then hypothesized that VEGF production by astrocytes after birth is critical for gliovascular maturation. To test this, we performed conditional, astrocyte-specific ablation of Vegfa starting at P0, in Aldh1l1-CreERT2;Vegf flox/flox mice (or "Vegf DAstro"). Preliminary data showed that Vegf DAstro mice exhibited a significant reduction in VEGF protein levels and reduced levels of blood-brain barrier markers such as beta-catenin and occludin at P7, compared to control littermates. Vegf DAstro mutant mice exhibited a 2-fold reduction in vascular area in the cerebral cortex as well as reduced pericyte area coverage. Moreover, astroglial gap junction protein Connexin43 displayed aberrant cortical distribution in Vegf DAstromice. This study suggests that astrocyte-derived VEGF is critical for postnatal vascular development.

P2-A-09 - Ehmt2-dependent gene regulation during zebrafish neurodevelopment

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Epigenetic silencing coordinates the downregulation of genes expressed in stem and progenitor cells to facilitate cell cycle exit and differentiation. Ehmt2 is thought to have a key role in initiating early repressive modifications, where it deposits a dimethylation mark at Histone 3 Lysine 9 (H3K9me2) to recruit other repressive complexes and promote gene silencing. However, studies of the developing nervous system indicate that Ehmt2 possesses a multifaceted role in neural tissues including transcriptional activation and exon splicing, to encourage proper cell differentiation and survival. Our research aims to understand how Ehmt2 regulates the genome wide changes that underlie the transition from progenitor cell to terminally differentiated cell during neurodevelopment. We generated a zebrafish Ehmt2 loss of function mutant which exhibited delayed growth of neural tissues. Immunostaining and live imaging in the retina revealed this growth deficit was due to prolonged progenitor cell cycling. Interestingly, RNA-Seq revealed that Ehmt2 progenitor gene expression was minimally altered in our mutant, and that Ehmt2 loss of function was correlated with downregulation of lineage specification genes. These findings suggest a function for Ehmt2 in activation or enhancement of lineage specification gene expression during neurodevelopment. Understanding the role of Ehmt2 in progenitor differentiation will provide insight into the mechanisms of neural development and how defects in these mechanisms may lead to neurodevelopmental disorders. Research supported by a grant from CIHR.

P2-A-10 - Standardization of the limited bedding and nesting materials model for early life stress

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Experiencing early life stress (ELS) can alter the development of cognitive and socioemotional behaviours and the neurobiology responsible for regulating these behaviours. To assess the

mechanisms driving these differences in neurobiological structure and function, the use of preclinical rodent models enables the careful manipulation of stress exposure during development. Evidence suggests that stressors ranging in type and severity influence neurodevelopment, though the underlying mechanisms likely differ across ELS manipulations. The limited bedding and nesting materials (LBN) model, which induces a stressful environment for dams and alters maternal care by restricting access to bedding and nesting materials, has been used in various studies with considerable variability in low-resource apparatus designs. Here, we share open-source resources to build LBN cages, which induce stress in dams as detected by elevated fecal corticosterone, and sufficiently alters maternal care patterns, producing more fragmented bouts of maternal care despite similar time spent in nest as standard housing controls. This LBN model also appears to facilitate a significant reduction in blood serum corticosterone in pups at postnatal day 10, and lower body weight when reared under LBN conditions. With open-source equipment and carefully documented experimental conditions, our standardization of the LBN model will enhance both the accessibility and reproducibility of research using this type of ELS.

P2-A-11 - Development and neural stem cell dynamics of the zebrafish rostral migratory stream

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The rostral migratory stream (RMS) is characterized by the continuous migration of neuroblasts from neural stem cells (NSCs) of the forebrain to the olfactory bulbs (OB), where they differentiate into neurons. First described in mammals, the RMS has been found to be conserved in the highly neurogenic zebrafish. Currently, we lack knowledge on the formation and dynamics of the RMS as a distinct migratory route from larval to adult stages, and how it contributes lifelong to the organization of OB neurons. To characterize the development and maturation of the zebrafish RMS over ontogeny, I studied cellular and migratory features in the telencephalic subpallium and OB across larval, juvenile, adult, and senescent life stages. Stemness markers, electron microscopy and proliferation (PCNA, EdU) experiments revealed a progressive and life-long shift in NSC phenotype and proliferative activity at the subpallial origin of the RMS. Labeling for the migration marker PSA-NCAM in our Tg(Fli1a:GFP) endothelial line showed that vasculature needs to develop until adulthood before serving as a support for RMS-like migration. Additional pulse-chase experiments highlighted that, by adulthood, progenitors shift from lateral migration into the adjacent parenchyma to migrating towards the OB where they differentiate into interneurons. Despite the establishment of the RMS in adults, neurogenesis in the OB decreased significantly after early development. Findings from this study offer novel insight in understanding the NSC mechanisms and structures supporting neurogenic migration along the RMS.

P2-A-12 - Store-operated calcium entry triggers the activation of cAMP response element binding protein through the calcium/calmodulin-dependent protein kinase pathway in neural progenitor cells

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In neural progenitor cells (NPCs), intracellular calcium (Ca2+) is primarily regulated by store-operated Ca2+ entry (SOCE)—a mechanism that promotes Ca2+ influx through ORAI channels when intracellular Ca2+ stores are empty. In addition to the filling of Ca2+ stores, SOCE has been implicated as having downstream effects on NPC proliferation and differentiation. Therefore, our lab sought to identify the cellular signalling pathways that connect SOCE to NPC function. We first found that the induction of SOCE in human induced pluripotent stem cell (iPSC)-derived NPCs altered gene expression, especially those related to development. We then probed whether SOCE activated cAMP Response Element-Binding Protein (CREB), a calcium (Ca2+)-sensitive transcription factor that regulates neurodevelopment. Indeed, our efforts suggest that SOCE induction results in the phosphorylation of CREB at serine 133, thereby activating it. Next, we established the signaling pathway responsible for this activation of CREB using inhibitors of the ERK1/2, Akt, and CaMK pathways. Unsuspectedly, only pharmacological inhibition of the CaMK pathway significantly blocked the activation of CREB, suggesting that this is the main pathway that drives CREB activation following SOCE induction in NPCs. To further confirm this, we are creating CaMK knockouts using a CRISPR-Cas system to probe CREB activation following SOCE. Taken together, these findings provide a better understanding of SOCE-activated signaling during the initial stages of neuron growth and differentiation.

P2-A-13 - Impact of cannabis smoke exposure in utero on the development of enteric glia

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Cannabis legalization in Canada highlights the relevance of investigating the impacts of in utero cannabis exposure on postnatal neurodevelopment. It is increasingly recognized that the endocannabinoid system (ECS) is expressed in enteric neurons and glia. We tested the hypotheses that cannabinoid receptor 1 (CB-1) is expressed by enteric glia during development and that enteric glia can be affected by in utero exposure to cannabis. Timed-pregnant CD-1 mice were sacrificed at embryonic day 18 (E18) and pups were sacrificed at postnatal days P1, P7 and P28. We developed a "real world" smoke inhalation model where pregnant dams were exposed daily to a delta-9-tetrahydrocannabinol (THC)-dominant strain of cannabis from E6 to E18. Control mice were placed in a restraining cage and exposed to room air for the same duration. Fetal (E18; stomach to distal colon) and pup (P21; small intestine and colon) tissues were collected and processed for RNA extraction (n=per litter; 5-8 cannabis-exposed and control). CB-1 expression in S100B positive cells was detected using immunohistochemistry in small intestine from E18 until P28. Differential expression of key markers of glial development was determined with NanoString and nSolver Analysis. At E18, we found an increase in expression of both Sox10 (p<0.007) and S100b (p<0.0001) in cannabis-exposed intestine. At P21, the expression of S100b remained elevated in cannabis-exposed intestine (p<0.05), but not the expression of Sox10. Together, these results suggest that exposure to cannabis in utero can impact enteric glia development.

P2-A-14 - Investigating the role of Baf53b in mouse neuronal gene expression and autism behaviours

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Autism spectrum disorder (ASD) is a prevalent neurodevelopmental disorder. It is well established that ASD is heritable, but 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.

P2-A-15 - Characterization of transcriptional blood-brain barrier postnatal development

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The blood-brain barrier (BBB) is a specialized structure, formed by endothelial cells, pericytes and astrocytes, crucial for CNS homeostasis. It regulates the exchange of substances between the bloodstream and the brain, provides trophic support and ensures neural protection. Brain vascular maturation takes place days to weeks after birth. Some of the changes imply modification in morphology, cell proliferation, and functionality. For instance, in mice, it was shown that vascular density increases between birth and weaning[CM1] , pericyte maturation and its endothelial coverage progresses during the first postnatal weeks, and astrogenesis takes place during the second postnatal week. Despite the identification of these brain development sensitive periods, it is yet to be determined when the BBB acquires adult-like functionality and if the BBB is more vulnerable to environmental challenges during sensitive development periods. Using brain punches collected from female and male C57BL/6 mice every 7-days from postnatal day (PD) 7 to PD63, we evaluated expression levels of genes related to endothelial cell, astrocyte and pericyte functions. As expected, most of the genes had a peak of expression by PD21 compared to PD7, however, during adolescence several patterns[CM2] of expression emerged indicating that the postnatal development of the barrier is dynamic and time sensitive. These findings suggest that the BBB undergoes significant molecular and cellular changes through life, which may have implications for various neurological and mental health disorders.

P2-A-16 - Maternal high fat diet and neonatal lipopolysaccharide exposure alters ultrasonic vocalization patterns in neonatal rats

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Rodent pups emit ultrasonic vocalizations (USVs) to elicit maternal care and improve survival. Inflammatory stressors, including maternal high-fat diet (mHFD) and neonatal lipopolysaccharide (nLPS) exposure, have been shown to affect neonatal neurodevelopment. While the production of USVs is widely used to study neurodevelopment, limited research has examined the biological significance of their sonographic and syntax characteristics. DeepSqueak, a deep learning system, enables automated USV detection and classification. This study was designed to validate DeepSqueak's accuracy in unsupervised USV classification and explore temporal and syntax changes in neonatal USVs after mHFD and nLPS exposure. Adult Long Evans rats were fed a high-fat or control diet for three weeks before mating and throughout gestation and lactation. Offspring received 0.05mg/kg of intraperitoneal nLPS or saline injections (n=5–6 per condition) on postnatal days (PND) 3 and 5. On PND 7, USVs were recorded after maternal separation. DeepSqueak was similar to manual USV classification for total and mean duration, mean frequency and call type percentages, demonstrating its accuracy for standard call features. Additional temporal analyses enabled by DeepSqueak revealed that mHFD and nLPS exposures significantly impacted sequence and bout parameters. These findings provide insights into the significance of USV structure in understanding how early-life inflammatory stressors affect social and emotional communication, potentially influencing pups' ability to solicit maternal care.

P2-A-17 - Maternal vitamin d deficiency impacts body development, locomotor activity, and ultrasonic vocalizations in female and male Wistar rat offspring

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Vitamin D is involved in many aspects of development and brain health. It is produced in response to sunlight, thus in northern communities where sun levels vary substantially, vitamin D deficiency (VDD) is common. Further, recent changes in northern dietary patterns (e.g., lower consumption of salmon) may be contributing to insufficiency. In this study, we sought to better understand the neurological consequences of VDD. Naïve adult female Wistar rats were fed either a control (n=6) or VDD-deficient (n=7) diet for six weeks before breeding, and until weaning. Female and male offspring were assessed on weight, righting reflex, negative geotaxis, incisor eruption, grip strength, blood glucose, ultrasonic vocalizations (USVs), and performance during an open field test (OFT). At PND22/23, offspring were sacrificed and cortical, hippocampal, and hypothalamic samples collected. VDD offspring were significantly smaller than control-fed offspring, however no differences in incisor eruption, righting reflexes, negative geotaxis, grip strength, or blood glucose were observed. At PND16, VDD rats produced more USVs relative to control. During the OFT, females were more active than males, and VDD offspring were more active than offspring fed a control diet. Brain and tissue analysis is in progress. Together these data demonstrate body size differences and behavioural changes in VDD offspring compared to control diet animals. Further, these experiments provide the foundation for additional model work assessing changes related to diet and development in northern and circumpolar environments.

P2-A-18 - Msi1a and msi1b function redundantly to regulate Müller glial cell reprogramming during retinal regeneration

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Müller glial (MG) cells in zebrafish exhibit a remarkable capacity for retinal regeneration. In response to acute injury, MG cells undergo a genomic reprogramming event that enables them to produce new cells for tissue repair. This reprogramming involves the activation of a multipotency gene program and the suppression of genes that maintain quiescence. To initiate and regulate these changes, MG cells require dynamic control at both transcriptional and translational levels. While many transcriptional regulators of this process have been identified, relatively few translational regulators have been characterized. The Musashi (msi) family of RNA-binding proteins are well-known translational regulators involved in stem cell maintenance, but their role in MG-mediated regeneration remains unexplored. In this study, we characterized the expression of msi family members during photoreceptor lesion induced retinal regeneration and identified msi1b as specifically upregulated following retinal injury. To investigate its function, we generated an msi1b CRISPR mutant. Surprisingly, the loss of msi1b alone did not impair MG reprogramming or proliferation. However, double loss of function mutants for msi1a and msi1b revealed that these paralogs function redundantly to regulate MG cell reprogramming and cell cycle re-entry in response to a lesion. Our findings establish a critical, cooperative role for Musashi proteins in facilitating MG reprogramming during retinal regeneration. Supported by CIHR.

P2-A-19 - Investigating the effects of hypoxia on transposable element expression during development of brain organoid models

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Transposable elements (TEs) are sequences that translocate within the genome. Since TEs can cause DNA damage, their expression is tightly regulated via epigenetic mechanisms such as DNA methylation and RNA silencing. Interestingly, evidence suggests that TE expression is increased during neurogenesis in vitro and the intact adult hippocampus. Additionally, studies in plants and drosophila have shown that TE expression is altered due to environmental stresses. Accumulating evidence shows that human endogenous retroviruses (HERVs) are retrotransposons that are dysregulated during neurodegeneration and due to stressors, such as reactive oxygen species. However, little is known about how hypoxia affects HERV expression during neurogenesis. Consequently, we investigated how hypoxia alters HERV expression during neurogenesis under normoxic and hypoxic conditions. Here, we present HERV expression measured via RNA sequencing and qRT-PCR in cultured cerebral organoids and neurons. We found that HERV expression initially increases as the neuron begins to differentiate and decreases in more mature neurons. We also observed alterations in this expression pattern upon exposure to hypoxic conditions. Together, these results suggest that the epigenetic regulation of these HERVs is altered due to hypoxia. Widespread changes in the epigenetic regulation of TEs have been implicated in neurodevelopmental disorders such as schizophrenia and autism. Thus, deciphering the HERV expression can provide valuable insights into the pathogenesis of these conditions.

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P2-A-20 - Developmental recruitment of medial prefrontal cortex to amygdala pathway by fear learning

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The rodent medial prefrontal cortex (PFC) undergoes substantial anatomical and synaptic changes in early development. The prelimbic cortex (PL) subregion of the PFC and its connections to the basolateral amygdala (BLA) are particularly important for driving fear expression and retrieval in adult rodents. However, fear learning is developmentally regulated, and some evidence suggests that younger rodents do not require the PFC for fear processing. Since there is limited research on the role of the immature PFC in fear processing, we investigated the timing of PL and PL-BLA pathway recruitment for fear conditioning. We first used chemogenetics to inhibit the PL and PL-BLA pathway in infancy (postnatal day (P) 15) and adolescence (P30) during fear training and tested for fear retrieval 24h later. We found that while P30 mice showed impaired retrieval when the PL and PL-BLA pathway were inhibited, P15 mice were unaffected by this manipulation, suggesting that the PL-BLA pathway is not required for auditory fear during infancy. Additionally, using a combination of optogenetics and whole-cell patch clamp electrophysiology, we found that while fear conditioning in P30 mice led to an increase in AMPA:NMDA ratios in PL-BLA synapses, P15 mice showed an absence of fear-induced synaptic plasticity. Lastly, we found that early life stress can accelerate PFC recruitment for fear processing as early as P15. Overall, our results indicate that onset of the PL and PL-BLA pathway recruitment to fear encoding occurs between infancy and adolescence, and stress can alter this trajectory.

P2-A-21 - Synaptopodin: A novel therapeutic target for treatment of ASD

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Autism Spectrum Disorders (ASD) is a heterogeneous group of neurodevelopmental disorders accounting for 1% of the world population and currently has no cure. Evidence shows that ASD-related genes play roles in synaptic function, suggesting that targeting synaptic proteins could potentially be a therapy for ASD. With over 800 genes associated with ASD, developing therapeutics for each is impossible. Therefore, identifying shared molecules in ASD is a promising approach. We propose an actin associated protein Synaptopodin (SP) as a possible novel therapeutic target for treatment of ASD. SP is found in excitatory synapses in limbic brain regions. Our lab has previously reported that SP is required for Hebbian plasticity as well as learning and memory. We recently found that there are changes in the hippocampal SP levels in the Fragile X Syndrome autistic model, Fmr1-/y mice, and Tuberous Sclerosis Complex autistic model, Tsc2+/- mice. Hence, we hypothesize that changes in SP levels during development lead to aberrant synaptic connections and consequently result in autistic traits. To address this hypothesis, we first investigated if the loss of SP is sufficient to induce ASD behaviors. By using mouse behavioral studies, my results indicate that by knocking out SP, these mice display significant autistic traits including repetitive behaviors, higher anxiety and social interaction deficits. Taken all results together, we anticipate that SP could a shared molecule in ASD's pathology and thus, opens up a novel avenue for potential therapeutic target for multiple forms of autism.

P2-A-22 - Assessment of the role of adaptor protein SHcD in murine adult neurogenesis

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Adult neurogenesis modulates existing neuronal circuits that influence cognitive functions by preserving the neural stem cell (NSC) pool in specific brain regions. Defects in neurogenic functions including olfaction and cognition are common symptoms of neurodegenerative disorders, thus understanding the molecular pathways regulating neurogenesis is crucial. Our lab has discovered that the adaptor protein ShcD is enriched in the brain – specifically in the olfactory bulbs (OB) and the dentate gyrus (DG) of the hippocampus, which are both adult neurogenic niches. ShcD can associate with multiple signaling pathways that influence NSC growth, but the role of ShcD in neurogenesis is not established. Previous work with ShcD knockout (KO) mice has shown that loss of ShcD results in reduced cell volume in the OB and DG, with altered olfactory function. This evidence suggests an important role for ShcD signaling in adult neurogenesis. To assess the role of ShcD in NSC growth, neurogenic niches of 6-8 week old ShcD wildtype (WT) and KO mice were dissected and cultured to generate a model of neurospheres in vitro, and the number and size were measured. Neurospheres were dissociated and seeded as single cells to terminally differentiate for four different time points to observe neuronal maturation. In parallel, the proliferation capacity of NSC in vivo was measured by BrdU injection and the number of migrating neuroblasts were quantified with doublecortin. As altered neurogenesis is connected to multiple neurodegenerative disorders, this work may establish ShcD as a potential target.

P2-A-23 - Adult hippocampal neurogenesis in response to early-life stressors: Potential benefits of environmental enrichment

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Introduction: Alcohol consumption during pregnancy causes lasting neurodevelopmental effects, known as Fetal Alcohol Spectrum Disorders (FASD). Children with FASD often experience co-occurring early-life stress, which exacerbates the risk of cognitive and mood impairments. Given the hippocampus's key role in these processes, this study aimed to investigate the impact of perinatal alcohol exposure and maternal separation (MS) on adult hippocampal neurogenesis (AHN) - a process highly sensitive to stressors - and to explore whether Environmental Enrichment (EE) during early adolescence can mitigate potential changes. Methods: Wistar rats were divided into eight experimental groups: control, MS, ethanol (EtOH), and MS + EtOH. Post-weaning, animals were housed in either standard or enriched environment, totaling eight groups. Neurogenesis was assessed via immunohistochemical staining for the immature neuron marker DCX. Data were analyzed using a three-way ANOVA. Ethics Committee approval number: 6980201116. Results: At 45 days of age, intense DCX staining was observed, with a strong trend towards an environmental effect ($p=0.056$). No significant differences were found regarding MS ($p=0.510$) and EtOH exposure ($p=0.527$). Conclusion: While no significant changes in AHN were identified, the observed trend highlights the potential of environmental enrichment to influence neurogenesis. Future studies are necessary to unravel the complex interactions between alcohol exposure, early-life stress, and hippocampal neuroplasticity.

P2-A-24 - Npat regulates nucleosome composition and retinal progenitor cell behaviour in the postembryonic ciliary marginal zone

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The ciliary marginal zone (CMZ) of the zebrafish retina is a stem cell niche mediating post-embryonic retinal neurogenesis. Its retinal progenitor cells (RPCs) balance proliferation to maintain their population with differentiation to produce neurons and glia. At every division, the new genome is packaged into chromatin. The basic unit of chromatin is the nucleosome, a structure comprising 1.7 turns of DNA around an octamer of histone proteins. Histones are encoded by clusters of replication dependent (RD) histone genes found in histone locus bodies (HLBs), subnuclear condensates seeded by NPAT and FLASH proteins. NPAT stimulates RD histone gene transcription during S phase, enabling the cell to quickly produce RD histone proteins for nucleosome assembly. rys is a mutant of npat with a disproportionately large CMZ in an undersized retina and RPC chromatin with altered electron density, raising the possibility of abnormal chromatin structure. We used EdU to mark rys RPCs and found them to be abnormal both in proliferation, which occurs on a delayed schedule, and differentiation, which fails entirely. We next assessed nucleosome structure in rys. By MNase-seq, we found unique nucleosome positions with different DNA sequence preferences and using 3'RACE-seq, we found changes in the relative abundances of transcripts encoded by many RD histone genes of the H2A and H2B classes. Together these findings suggest that NPAT has a role in regulating the pool of RD histone isoforms that determine nucleosome structure in RPCs and RPC behaviour. Supported by CIHR.

P2-A-25 - Modeling group 4 medulloblastomas (G4MBS) with mouse cerebellar organoids

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Organoids are 3-D, in vitro modeling systems that have been shown to recapitulate the genetic and cellular characteristics of a target organ. Since the early 2000s, various groups have successfully cultured pluripotent stem cells into cerebellar organoids. With their high-throughput nature and ease of manipulation, cerebellar organoids are uniquely suited as a modeling system for medulloblastomas, a cancer of the developing cerebellum. This work focuses on the subgroup called Group 4 Medulloblastomas (G4MBs), where much of their underlying pathology is still poorly understood. This project aims to 1) generate cerebellar organoids that recapitulate the genetic expression and cellular composition of an early cerebellum and 2) to overexpress hypothesized cancer drivers, such as Prdm6, Mycn, and Otx2, to model medulloblastoma development. Our results show the presence of early genetic markers of cerebellar development in the organoids. We also observe the presence of rosettes within these organoids, which are structures that mimic the early development of the neural tube. Additionally, we identify the presence of Tbr2+/Calb2+ dual-positive cells in the organoids, which indicate the presence of unipolar brush cells, the hypothesized cells of origin of G4MBs. Future experiments will continue to utilize these cerebellar organoids as tools for disease modeling and genetic screening.

P2-A-26 - The impact of chronic late adolescent stress on adult sociability in male and female mice

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Adolescents are more sensitive to stress than adults and experiencing stress during this developmental period can have lasting effects on behaviour, including increased depression-like and anxiety-like behaviours. However, its long-term impact on sociability has not been well studied. The present research aims to determine the effects of chronic late adolescent stress on adult peer-to-peer sociability using male and female, stressed and control mice. Male and female mice (n=48) were evenly split into stress and control groups. From post-natal day (PND) 48-60, we exposed stressed mice to a chronic predator stress paradigm while controls were left undisturbed in their home cages. Once mice reached adulthood (PND 96), we performed behavioural testing aimed at quantifying social dominance and social interest in same-sex and opposite-sex peers. Following behavioural testing, we sacrificed the mice and analysed brain tissues using qPCR analysis. Results suggest that chronic adolescent stress may lead to increased interest in same sex peers. These findings could inform research on lasting effects of human childhood and adolescent chronic stressors such as unsafe home environments and mental health struggles.

P2-A-27 - Long-term neuronal and molecular maladaptation in the mesocorticolimbic system produced by adolescent edible delta-9-tetrahydrocannabinol consumption

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Adolescent cannabis use may increase the risk of psychiatric illness later in life, yet rates of use among this group remain high. Exposure to Δ9-tetrahydrocannabinol (THC) during adolescence may disrupt mesocorticolimbic circuits (i.e. the prefrontal cortex [PFC], ventral tegmental area [VTA], and nucleus accumbens [NAc]), potentially leading to long-term consequences. Previously we found in rats that adolescent consumption of THC edibles produces sex-dependent effects on adult anxiety and cognitive behaviour. Therefore, we investigated the impact of adolescent THC consumption on the mesocorticolimbic circuits to better understand the underlying mechanisms. Adolescent male and female Sprague Dawley rats were given Nutella® edibles containing THC. In adulthood, in vivo electrophysiology revealed hyperactivity in the PFC and altered VTA dopamine (DA) firing in males and females. Matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) and Western blotting were used to examine the impact on the metabolism of glutamate, GABA and monoamine neurotransmitters in the PFC, NAc, and VTA. Preliminary MALDI-IMS analysis showed an altered distribution of glutamate, GABA, and DA in the PFC and NAc of both sexes, while norepinephrine was exclusively disrupted in females. Further MALDI-IMS and protein analysis are underway to explore the metabolism of these neurotransmitters in the PFC, VTA, and NAc. Alterations in the GABA, Glut, and monoaminergic systems within the mesocorticolimbic system may mediate the behavioural consequences of adolescent THC consumption.

P2-A-28 - Intestinal microbiome-modulation of microglia in the developing brain

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As resident macrophages of the central nervous system, microglia protect against pathogens and regulate brain development. Microglial morphology changes based on the brain's immune status. In homeostasis, microglia have long branches to survey the brain; in response to inflammatory stimuli, these cells adopt a spherical shape to promote pro-inflammatory cytokine release. Studies in mice lacking a microbiota (germ-free, GF) suggest that gut microbial metabolites cross the blood-brain barrier and bind to microglial receptors to influence the brain's immune status. However, it has yet to be determined how the microbiome impacts microglial morphology and function during development. In this study, we analyzed the cellular morphology of microglia from mice with or lacking a microbiome to assess the influence of intestinal microbes on microglia function during brain development. Experiments were conducted in male and female conventional (specific-pathogen free) and GF C57Bl/6J mouse pups 7-, 14-, and 21-days post-birth, key time points for microglial proliferation and maturation. Immunohistochemistry was performed to stain IBA1+-microglia and morphology was quantified with MicrogliaMorphologyR, an open-source ImageJ and R analysis tool. Morphological differences were observed across mice of different microbiome, ages, and sexes. These data demonstrate that the intestinal microbiome modulates microglial morphology in early-life, highlighting a potential mechanism for microbiome-mediated microglial dysregulation in neurodevelopmental disorders.

B - NEURAL EXCITABILITY, SYNAPSES, AND GLIA: CELLULAR MECHANISMS

P2-B-29 - Investigating how TREM2 regulate the response of macrophages and microglia to oxidized phosphatidylcholine in multiple sclerosis

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Multiple sclerosis (MS) is a chronic neuroinflammatory disease characterized by demyelination and neurodegeneration in the central nervous system (CNS). During MS, the oxidation of myelin associated lipids can lead to the production of cytotoxic molecules such as oxidized phosphatidylcholines (OxPC). We recently found that OxPC accumulate in MS lesions and that their deposition in the spinal cord white matter of mice promotes demyelination and neurodegeneration. Notably, triggering receptor expressed on myeloid cells 2 (TREM2) expressing microglia/macrophages are the primary immune cells that respond to OxPC mediated neuroinflammation and neurodegeneration. Since we previously found that TREM2 deficiency exacerbates OxPC-mediated neurodegeneration, TREM2 expression by microglia/macrophages may be critical for OxPC mitigation. Thus, we aim to assess how TREM2 mediated signaling modulates macrophage/microglia response to OxPC remains unknown. Specifically, I will treat microglia and bone marrow derived macrophages derived from TREM2+/+ and TREM2-/- mice with OxPC and analyze changes in cellular behavior and morphology through live cell imaging, quantitative fluorescent microscopy, and transcriptomic analysis. By understanding the role of TREM2 in the response of microglia/macrophage to OxPC, we will gain new knowledge on the therapeutic potential of targeting TREM2 in MS.

P2-B-30 - Elucidating the unexpected memory suppression role of a vesicle trafficking protein, sec22

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Decades of research have identified numerous genes required for normal memory processes. However, recent studies show that genes acting to suppress memory also exist. Through a small-scale memory screen conducted using Drosophila melanogaster, sec22 was identified as a novel memory suppressor. sec22 plays a pivotal role in the budding and fusion of vesicles transported between the endoplasmic reticulum and Golgi apparatus. Although the role of sec22 in vesicle transport is well-known, its role in learning and memory is still unclear. Using the aversive olfactory conditioning assay, we found that knockdown (KD) of sec22 in all neurons, dopamine neurons and mushroom body neurons (MBNs), significantly improved memory due to a specific enhancement of learning. To investigate sec22's mechanism of action, we assessed whether it affected MBN neurophysiology using GRABAch and GCaMP6 in vivo imaging and found that sec22 KD in MBNs increased acetylcholine release but decreased intracellular calcium (Ca2+) in response to odors. This demonstrates that the two properties of neurophysiology can be decoupled as neurotransmitter release can be increased while intracellular Ca2+ levels are decreased. Consistent with this, immunostaining and confocal imaging showed that sec22 KD increased synaptic protein levels in MBNs without altering its presynaptic sites, suggesting an increase in synaptic vesicle numbers. Characterizing sec22 as a novel memory suppressor could reveal key insights into cellular mechanisms of memory and may help reveal therapeutic targets for memory-associated disorders.

P2-B-31 - Alternative splicing of NMDA receptor subunit GluN1 controls the kinetics of blockade by ketamine

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NMDA receptors are heterotetrameric cation channels composed of two GluN1 subunits and two GluN2 subunits. The obligatory GluN1 subunit is expressed as eight distinct splice variants, with four of these containing the N1 cassette, GluN1-(1a-4a), and four in which N1 is absent, GluN1-(1b-4b). The N1 cassette is known to affect NMDAR function, and its absence has been implicated in neuropsychiatric disorders. Here, we examined impact of alternative splicing of GluN1 exon 5 which encodes the N1 cassette on action of ketamine, an open channel blocker of NMDARs. We found that there is no difference on inhibition of ketamine at 3 μM on magnitude of NMDAR currents between GluN1a and GluN1b in mouse hippocampal neurons. However, ketamine had more rapid effects on NMDARs in cultured GluN1b neurons compared with GluN1a neurons in both blocking (5.14 ± 0.40 s versus 7.47 ± 0.53 s, n=10 and 13, respectively; P<0.01) and unblocking (4.43 ± 0.36 s versus 9.21 ± 1.07 s, P<0.01). The more rapid blocking and unblocking by ketamine in GluN1b- versus GluN1a-containing NMDARs were recapitulated with recombinant receptors expressed in HEK 293 cells co-transfected with GluN2B or with GluN2D. In addition, voltage-dependent relief of ketamine-induced blockade was more rapid in GluN1b compared with GluN1a in cells co-transfected with GluN2B (0.57 ± 0.07 s versus 1.49 ± 0.33 s, n=10 each; P<0.05). These findings demonstrate that alternative splicing of exon 5 of NMDAR GluN1 controls the kinetics of the blockade and unblock by ketamine, which may have significance in therapeutic use of ketamine.

P2-B-32 - Characterizing Mef2 transcription factors in healthy adult microglia

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Microglia are the resident macrophages of the central nervous system, essential for regulating synaptic plasticity, clearing apoptotic neurons, and repairing neuronal damage. These functions depend on transcription factors (TFs) to modulate gene expression. Among these, myocyte enhancer factor 2 (Mef2) proteins—Mef2a, Mef2c, and Mef2d—stand out, with enriched DNA-binding motifs in open chromatin under basal conditions, emphasizing their role in microglial homeostasis.To explore Mef2's function in microglia, a mouse model with a microglia-specific deletion of Mef2 was used. Behavioral tests showed impairments in memory, motor function, and anxiety. Microglia were analyzed using immunohistochemistry, RNA sequencing, and chromatin accessibility assays.Preliminary results show Mef2-deficient microglia contribute to anxiety and motor dysfunction, linked to changes in morphology and density in regions such as the hypothalamus, motor cortex, and substantia nigra. Mutant microglia exhibit an amoeboid shape, indicating activation, with broader CNS effects reflected in altered astrocyte density. Transcriptomic analysis identified over 600 differentially expressed genes and significant chromatin accessibility changes.In conclusion, Mef2 regulates chromatin accessibility and gene expression, shaping microglial morphology and function. Its loss disrupts microglial homeostasis, contributing to behavioral deficits like anxiety and motor dysfunction.

P2-B-33 - Investigating glia dysfunction in the 15q13.3 microdeletion syndrome

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Background: Copy number variations (CNVs) are major risk factors for neurodevelopmental disorders (NDDs). A common CNV is the 15q13.3 microdeletion, a 1.5-2 Mb deletion that leads to neurological deficits including intellectual disability, epilepsy, autism spectrum disorder, and schizophrenia. Deficits in neurons are well characterized in the microdeletion; however, little is known about the involvement of glia. Glial dysfunction, including a reduction in astrocytes and white matter abnormalities, has been implicated in NDDs. Thus, the objective of this project is to investigate the impact of the 15q13.3 microdeletion on glial development. Methods: We examined the expression of 15q13.3 genes in glia using publicly available single-cell sequencing datasets. We performed immunohistochemistry at multiple time points in the 15q13.3 mouse model to characterize the expression of glia. Results: In the cortex, 15q13.3 genes are expressed in astrocytes and oligodendrocytes. At P2, we found a reduction in glial progenitors and oligodendrocyte lineage progenitors in the 15q13.3 mice. There was no difference in the expression of astrocytes and oligodendrocytes in the adult cortex. Conclusion: The findings of this study suggest that the 15q13.3 microdeletion impacts the early development of glial cells. Although compensatory mechanisms may mitigate glial cell populations in the adult cortex, further research is needed to understand the functional implications of these early developmental disruptions.

P2-B-34 - Astrocytes drive sexually dimorphic effects of stress on orexin neurons and behavior

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Astrocytes regulate multiple processes in the brain that integrate local and distal synaptic signals to shape complex behaviors. In the lateral hypothalamus (LH), astrocytes tune the excitability of orexin neurons, which are essential for maintaining arousal states across day and night. In conditions of stress, astrocytes undergo structural and functional change, which directly alters neural circuitry. Here, we tested the effects of an early-life stressor on LH astrocytes and orexin neurons. We hypothesize that elevated blood glucocorticoids resulting from stress impact LH astrocytes to alter orexin neuron activity.We employed an early life stress (ELS) paradigm (maternal separation 4hr/day), which significantly increases blood glucocorticoids in adulthood. Our findings reveal striking sex-specific effects of ELS on astrocyte morphology, orexin firing, and behavior. ELS enhanced purinergic signaling in males, resulting in elevated orexin firing and wheel running. We observed reduced orexin firing in females through decreased L-lactate availability, resulting in decreased diurnal running wheel activity. Bath application of L-lactate rescued firing rates, highlighting a critical role for astrocyte-neuron lactate shuttling in stress-induced deficits in female mice. Finally, conditional deletion of astrocyte glucocorticoid receptors in the LH significantly ameliorated the synaptic and behavioral deficits caused by ELS, in both sexes.Our data suggest that ELS perturbs circuits and behaviour through astrocyte glucocorticoid signaling in a sex-specific manner. ELS disrupted the supply of key energy substrates in female mice and elevated purinergic receptor signaling in male. These disruptions underlie stress-induced behavioral dysfunction and emphasize the need to consider sex as a biological variable in understanding astrocyte-mediated mechanisms of stress.

P2-B-35 - Optogenetic stimulation of astroglia prevents dopamine neuron degeneration

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Astroglia play a key role in Parkinson's disease (PD) via their uptake of α-synuclein, regulation of oxidative stress and modulation of neuroinflammation. The overexpression of α-synuclein in astroglia alone is sufficient to induce motor deficits1. Moreover, the specific optogenetic stimulation of astroglia can increase functional repair following administration of MPTP and 6-OHDA2, suggesting a neuroprotective effect of astroglia in PD. Here, we further investigate the therapeutic potential of astroglia using optogenetics to stimulate astroglia in the substantia nigra (SN) of rats injected with 6-OHDA. Briefly, a unilateral 6-OHDA lesion was followed the next day by optogenetic stimulation of SN astroglia. Behavioral testing was conducted 8- or 21-days post-lesion. While there was no significant effect of the lesion 8 days post 6-OHDA, there were, as expected, significant motor deficits 21 days post-lesion. Interestingly, these deficits were attenuated with stimulation of SN astroglia. Furthermore, a significant decrease in tyrosine hydroxylase (TH) positive neurons was noted in lesioned animals that was also attenuated with SN astroglial stimulation. This suggests an astroglial-induced neuroprotective effect at a cellular and behavioral level. RNA-sequencing analysis of the SN revealed differentially expressed genes that are implicated in microglial activation and immune pathways, as well as gene expression changes in oligodendrocytes. Altogether, the results support the notion that multi-cellular crosstalk is integral to neurodegeneration and neuroprotective processes.

P2-B-36 - PVN astrocytes regulate corticosterone secretion and stress responses

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Glucocorticoids, such as corticosterone, are essential for maintaining energy balance and adaptive behaviour. Their secretion is tightly regulated by the balance between hypothalamic-pituitary-adrenal (HPA) axis out and glucocorticoid receptor (GR)-mediated negative feedback in the brain. While the activity of corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus of the hypothalamus (PVN) orchestrates this process, the involvement of PVN astrocytes remains poorly understood, despite their high GR expression and known role in modulating neuronal activity. We hypothesised that astrocytic GR signalling is critical for the regulation of corticosterone secretion.To test this, we used mice with a brain-wide astrocyte-specific GR deletion (AstroGRKO). They showed significantly elevated corticosterone levels in both sexes compared to controls, with no change in dexamethasone test and adrenal weight, suggesting a central origin of this phenotype. This change was associated with alterations in the number of cFos-positive cells in the PVN in both sexes and increased CRH mRNA expression in the PVN of male AstroGRKO mice. Interestingly, GR deletion specifically in PVN astrocytes replicated increased CORT phenotype we found in the whole-brain KO. Using these genetic models we will show further data elucidating the mechanisms by which astrocyte-neuron interactions in the PVN fine-tune circulating glucocorticoid levels.

P2-B-37 - Astrocyte calcium activity tunes fear memory specificity

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Early life experiences are critical for brain development and behaviour. Recently, we have shown that early-life-stress leads to fear generalisation in an amygdala-dependent learning and memory task. Fear generalisation was associated with impaired synaptic plasticity and increased neuronal excitability in the lateral amygdala. We revealed that the mechanisms underlying these phenotypes are not restricted to neurons and rely upon a complex interplay between distinct brain cell types including astrocytes. We found structural changes in amygdala astrocytes suggestive of astrocytic network uncoupling. Supporting a central role for astrocytes in the effects of stress, we found that a genetically-induced decrease in astrocyte network function was sufficient to replicate cellular, synaptic, and fear memory effects associated with early-life stress. Here, we will discuss recent 2-photon calcium imaging data investigating calcium dynamics in amygdala astrocytes. We found a striking sexual dimorphism in calcium activity in non-stressed mice that was suppressed by stress, resulting in a homogenous decrease of activity levels in both sexes.Interestingly, astrocytic expression of the calcium extruder pump CalEx closely mimics the stressed-induced decrease in calcium activity. This astrocyte manipulation was sufficient to recapitulate the effects of stress at synaptic, cellular, and behavioural scales.Collectively, these data support the hypothesis that astrocyte calcium signalling integrity is crucial for amygdala-dependent fear memory specificity.

P2-B-38 - Mpfc-VTA synaptic transmission drives cognitive performance and is disrupted by chronic stress: Transcriptional and synaptic deficits

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Cortical dysregulation of subcortical circuits triggers mood disorders in humans and animals. Recent findings showed that chronic stress induces functional and morphological changes to cortical neurons projecting to the VTA. Whether these modifications affect synaptic transmission at dopaminergic (DA) neurons in the VTA remains unclear. Optogenetic stimulation of cortical axons in VTA slices revealed short-term potentiation of glutamatergic transmission at DA neurons, which was significantly impaired by chronic stress in both males and females. Using pharmacological approaches, we discovered this facilitation is mediated by presynaptic Ca2+-interacting proteins. Underlying molecular mechanisms were investigated using RNA-seq on PFC neurons projecting to the VTA, revealing key targets involved in Ca2+ regulation at mPFC-VTA synapses. In females, the Calhm1 gene, encoding a Ca2+ homeostatic protein, was significantly downregulated after stress. Using CRISPR gene editing, we suppressed Calhm1 expression in naïve females, mimicking stress-induced plasticity alterations at mPFC-VTA synapses. Finally, we evaluated cognitive performances in Calhm1 KD mice and found impairments in working memory and cognitive assessment tasks, phenocopying chronic stress effects on cognitive performances. Overall, our findings highlight a novel form of plasticity at mPFC-VTA synapses underlying cognitive deficits induced by chronic stress. Therapeutic options targeting this pathway could improve stress-related cognitive deficits.

P2-B-39 - C9orf72-knockout mice show heightened susceptibility to excitotoxicity via dysregulated glutamate receptor

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Hexanucleotide repeat expansions in C9orf72, the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal degeneration (FTD), result in haploinsufficiency and synaptic dysfunction. Here, we identify C9orf72 deficiency as a critical driver of synaptic hyperexcitability and excitotoxic vulnerability through dysregulation of calcium-permeable AMPA receptors (CP-AMPARs). In C9-KO mice, we observed elevated surface GluA1 expression, reduced dendritic spine density, and enlarged spine heads in CA1 neurons, which correlated with enhanced CP-AMPAR-dependent long-term potentiation. Following kainic acid (KA)-induced excitotoxic stress, C9-KO mice exhibited heightened seizure severity, abnormal EEG spectral power, and persistent hippocampal GluA1 elevation. Selective CP-AMPAR antagonism effectively mitigated hippocampal hyperexcitability, underscoring the pathological role of CP-AMPAR dysregulation. At the network level, C9orf72 deficiency amplifies excitatory signaling, linking synaptic dysfunction to broader network instability and impaired cognitive and motor performance. These findings extend C9orf72's role as a key regulator of synaptic homeostasis and neuronal resilience. By identifying CP-AMPARs as central mediators of excitotoxicity, this study highlights CP-AMPAR as a therapeutic target for C9orf72-associated ALS/FTLD characterized by excitatory network dysfunction.

P2-B-40 - Disruption of TrkC-PTPσ complex formation impairs synapse maturation and alters glutamate release in CA1 pyramidal neurons

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The precise organization of pre- and postsynaptic terminals is critical for synaptic function in the brain. Postsynaptic tropomyosin receptor kinase C (TrkC), a neurotrophin-3 receptor tyrosine kinase, acts as an excitatory synaptic organizer through interaction with presynaptic receptor-type tyrosine phosphatase PTPσ. However, the role of the TrkC-PTPσ complex in synaptic transmission remained unknown. To isolate the synaptic organizer function of TrkC from its role as a neurotrophin-3 receptor, we generated mice carrying TrkC point mutations (TrkC KI) that selectively abolish PTPσ binding. Whole-cell patch clamp recordings from CA1 pyramidal cells revealed that TrkC KI mice exhibit a significantly lower AMPAR/NMDAR ratio at SC-CA1 synapses compared to WT mice. Furthermore, the coefficient of variance ratio (CV-NMDAR/CV-AMPAR) was reduced in TrkC KI mice, indicating an increase in silent synapses. Surprisingly, AMPAR-mediated miniature EPSC (mEPSC) frequency but not amplitude was elevated in TrkC KI mice. These findings suggest that active synapses in TrkC KI mice may have increased release sites or release probability without postsynaptic AMPAR alterations. Further analysis of fEPSPs revealed enhanced basal synaptic transmission in TrkC KI mice, while paired-pulse facilitation indicated reduced presynaptic release probability. Together, these results suggest that loss of TrkC-PTPσ complexes impaired excitatory synapse maturation characterized by an increase in silent synapses and aberrant active synapse function due to altered glutamate release dynamics.

P2-B-41 - Pannexin1 channels modulate excitation-inhibition balance and neural network dynamics in larval zebrafish

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Pannexin1 (Panx1) is an integral ion and metabolite membrane channel in the central nervous system that regulates synaptic plasticity. Despite its well-documented role in ATP release and neurotransmission, downstream signalling pathways that contribute to learning and memory remain largely unexplored. Using 6-day-old zebrafish larvae, we have previously shown that Panx1a, a homolog of Panx1, is critical for visual habituation, with knockouts (Panx1a-/-) showing persistent startle responses compared to wild-type larvae. We used post-hoc fosab labelling to identify brain activity hotspots that require Panx1a for habituation. Our results show higher baseline transcription and reduced activity-induced transcription in localized brain regions in the knockouts, suggesting the involvement of Panx1a in excitation-inhibition (E/I) balance. We demonstrate this further by pharmacologically modulating receptor signalling pathways. NMDA receptor antagonism partially rescued habituation deficits, and GABA receptor blockade disrupted habituation in both genotypes. Additionally, local field potential recordings and spike analyses in the dorsolateral pallium (hippocampus analog) and optic tectum (sensory center) indicate altered oscillatory activity across frequency bands in Panx1a-/- larvae post-habituation, suggesting disrupted network dynamics. The molecular, pharmacological, and electrophysiological evidence implies that loss of Panx1a shifts the excitation-inhibition balance during learning and memory processes. Funded by NRSC DG RGPIN-2019-06378 (GRZ).

P2-B-42 - Sex differences in a stress induced depressive phenotype: a time course of behavioural and central effects

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Females show a two-fold increased prevalence of mood and anxiety disorders. Similarly, there are sex differences in cognitive and emotive behaviours following exposure to acute and chronic stressors in rodents. We have previously shown that astroglial cells respond to five weeks of chronic variable stress (CVS) by upregulating perineuronal net (PNN) components, leading to an upregulation of PNNs themselves surrounding interneurons in the mPFC of male mice. However, whether sex differences occur in the timing of the onset of these changes in response to CVS is unknown. We employed a time course paradigm whereby male and female mice were exposed to CVS for one to five weeks in duration, followed by a battery of behavioural testing to assess emotive behaviours and histopathological analyses of their brains. Samples of dissected PFC were processed for single-nuclei sequencing to build a complete phenotypic gene expression profile of every cell type in response to stress exposure in males and females. We found sex differences in emotive behaviours over time whereby males show increases in emotive behaviours at one week following the onset of stress whereas females only show increases at weeks two or three, depending on the behaviour assessed. We also found sex differences in almost all cellular phenotypic changes, including oligodendrocytes, microglia, astrocytes and neurons, with peak sex differences in cellular phenotype observed after one week of stress. Altogether, these data show sex differences in behavioural and cellular patterns of response to chronic stress over time.

P2-B-43 - The HCN channel modulates muscarinic acetylcholine receptor function in the juvenile mouse entorhinal cortex

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The entorhinal cortex (EC) supports the cognitive functions of spatial learning and memory, which are known to differ between sexes. Acetylcholine facilitates EC-supported cognitive functions through activation of its muscarinic class of receptor (mAChR). Previous work found that mAChRs modulate the function of specific ion channels that regulate neuron excitability, like the hyperpolarization-activated cyclic nucleotide-gated (HCN) channel. It is not known whether this relationship is bidirectional, or whether this relationship contributes toward mechanisms underlying sex differences in EC-supported cognitive functions. We sought to determine whether HCN channels modulate mAChR function in pyramidal neurons located within layer 5 of the EC, which is the primary output layer for this region. Using whole-cell electrophysiology in acute brain slices from male and female juvenile mice, we measured effects of the selective HCN channel antagonist ZD7288 (10 mM) on neuronal responses to mAChR activation by muscarine (30 mM). For neurons held at a subthreshold membrane potential, ZD7288 significantly reduced muscarine-induced inward current, depolarization, and excitability (firing) responses. Initial mAChR responses were greater in males, and this sex difference was normalized after application of ZD7288. Findings from this study suggest that HCN channels augment mAChR responses in EC layer 5 neurons in a sex-dependent manner. This work adds to our fundamental knowledge of the EC and potential mechanisms underlying sex differences in EC-supported cognitive functions.

P2-B-44 - Success in transitive inference is associated with the activation of the medial prefrontal cortex in mice

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Transitive inference (TI) allows to predict indirect relationships between items based on previously learned set of overlapping premises. Past lesion studies have shown that TI relies on the integrity of the medial prefrontal cortex (mPFC) and hippocampus in both humans and rodents. However, the precise anatomical pathways underlying this essential component of intelligence remain poorly understood. By combining gene-based circuit mapping with a newly developed automated TI task for mice, we investigated brain regions activated during the recall of direct and indirect item relationships. During the recall test, all mice chose correctly in the direct relationships; however, ~60% made correct transitive judgments on indirect relationships (good performers), while the remaining mice could not (bad performers). Analyses of cFos expression revealed that the prelimbic and infralimbic subregions of the mPFC were more strongly activated in the good performers than the bad performers. In contrast, the cell activation in the CA1 subregion and dentate gyrus of the dorsal hippocampus was comparable between the two groups. All except dentate gyrus showed more robust cell activation in mice that underwent the test than those that stayed in their home cage. Currently, we are examining cFos expression in several other regions and overlaps between cFos expression and efferent projection targets. These results uncover the brain activity patterns associated with the ability to organize separately learned information into a mental model and use it for future adaptive behaviour.

P2-B-45 - Comparative optomapping of V1 and M1 reveals layer-specific microcircuit structure

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Microcircuit structure determines function, which means mapping circuits helps to clarify their function. However, state-of-the-art mapping techniques like multiple patch clamp are challenging and low-throughput. We therefore created optomapping, a high-throughput circuit mapping method that relies on 2-photon optogenetics and patch-clamp electrophysiology. Here, we investigated how microcircuits differ in primary visual (V1) and motor cortices (M1), two classical input and output areas, respectively. To express the soma-targeted opsin, ChroME, in neocortical pyramidal cells (PCs), we performed viral injections in neonatal Emx1-Cre mice. In postnatal day 18-26 acute slices, we made ChroME-expressing PCs spike using 1040-nm Ti:Sa-laser spiral scans. We patched PCs, basket cells (BCs), and Martinotti cells (MCs) in V1 and M1 while sequentially activating hundreds of surrounding PCs to find inputs from all cortical layers, with layer boundaries verified by in-situ hybridization. In both V1 and M1, the L5 PC to L5 BCs pathway was strongest, although it was stronger in V1. Lateral connectivity decayed faster for PC to PC than for PC to BC/MC synapses in V1, with preliminary data suggesting the same for M1. Excitatory inputs strengths onto PCs, BCs, and MCs distributed log-normally in both V1 and M1. The L4 PC to L2/3 PC pathway was denser ($p < .001$) and stronger ($p < .01$) in V1 than M1, which makes sense for an input area like V1. Thus, although V1 and M1 microcircuits appear grossly similar, optomapping revealed previously unappreciated layer-specific differences.

P2-B-46 - Reduction of MDGA2 in Mus musculus impairs synaptic long-term depression during the synaptic pruning critical period

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Autism Spectrum Disorder (ASD) is a heterogeneous neurodevelopmental disorder characterized by impaired social interactions, repetitive behaviour, and sensory sensitivities. Mutations in genes encoding synapse organizer proteins have been linked to ASD, including MAM domain-containing glycosylphosphatidylinositol anchor 2 (MDGA2) protein, a negative regulator of synaptogenesis. MDGA2 binds to postsynaptic neuroligin, blocking its interaction with neurexins via steric hindrance, preventing the formation of synaptic adhesion complexes. Mdga2+/- mice express behavioural phenotypes akin to ASD and in adulthood have elevated excitatory synaptic density and neurotransmission, and altered LTP profiles. However, the effects of reduced MDGA2 have not been explored in critical neurodevelopmental periods. This project aims to assess synaptic long-term depression (LTD) and evaluate the proteomic profile of isolated neuro-synaptosomes during and after the synaptic pruning period. We hypothesize that a reduction in MDGA2 will lead to premature synapse maturation, making synapses less susceptible to activity-dependent weakening. It has been found that acute hippocampal slices from Mdga2+/- mice display impaired NMDA receptor-mediated LTD induction and expression across the dorsal-ventral axis. We further aim to link this deficit to changes in the synaptic proteome through timsTOF mass spectrometry. This project will provide insight into the role of MDGA2 in synapse maturation and development and contribute to our understanding of ASD pathology during early neural development.

P2-B-47 - TRACR: a molecular tool to investigate the retinal connectivity

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To investigate retinal connectivity in photoreceptor transplantation, degeneration, and development, we use TRACR (TRanssynaptic Anterograde Circuit Readout), a ligand-receptor system based on a synthetic Notch receptor. TRACR detects synaptic interactions using (i) a GFP ligand at the presynaptic terminal (Sender), (ii) a postsynaptic Notch-based Receiver with an anti-GFP nanobody and tTA, and (iii) a Reporter gene under a TRE promoter. Synaptic interactions trigger tTA release, activating Reporter expression in postsynaptic cells. We hypothesize that TRACR enables high-throughput detection of photoreceptor synapses.Methods:Sender and Receiver AAVs were injected subretinally in neonatal Reporter mice. Eyes were sectioned, and markers were assessed via immunohistochemistry.Results: Sender was selectively expressed in photoreceptors and Receiver in ON bipolar cells. Reporter activation in Receiver+ cells required Sender expression in presynaptic photoreceptors. Eliminating Sender cells via MNU treatment or in RD1s significantly reduced Reporter expression. Reporter activation was also attenuated by rod-selective neurosecretion inhibition.Conclusion: Reporter activation depends on trans-synaptic interactions and sustained neurosecretion. TRACR provides a robust, high-throughput method to study synapse formation, degeneration, and transplant-associated synaptogenesis.

P2-B-48 - Targeting SRSF3 modulates microglia immune response and improves cognitive function in Alzheimer's Disease model

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Chronic deregulation of innate immunity likely plays a key element in Alzheimer'sDisease (AD) pathobiology, yet the precise mechanisms underlying the transition ofmicroglia from beneficial to harmful phenotypes remains unclear. Evidence hasemphasized that rebalancing and/or strengthening the innate immune response may betherapeutically relevant. We recently described a novel ribosome-based regulatorymechanism controlling innate immune gene translation in activated microgliaorchestrated by the RNA binding protein SRSF3. Here, we investigated SRSF3's role asa regulator of microglial innate immune response in AD using the APPswe/PS1 mousemodel. Expression levels of SRSF3, its active form pSRSF3 and disease-associatedmicroglial markers were analysed at different time point of disease. Levels ofpSRSF3/SRSF3, Aβ, phagocytic, pro-inflammatory and neuronal markers wereevaluated by western blot and ELISA while mice cognition was assessed by behaviouraltests. Our results revealed that the observed increase in pSRSF3/SRSF3 levelscorrelated with disease progression and was restricted to IBA1 positive cells. TargetingSRSF3 by intranasal antisense morpholino delivery induced a marked knockdown ofendogenous protein (50-60%). Anti-SRSF3-Morpho treatment initiated at 12 months olddecreased the levels of Aβ peptides and increased the expression of microglial markersLILRB4 and TREM2, increased expression of TLR2 and restored the recognitionmemory. Together, our findings suggest that targeting SRSF3 may open new avenuesfor therapeutic modulation of microglial response in AD.

P2-B-49 - Enhancement of synaptic transmission onto somatostatin interneurons under acidic conditions is mediated by mGLUR7

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Healthy brain function relies on a balance between excitation and inhibition. Activation of inhibitory somatostatin (SOM) interneurons is regulated by the synaptic proteins, Elfn1 and mGluR7, and their loss results in epilepsy. These proteins regulate excitation of SOM interneurons, creating an inhibitory "emergency brake" during high excitability. Accordingly, Elfn1 knockout (KO) mice experience seizures. Increased excitability and seizures can alter extracellular pH. Noting that mGluRs are acid-sensitive, we hypothesized that acidic conditions would act on the Elfn1/mGluR7 complex to increase synaptic transmission. Previously we found that a mGluR7 antagonist increases excitatory post-synaptic current (EPSC) amplitude and enhances activation of SOM interneurons. Here, we find that acidic conditions also increase EPSC amplitude in whole-cell patch clamp recordings taken from labelled SOM interneurons from both male and female transgenic mice. We also observed a trend towards a decreased paired-pulse ratio in acidified conditions, consistent with previous findings. These results suggest that acidification inhibits mGluR7 to enhance synaptic transmission and increase SOM interneuron activity. Future experiments with Elfn1 KO mice and pharmacological blockade will determine if this effect is mediated by Elfn1/mGluR7. This study advances understanding of how these proteins influence synaptic plasticity in acidic environments with implications for understanding epilepsy.

P2-B-50 - The role of cadherin-13 in cortical interneurons and cognitive function

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Cadherin-13 (Cdh13), a glycosylphosphatidylinositol-anchored cadherin family member, has been identified as a risk gene for neurodevelopmental and psychiatric disorders, including attention-deficit/hyperactivity disorder. In the hippocampus, Cdh13 expression is restricted to GABAergic interneurons (INs), and its global deletion impacts basal inhibitory transmission. GABAergic neurons are highly diverse, but the role of Cdh13 in specific IN populations and its effects on cortical network function and cognition remain unclear.Using single-cell transcriptomics, we found that Cdh13 mRNA is highly enriched in cortical somatostatin-expressing (Sst) GABAergic INs in adult mice. RNAscope analysis revealed that both parvalbumin-expressing (Pv) and Sst INs express Cdh13, though at varying levels. CDH13 protein expression in sensory cortices peaked during the second postnatal week. To explore whether Cdh13 expression in IN subtypes contributes to cognitive deficits, we generated conditional knockout (cKO) mice with Cdh13 selectively deleted in Sst (Sst-Cre; Cdh13LoxP/LoxP; Sst-cKO) or Pv (Pv-Cre; Cdh13LoxP/LoxP; Pv-cKO) INs. In the Barnes maze, Sst-cKO mice exhibited spatial learning and memory deficits, while Pv-cKO mice performed normally. At postnatal day 15 (P15), VGAT intensity in axonal boutons of Sst INs in visual cortical layer 1 was reduced in Sst-cKO mice. Adult analysis is ongoing. Understanding cell-specific alterations caused by Cdh13 deficiency could clarify mechanisms underlying psychiatric symptoms.

P2-B-51 - Level of circulating estradiol impacts the cholinergic modulation of excitatory synaptic transmission in the entorhinal cortex

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Cognitive function has been shown to vary depending on the reproductive cycle in both rats and humans. These fluctuations are thought to be mediated in part by the estradiol-induced enhancement of activity in cholinergic neurons. Cholinergic neurons provide an important input to the entorhinal cortex (EC), a key structure in memory function. We previously found that estrogen replacement prevents the disruption of cholinergic function in the EC following ovariectomy, and the present study has investigated the impact of low and high levels of 17β-estradiol (E2) that correspond to rat reproductive phases. Rats underwent ovariectomies and were implanted with capsules to induce either high or low circulating E2. After one week, excitatory synaptic field recordings were obtained from the lateral EC in vitro, and slices were exposed to the acetylcholinesterase inhibitor eserine which increases endogenous acetylcholine availability and reduces excitatory transmission by inhibiting glutamate release. Preliminary results demonstrate a reduction in EPSP amplitude after 30 minutes of eserine application to 73.3% of baseline levels in the low E2 group and to 81.9% of baseline in the high E2 group. Results indicate that changes in circulating levels of E2 can modulate cholinergic function and suggest that prolonged high levels of E2 may attenuate cholinergic transmission in the EC. Ongoing studies are investigating the effects of more transient increases in E2 on excitatory transmission via acute injections of E2.

P2-B-52 - Neurosteroid modulation of muscarinic acetylcholine responses in the mouse medial prefrontal cortex

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The medial prefrontal cortex (mPFC) plays an important role to support cognitive functions such as attention and decision making. Acetylcholine facilitates mPFC-dependent activity through its muscarinic acetylcholine receptors (mAChRs). Neurosteroids are endogenous modulators of neurotransmission which act at numerous sites. Recent studies provide evidence that neurosteroids can allosterically modulate mAChRs in vitro. We sought to determine whether neurosteroids also modulate mAChRs expressed on living neurons from the mPFC. Using whole-cell electrophysiology in pyramidal neurons located within layer V of the young postnatal mouse mPFC, we measured the ability of progesterone (PROG; 100 nM and 1 µM) and its metabolite allopregnanolone (ALLO; 10 nM and 100 nM) to modulate mAChR-induced neuron excitation responses. PROG had no effect on mAChR responses at 100 nM, but potentiated mAChR responses at 1 µM in males only. ALLO potentiated mAChR responses at both 10 nM and 100 nM in males and females. This effect of ALLO persisted in the presence of antagonists to AMPA, kainate, and GABA(a) receptors, suggesting that the mechanism of this potentiation does not occur indirectly via these receptors. Overall results from this study suggest that PROG and ALLO potentiate mAChR responses in the mPFC, implicating these endogenous neurosteroids in the modulation of acetylcholine-dependent cognitive functions via this receptor class.

P2-B-53 - Elucidating the role of distinct neural stem cell lineages 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 NSCs characterized by the expression of PDGFRβ was discovered in the adult subventricular zone (SVZ), these cells had a gliogenic potential and gave rise to astrocytes and oligodendrocytes. This contrasts with Nestin+ lineage of NSCs which is mostly neurogenic under homeostatic conditions. Our study aims to investigate the dynamics of PDGFRβ- and Nestin-expressing NSCs and the fate of their progeny after stroke. To this end, we use the PDGFRβCreErt2-Tdtomato and NestinCreErt2-YFP mouse lines for lineage tracking after stroke. Mice are subjected to ischemic stroke via middle cerebral artery occlusion.Our results revealed an increased number of PDGFRβ-TdT+ cells in the SVZ and the striatum of the ipsilateral hemisphere at 7 and 30 days after stroke. Interestingly, these PDGFRβ-TdT+ cells were CD13 negative, suggesting that these cells were not pericytes. Moreover, after stroke, these cells became active and acquired mostly gliogenic rather than neurogenic fate, characterized by the higher proportion of GFAP+/TdT+ and Olig2+/TdT+ cells as compared to Dcx+/TdT+. Our ongoing analysis suggests that Nestin+ NSCs contribute mostly to neurogenic responses in post-stroke recovery in the striatum. Our study revealed the contribution of distinct lineages of NSCs in the post-stroke responses.

P2-B-54 - Deciphering the dynamics of adult neural stem cells activation and their regenerative potential in freely behaving mice

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Neural stem cells (NSCs) in the subventricular zone (SVZ) of mammalian brain transit from a quiescent to activated states to generate new progeny. The mechanisms regulating this transition under homeostatic and regenerative conditions, as well as the dynamics of NSCs activation in freely behaving mice are still poorly understood. We used mini-endoscopic imaging of adult NSCs in the SVZ of freely behaving mice to decipher sex-dependent and region-specific differences in their activation dynamics and to link it to animal behavior. We have also studied the regenerative potential of adult NSCs in response to 7 days antimitotic drug (AraC) treatment. We demonstrate that AraC-induced elimination of rapidly dividing progenitor cells affects Ca2+ signaling in NSCs, by decreasing the frequency of Ca2+ transients and increasing the steady-state intracellular Ca2+ level. This Ca2+ signature is consistent with NSCs transition towards the activation state. In addition, we demonstrated that modulation of Ca2+ dynamics in NSCs after AraC treatment by in vivo optogenetics mimicking their quiescent state impacts NSCs activation and their regenerative potential. Altogether, our results highlight the importance of Ca2+ signalling in the regulation of proliferative vs quiescent states of NSCs under homeostatic and regenerative conditions.

P2-B-55 - Microglial dynamics in response to recurring stimulus in vivo

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The brain is equipped with a diverse array of cells that collectively protect its function and integrity, with microglia playing a role as the central nervous system's resident sentinel. These highly dynamic cells respond to injuries, such as cerebral microbleeds (CMBs), by migrating to the area of insult. While an individual CMB is asymptomatic (ie. no overt behavioural manifestations), CMBs can accumulate over the lifespan, especially in disease states, which has been associated with cognitive decline. Currently, there is a lack of understanding of microglial response to repeated microbleeds in vivo. In this study, we used two-photon microscopy to evaluate the dynamics of microglia in response to recurring microbleeds within the somatosensory cortex. To accomplish this, we performed chronic cranial window implantation on adult mice that express Cre-dependent fluorescent reporter tdTomato, and tamoxifen-inducible microglia specific Cre recombinase for real time imaging of microglia. Following recovery, a microbleed was induced by transiently rupturing a single capillary using the imaging laser. The induction of recurrent CMBs were followed over a four-week period. Preliminary findings indicate an increase in percentage of mobile microglia as subsequent CMBs were elicited. In particular, this elevated fraction of mobile cells was attributed to their movement 48 hours post-injury. Continuing experiments are focused on further evaluation of microglial migration, phagocytic activity, and morphological changes with repeated microbleeds.

P2-B-56 - Noradrenaline recruits hypothalamic PVN astrocytes to regulate CRH-PVN neuron activity during fear learning

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Noradrenaline (NA) released from the brainstem powerfully modifies the neuroendocrine stress response by altering the activity and plasticity of hypothalamic CRH-PVN neurons. Extrasynaptic

NA drives intracellular calcium elevations in nearby astrocytes, which may release neuroactive substances like ATP/adenosine to modulate neuronal activity. Whether PVN astrocytes process stress-related information, and subsequently influence CRH-PVN activity, remains unknown.We used head-mounted microscopes and fiber photometry to investigate the dynamics of PVN astrocyte Ca2+ activity, in relation to NA afferent and CRH-PVN neuron activity, during acute stress and across fear learning. To probe astrocyte-neuron interactions, we expressed a plasma membrane Ca2+ ATPase to reduce PVN astrocyte Ca2+ activity in freely behaving mice and measured CRH-PVN neuronal activity and plasma CORT levels. We find that PVN astrocytes are engaged by different aversive stimuli in a spatially homogeneous yet temporally heterogeneous manner, and their intracellular Ca2+ activity is correlated with defensive behavior. NA afferent and PVN astrocyte activity, and extracellular adenosine levels, show evidence of associative fear learning. Preliminary findings indicate that PVN astrocyte Ca2+ responses to mild footshock require intact alpha1a-adrenergic receptor signaling, and that PVN astrocyte Ca2+ activity constrains CRH-PVN neuronal activity.Our observations support the idea that PVN astrocytes process stress-related signals on a slower timescale to adjust local circuit activity in a context-specific manner.

P2-B-57 - Optogenetic silencing of medial septum glutamatergic neurons distorts grid cell spatial firing and alters velocity coding in the medial entorhinal cortex

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Grid cells in the medial entorhinal cortex (MEC) exhibit firing at locations that form a repeating hexagonal array throughout an environment and may play a pivotal role in spatial navigation by providing idiothetic cues for path integration. This spatial periodicity of grid cells relies on input from the medial septum (MS). The medial septum plays a pivotal role in spatial and speed coding in the MEC and has also been linked to locomotion behaviors.There are two types of speed coding in the MEC: (1) firing frequency vs. running speed and (2) firing rhythmicity speed signal. The MS consists of three separate populations: cholinergic, GABAergic, and glutamatergic neurons. Here, we evaluate the contribution of glutamatergic neurons to grid cell firing. We have specifically targeted MS glutamatergic neurons using optogenetic activation of Archaerhodopsin to selectively silence this population while recording grid cells in the MEC. Light delivery was achieved using an optic fiber placed above the MS, and a four-tetrode microdrive was implanted into the MEC for grid cell recordings. We used a stimulation protocol of 30-second inactivation followed by a 30-second recovery period throughout the recording session. Our results show that silencing septal glutamatergic neurons did not affect MEC theta oscillations; however, silencing this population resulted in a distortion of the central grid fields. In addition, we found that silencing septal glutamatergic neurons also alters velocity coding in a subset of neurons. Together, these data point to an important role for septal glutamatergic neurons in both spatial and speed coding in the MEC.

P2-B-58 - Single-nucleus transcriptomic and chromatin accessibility analysis of rat hippocampal cells following amyloid-beta oligomers injections

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Background: Alzheimer's disease (AD) is a neurodegenerative disease characterized primarily by the aggregation of amyloid-beta (Aß) oligomers and neurofibrillary tangles. Accumulation of Aß oligomers is detected 10 to 15 years before the appearance of the first clinical symptoms of AD. Studies have shown that Aß oligomers are involved in synaptic and neuronal losses observed in the hippocampus. Thus, we are interested to study the impact of Aß oligomers on the different cell types present in the hippocampus using a rat model via single-nucleus transcriptomic and chromatin accessibility profiling. Methods: Aß oligomers were injected daily into the hippocampus for 0 (control), 2, 4 or 6 days. The brains were collected and flash freeze. Cells located directly under the injection site were cryosection at 50 micrometers then collected by laser microdissection. The nucleus was isolated, and we performed an analysis on each of these nucleus of neurons, astrocytes, oligodendrocytes, pericytes, endothelial cells and microglia, using the single nucleus RNA-sequencing and ATAC-sequencing technics (10xGenomics). Results: Neuronal loss was observed at the granular layer of the dentate gyrus caused by Aß oligomers injection. We observed a significant accumulation of Aß in rat's hippocampus, consecutively injected with Aß 6 consecutive days. The immunofluorescence's results with NeuN and GFAP showed a neuronal loss after repetitive injections. Moreover, our test sample's sequencing profiles identified different clusters with different interesting gene expression changes over time. Conclusion: This study will allow us to determine the gene expression and epigenetic changes induced specifically by A oligomers in each hippocampal cell type during the progression of Aß pathology.

P2-B-59 - Uncovering the role of primary cilia in astrocyte reactivity following adolescent mild traumatic brain injury

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Mild traumatic brain injuries (mTBIs), caused by forces to the head which result in transient disturbances in brain function, can lead to long-term, debilitating, neurobehavioral deficits. In Canada, due to their frequent involvement in contact sports, adolescents are especially prone to repetitive mTBIs, which can interrupt healing and induce cumulative damage in the brain. In response to insults/injury, astrocytes are activated in a process termed reactive astrogliosis to protect neurons and promote healing. However, if dysregulated, reactive astrocytes can induce secondary damage. Currently, the molecular mechanisms governing astrocyte responses to injury are poorly understood, though it is agreed that environmental cues play an important role. Recently, we found that primary cilia, antenna-like sensory signalling organelles, are involved in reactive astrocyte modulation. Using an adolescent mouse model with inducible, astrocyte-specific cilia dysfunction, we show that in response to repetitive mTBIs, (1) mice with impaired astrocyte primary cilia have enhanced functional recovery, as compared to controls, and (2) that astrocyte reactivity is suppressed in cilia mutant mice. Our results suggest that primary cilia signalling does instruct astrocyte responses to brain injury, though why neuroprotection is observed in cilia mutant mice is being currently explored. This study reveals a novel function of primary cilia signalling in the regulation of astrocyte reactivity.

P2-B-60 - CREB-induced PDE10A expression is disrupted in humans by LINC00473

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The long noncoding RNA (lncRNA) LINC00473 is newly-evolved in primates and is expressed in response to cyclic AMP (cAMP) signaling in activated neurons. LINC00473's nearest chromosomal neighbor is the cAMP signaling inhibitor PDE10A, and despite their proximity and functional convergence their regulatory interplay is unclear. Although mice lack a LINC00473 gene, we found that the cAMP-inducible promoter of LINC00473 was conserved in mice, where it is predicted to regulate an alternative Pde10a transcript. To gain insight into the gene regulatory consequences of LINC00473 acquisition in humans, we used CRISPR activation (CRISPRa) to test the function of this conserved promoter in mouse and human cells. In human cells, activation of this promoter increased expression of LINC00473 as expected, while PDE10A was unchanged. Conversely, activation of the conserved promoter in mouse cells stimulated production of both Pde10a mRNA and protein. Consistent with these findings, cAMP signaling activated Pde10a expression in mouse neural progenitors and neurons, but not in human cells. This cAMP-induced expression of Pde10a suggests that mice may have a negative feedback loop that is absent in humans. Notably, pharmacological inhibition of Pde10a led to increased expression of cAMP-inducible gene expression, confirming its potential as a feedback inhibitor. Together, these findings suggest that the newly evolved LINC00473 gene has hijacked the cAMP-inducible Pde10a promoter in primates, thereby ablating a negative feedback loop that may otherwise constrain cAMP-induced gene expression.

P2-B-61 - G-protein mediated regulation of glial activation and TNF production

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Neuromodulators generally act through G-protein-coupled receptors, but their effects on glia are not well defined. Here we examine the impact of various G-protein-coupled receptors on glia, using the production of the pro-inflammatory cytokine Tumor necrosis factor alpha (TNF) as a measure of activation. TNF is a major part of the innate immune response but is also an important regulator of synaptic function and can be released by both astrocytes and microglia. We characterized the response to activation of the Gi, Gq, and Gs signaling pathways. Through a series of experiments involving Designer Receptors Exclusively Activated by Designer Drugs (DREADDs)-based and pharmacological approaches in rat astrocyte and microglia cultures and human induced pluripotent stem cells (hiPSCs) derived astrocytes, our findings showed distinct actions of different G protein pathways. Activation of Gs-GPCRs results in a decrease in TNF expression in both types of glial cells. Similarly, activation of Gq pathways also results in a reduction in TNF mRNA levels. Conversely, Gi activation in astrocytes and microglia increases TNF levels. This is reversed from the activation or inhibition seen in neurons to these same G-protein cascades. Overall, this work demonstrates that G protein-mediated activation and inhibition in glia should be considered separately from the effects seen in neurons.

P2-B-62 - Sex differences in the effect of acute cannabis exposure on microglia in the prelimbic cortex in adult mice

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Microglia are the brain's resident immune cells—they actively maintain brain health via their many physiological roles across the lifespan. They possess cannabinoid receptors and respond to cannabinoids, such as the major phytocannabinoids in cannabis plants, including delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD). However, the effects of a highly translationally relevant type of exposure, inhalation, on the physiological functions of microglia that could modulate the brain inflammatory balance, but also promote neuroprotection and neural circuit integrity, remain unexplored, particularly across sexes. Here, whole cannabis plant was administered to young adult, male and female, C57BL/6J mice for 15 min (one 15 second puff every 5 min; 3 puffs total; 0.15 g flower/puff). Four groups were utilized, mice that received control air vapor, and mice that were exposed to either: high CBD/low THC [CBD], high THC/low CBD [THC], or balanced THC/CBD [Balanced] cannabis chemovars. We find an effect of brain region and sex on microglial cell density and distribution. Within the prefrontal cortex, in males, but not females, there was a reduction in the nearest neighbour distance of IBA1+ cells in the prelimbic region with exposure to all cannabis chemovars—indicating a difference in microglial distribution in this brain region. Additionally, we have found that in the prelimbic area, there was a change in microglial morphology in males but not females. We will next use scanning electron microscopy to investigate potential changes in microglial organelles and interactions with parenchymal elements. This work will lay the foundation for understanding how vaporized cannabis exposure alters microglial form and function. Future work will focus on the duration of these changes as well as the outcomes following chronic exposure.

P2-B-63 - The Role of Endogenous Retroviruses in Regulating Microglial Activation in the Hippocampus

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Endogenous retroviruses (ERVs) are remnants of ancient retroviruses that are genome-encoded and are implicated in chronic neuroinflammatory diseases. Microglia, the resident innate immune cells of the central nervous system, are essential for development, injury repair, and immune surveillance. They are also known to cause inflammation through the prolonged release of inflammatory mediators. Whether ERVs are involved in microglial activation and inflammation in the brain is not known. This study investigates the potential impact of ERVs on microglial activation within the hippocampus. Experiments were conducted in male and female age-matched toll-like receptor 7 (TLR7)-deficient (Tlr7 -/-) mice, which exhibit increased ERV expression, ecotropic murine leukemia virus ERV (Emv2)-deficient (Emv2-/-) mice, and TLR7 and Emv2 double-knockout (Tlr7-/-Emv2-/-) mice. Our results showed no significant shift in microglial morphology in Tlr7-/- mice compared to wildtype mice of either sex. However, microglia from male Emv2-/- and Tlr7-/-Emv2-/-mice exhibited a shift toward an inflammatory state, characterized by an increase in ameboid and a decrease in ramified microglia morphology. This suggests that Emv2-derived ERV helps maintain microglia in a resting state in male mice and prevents activation of microglia. Our results reveal a novel role for Emv2 in modulating microglial activation and underscore the complexity of ERV-mediated neuroimmune interactions. These findings highlight the therapeutic potential of targeting Emv2 in neuroinflammatory diseases, including Alzheimer's disease.

P2-B-64 - Primary cilia signaling shapes excitatory synaptic connectivity

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In the mouse cerebral cortex, pyramidal neurons elaborate a tree-like structure called a dendrite, which contains thousands of small protrusions called dendritic spines to receive synaptic inputs from other neurons. The appropriate density, size, shape and dynamics of dendritic spines are key determinants of how neurons receive, integrate, and encode circuit information. Knowing how dendrites and dendritic spines acquire their form during development is thus essential to understanding the emergence of functional neural circuits. We recently made an exciting discovery: Neuronal primary cilia serve as centralized signaling hubs regulating dendritic spine morphogenesis and synaptic functions. Specifically, we generated mice with ciliary gene deletion specifically in glutamatergic excitatory neurons. We found that ciliary mutant mics show altered dendritic spine morphogenesis and synaptic transmission defects. Further, mice with neuronal cilia defects acutely induced at adolescent or adulthood display changes in motor and cognitive behaviors. Collectively, these findings paints an intriguing picture that neuronal primary cilia serve as an undefined, extra-synaptic mechanism for the modulation of spines and synapses. Upon completion, our efforts will help uncover hitherto undefined signaling mechanisms fundamental for functional wiring of the brain and provide important insights into the pathology of primary cilia relevant neurodevelopmental disorders including ciliopathies, autism spectrum disorders, and intellectual disabilities.

P2-B-65 - Sensory cortex-specific differences in parvalbumin interneurons intrinsic and morphological properties

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In the mammalian brain, the primary somatosensory (S1) and auditory (A1) cortices comprise tightly coupled excitatory and inhibitory neuron networks that process tactile and sound stimuli, respectively. Among GABAergic inhibitory neurons, parvalbumin positive (PV+) cells provide fast inhibition onto excitatory cell bodies and proximal dendrite. Such precise synaptic localization allows powerful control of postsynaptic targets, restricting the temporal window for integration of excitatory synaptic inputs. While the functional role of PV+ cells in behavior and cognition has been studied in adult mice, their cellular properties have been investigated mostly in preadolescent mice. Therefore, little is known about PV+ cells intrinsic and morphological properties in adult sensory cortices. Here, we analysed intrinsic properties of PV+ cells in A1 and S1 of adult PV-Cre:RCE mice, where GFP is expressed specifically in PV+ cells, by whole-cell current clamp recordings. We found that, compared to PV+ cells in S1, PV+ cells in A1 fired less action potentials in response to somatic depolarization, with a strong spike amplitude accommodation ratio. In addition, soma size of PV+ cells in A1 was smaller compared to those in S1. Taken together, all these data suggest significant differences in intrinsic properties of PV+ cells depending on their sensory cortical location, which could in turn underlie different roles in cortical processing depending on the sensory modality. Further studies will characterize the molecular mechanisms underlying these differences.

P2-B-66 - Estrous cycle regulation of cortical inhibitory neurons and stimulus perception in the adult mouse auditory cortex

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Gonadal sex steroids such as estradiol modulate brain function via signaling at steroid hormone receptors. While this signaling is well studied in the context of limbic and hypothalamic function, hormone effects on cortical sensory perception are poorly understood. Using the adult mouse estrous cycle as a naturalistic model of changing gonadal hormone signaling, we characterized effects on auditory cortical function using chronic in vivo EEG recording in female mice, discovering select estrous phase-dependent modulation of cortical processing. Given the known sensitivity of inhibitory parvalbumin (PV) neurons to estradiol and the estrous cycle, we examined markers of PV plasticity, revealing auditory cortex-specific regulation of perineuronal nets. Using patch-clamp electrophysiology, we also noted phase-dependency in PV neuron passive and active properties. To better define the role of PV neurons in mediating estradiol-dependent signaling in brain function, we generated a conditional loss of function model for ESR1 (encoding estrogen receptor alpha) in PV neurons. Mouse phenotyping revealed specific behavioural deficits in conditional PV-Cre:ESR1fl/fl male and female mice. Future experiments dissecting the role of PV neuron ESR1 signaling in estrus cycle-dependent auditory perception will build a foundation to understand the architecture of estrogenic brain modulation. The outcome of this study will help define the basis of how sex steroid hormones influence sex and gender differences in the development of neurological disorders, thereby informing targeted treatment.

P2-B-67 - Suppressive mechanisms of amyloid beta on NMDAR-induced excitotoxicity

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Alzheimer's disease (AD) is the most common form of dementia, characterized by a gradual decline in memory and other cognitive skills. The buildup of Amyloid beta (Aβ) protein is suggested to play a key role in AD pathology. While mutations that increase Aβ levels are linked to familial AD, over 95% of AD cases are sporadic, indicating that environmental risk factors and co-morbidities may contribute to Aβ buildup. One such factor is ischemic stroke, which potentially contributes to AD pathogenesis by increasing Aβ levels.Our data challenges this concept and reveals that Aβ, at the pico- and nanomolar range, reduces excitotoxicity seen during ischemia, indicating a protective role, at least in the acute phase. This protective effect seems to work by indirectly inhibiting the activity of a channel, called Pannexin 1 (Panx1), which is crucial for sustaining excitotoxicity. This project has aimed to investigate how this suppression occurs in primary hippocampal neurons and brain slices. Specifically, we have studied the changes Panx1 undergoes on the plasma membrane following Aβ application, employing microscopy, including TIRF. Additionally, the signaling pathways involved in these changes have been elucidated utilizing molecular biology techniques.Overall, the current project will challenge the traditional view of Aβ as toxic, offering a deeper mechanistic understanding of its role in ischemic stroke, AD, and other neurodegenerative conditions with increased Aβ levels.

P2-B-68 - Poly-unsaturated lipids enhance membrane associated condensates to rescue morpho-functional deficits of cerebellar Pinceau terminals in a mouse model of autism

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Synaptic proteins form intracellular condensates with protein scaffolds, but uncertainty remains how essential lipids transform dynamic cytosolic condensates into stable, functional macromolecular assemblies. We found that docosahexaenoic acid (DHA), a key polyunsaturated fatty acid (PUFA), facilitates the re-localization of cytosolic "full-droplet" condensates composed of the key synaptic elements PSD95 and Kv1.2 to the plasma membrane as "half-droplet", independent of canonical signalling pathways. To exploit the therapeutic potential of DHA, we briefly placed juvenile wild-type (WT) and Fmr1 KO mice, a model of human fragile X syndrome (FXS), under DHA-enriched or PUFA depleted diets. DHA was able to reverse the inhibitory overtone by promoting the re-localization of presynaptic Kv1.2-PSD95 condensates towards the internal surface of presynaptic interneuron membranes. This corrected morpho-functional synaptic defects and stereotypical behaviours seen in Fmr1 KO mice. Our findings reveal an unexpected role of PUFAs in transforming dynamic intracellular condensates into stable presynaptic condensates, providing long-lasting benefits for rectifying excitation-inhibition imbalance in FXS and likely other neurodevelopmental disorders. This work is supported by the CIHR.

P2-B-69 - Exploring immune-mediated modulation of striatal dopamine release in a genetic mouse model of Parkinson's disease

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Parkinson's disease (PD) is a progressive neurodegenerative disorder, affecting ~10 million people worldwide. It is characterized by a loss of dopaminergic (DA) neurons in the substantia nigra, leading to motor and cognitive impairments. While the mechanisms of neurodegeneration in PD remain unclear, genetic risk factors such as mutations in the leucine rich repeat 2 (LRRK2) protein, and immune-induced inflammation may contribute to disease progression. To investigate this, we aim to study the impact of immune activation on DA neuronal activity using wild type (WT) and LRRK2-G2019S (GKI) mutant mice. Brain slices from mice expressing a D1 dopamine receptor-based fluorescent reporter (dLight) were prepared and incubated in artificial cerebrospinal fluid containing either inflammatory cytokine interferon gamma (IFNγ) or an untreated control (CTRL). DA release was then electrically stimulated in the dorsolateral striatum to measure its changes. We observed a sex-specific effect on DA release following IFNγ treatment in WT mice as DA release was increased in male but not female mice. Additionally, males showed reduced paired-pulse ratios independent of IFNγ treatment, suggesting increased D2-mediated negative tuning of dopamine release and slower DA reuptake. This preliminary work will be continued in GKI mutant mice to further study the combined effects of genetic and environmental factors in PD. Our findings may reveal mechanisms driving neurodegeneration and disease risk.

P2-B-70 - Characterization of α7 and α4β2 nicotinic cholinergic responses in layer 1 medial prefrontal cortical neurons

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The medial prefrontal cortex (mPFC) plays a crucial role in cognitive functions such as attention and working memory with cholinergic activation of nicotinic acetylcholine receptors (nAChRs) being an important modulator of neuronal excitability. Using whole cell patch clamp recordings in layer 1 neurons of the mPFC and optogenetic stimulation of acetylcholine (ACh) release, we identified two distinct nAChR mediated currents: a rapid transient inward current inhibited by the α7 nAChR antagonist MLA and a prolonged current abolished by the α4β2 antagonist DHβE.   The α4β2 current was significantly reduced by the slow Ca^{2+} chelator EGTA-AM, whereas α7 responses remained unaffected, indicating strong coupling between the presynaptic Ca^{2+} source and sensor for ACh exocytosis for α7 containing synapses and looser coupling for α4β2 synapses. Meanwhile, the fast Ca2+ chelator BAPTA-AM significantly reduced both α4β2 and α7 nAChR currents. Delayed asynchronous events were abolished by DHβE but persisted with MLA, confirming that asynchronous responses were mediated by α4β2 receptors. Additionally, spontaneous activity before stimulation was insensitive to MLA or DHβE but was significantly reduced by EGTA-AM and the L-type Ca^{2+} channel blocker (nifedipine), highlighting a role for L-type channels in spontaneous neurotransmission.   These findings suggest distinct presynaptic mechanisms governing α7 and α4β2 responses, with implications for cholinergic modulation of cortical circuits in cognitive processes.

P2-B-71 - The extent of Ca2+ influx limits the role of handling mechanisms in controlling Ca2+ channel Ca2+-dependent inactivation in Aplysia bag cell neurons

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Cytosolic free Ca2+ is controlled through channels and pumps at both the plasma membrane and within organelles. Neuroendocrine bag cell neurons of the sea snail, Aplysia, contain voltage-gated Ca2+ channels which undergo use-dependent inactivation (aka rundown) during a prolonged afterdischarge. This burst of action potentials follows a brief synaptic input, and consists of a ~5-Hz, ~1-min fast-phase, then a ~1-Hz, ~30-min slow-phase, which triggers the Ca2+-dependent secretion of egg-laying hormone. We used whole-cell voltage-clamp of cultured bag cell neurons with solutions that isolate Ca2+ currents to investigate rundown during a 1-Hz, 1-min slow-phase-like train-stimulus of 75-ms steps from -40 to 0 mV. In Ca2+-based external solution, there was more rundown (~63% current remaining) than in Ba2+ (~86% remaining), indicating Ca2+-dependent inactivation. Pharmacologically eliminating the mitochondrial uniporter (Ru360), as well as the Ca2+-ATPase pump at the membrane (carboxyeosin) or the endoplasmic reticulum (cyclopiazonic acid), did not alter rundown, suggesting that slow-phase Ca2+ influx is not handled by these mechanisms. Furthermore, Ca2+ imaging with fura confirmed that mitochondrial Ca2+ is not increased during slow-phase stimulation. As well, there was less Ca2+ influx to slow-phase (~168% change) compared to fast-phase (~303% change) stimulation. The change in Ca2+ during the slow-phase is likely insufficient to engage additional handling mechanisms, thus permitting Ca2+-dependent inactivation, and thereby potentially influencing the extent of secretion.

P2-B-72 - Molecular and cellular mechanisms of the long-lasting effects of psychedelics and their non-hallucinogenic derivatives

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Major depressive disorder (MDD) affects 280 million people worldwide, with 80 million experiencing treatment-resistant depression (TRD). Axon hypomyelination and neuronal hypotrophy in the prefrontal cortex (PFC) are hallmark features, underscoring the need for treatments targeting these deficits. Psychedelics, like psilocybin, produce rapid antidepressant effects via 5-HT2A receptor activation, but their hallucinogenic properties limit widespread use. This study explores whether 2-Bromo-LSD (2-Br-LSD), a non-hallucinogenic analog, promotes neuroplasticity and myelination.Adult C57BL6/J mice received saline or 2-Br-LSD (0.3, 1.0, 3.0 mg/kg) and were tested in the open field and forced swim 24 hrs later. Chronic stress-exposed mice received saline, 2-Br-LSD (1–3 mg/kg), or psilocybin (1 mg/kg) and underwent behavioral assays. Post-treatment, brains were processed for Golgi staining and bulk RNA sequencing, with cell-type deconvolution via CIBERSORTx.2-Br-LSD promoted active coping in the forced swim test, effects blocked by volinanserin (5-HT2A antagonist). It reversed stress-induced behaviors akin to psilocybin. Transcriptomic analysis identified differentially expressed genes related to synaptic plasticity and myelination, particularly in PFC oligodendrocytes. These findings suggest 2-Br-LSD mimics classic psychedelics' antidepressant effects via 5-HT2A, supporting its potential as a novel MDD therapy.

P2-B-73 - A role for phosphotyrosine in gating of a nonselective cation channel in Aplysia neuroendocrine cells

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Alongside the more common serine and threonine phosphorylation, ion channels are also regulated by the state of phosphotyrosine. In the hermaphroditic sea snail, Aplysia californica, the neuroendocrine bag cell neurons trigger egg-laying behaviour with a period of persistent firing, termed the afterdischarge. This is driven by the 2nd messenger-dependent gating of nonselective cation channels. Prior immunostaining established that phosphotyrosine is reduced following an afterdischarge, while single-channel recordings confirmed tyrosine dephosphorylation promotes cation channel gating. Thus, we employed whole-cell recording from cultured bag cell neurons to test the effects of tyrosine dephosphorylation on macroscopic current and membrane potential. In standard Na+-based external, with a Cs+-based internal, the tyrosine kinase inhibitors, PP1 (25 μM) and SU6656 (10 μM), evoked inward currents of ~980 pA and ~526 pA, respectively. Both currents were associated with an increase in membrane conductance, linear current between -60 and +40 mV, and presented reversal potentials of ~-30 mV, consistent with the opening of a voltage-independent nonselective cation channel. Moreover, under current-clamp, PP1 triggered an ~40 mV depolarization. Lastly, the PP1-induced current was potentiated by prior exposure to H2O2, a reactive oxygen species that is increased during the afterdischarge. Therefore, tyrosine dephosphorylation-induced cation channel gating may contribute to the depolarization that maintains the afterdischarge, suggesting a role for phosphotyrosine in persistent firing.

C - DISORDERS OF THE NERVOUS SYSTEM

P2-C-74 - Striatal dopamine dynamics and motor learning in the YAC128 model of Huntington disease

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Impaired motor learning is a major symptom in Huntington Disease (HD), and past research has attributed this deficit to altered neuromodulatory signaling in the striatum. Many studies suggest an increase in dopamine (DA) transmission in the early stages of HD, followed by a decline with disease progression. However, there is a lack of studies that directly assess DA signaling during the performance of behavioural tasks in HD, particularly at the premanifest stage prior to overt motor phenotypes. Thus, we aim to measure striatal activity and DA release in vivo during motor learning tasks in the YAC128 mouse model of HD and compare behavioural and neural patterns with wild-type (WT) littermates. Using optogenetic probes and fiber photometry, we simultaneously measured striatal calcium transients and DA signaling in dorsal striatum of YAC128 and WT mice during the rotarod, cylinder and string-pulling tasks. Pilot data suggests that there is a lower correlation between DA and striatal activity in premanifest YAC128 mice during performance of the rotarod and string-pulling tests compared to WT, consistent with the hypothesis that DA dynamics are hyperexcitable at this early disease stage and are potentially impaired in modulating motor learning. This study is the first to combine real-time imaging of striatal DA with behaviour in an HD rodent model. Identifying altered DA signaling patterns that occur during both the execution of specific motor movements and more broadly in the regulation of motor learning are crucial for guiding therapeutic efforts in correcting uncontrolled movements in HD.

P2-C-75 - Multi-parameter screening assay for TDP-43 patient derived motor neurons for ALS therapeutic development

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Amyotrophic lateral sclerosis is a common neurodegenerative disorder and the most prevalent motor neuron disease in adulthood. TDP-43 is a major pathological marker of ALS, identified as both a component of proteinaceous inclusion bodies in the large majority of patients (>95%) and a genetic factor of disease with mutations in sporadic and familial cases. Major advances in our understanding of ALS pathogenesis have been achieved due to the development of patient induced pluripotent stem cell (iPSC) differentiated into motor neurons (iMNs). However, phenotype heterogeneity and lack of automated morphological and functional read-outs have largely precluded their use in high content drug screening. In our study, we addressed these limitations by developing multi-parameter automated assays for analysis of iPSC-derived motor neurons from ALS patients with TDP-43 mutations. Our read-outs encompass several crucial aspects of motor neuron pathology that include neurite outgrowth and neuronal population organization, survival assays, electrophysiological parameters, as well as live automated confocal imaging of subcellular components. Our assays revealed specific aspects of TDP-43 iMN vulnerability which were exploited for the development of a robust and reproducible drug screening platform that was benchmarked against the protective effect of the ALS drug, riluzole. In conclusion, automating the analysis of the TDP-43 mutant iMN phenotypic features opens novel insights of disease mechanisms and accelerates the identification of therapeutics for ALS and related TDP-43 pathologies.

P2-C-76 - Effects of genotype and age on olfactory discrimination and reversal learning performance in neurexin1 +/- transgenic mice

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Mohammed Ali Ahmed, Simone Shaw-Litzgus, Wyatt Ortibus, and Richard E. BrownDepartment of Psychology and Neuroscience, Dalhousie University,Halifax, Nova Scotia, Canada B3H 4R2 Rodent models allow us to investigate genes implicated in age-related cognitive decline. The neurexin genes (nxrn) encode the NRXN family of membrane proteins which bind to post-synaptic neuroligins and act as synaptic organizers to regulate synapse development and function. Neurexins have been associated in autism spectrum disorder (ASD) and we investigated the effects of aging on cognitive functions in male and female Nrxn1+/- transgenic mice (N= 33) and their wildtype (C57BL/6J) controls (N = 33) from 2 to 18 months of age. Using an operant olfactometer, mice were trained on an initial odor discrimination task to obtain a sucrose reward by responding to Odor A (S+) and not to a non-rewarded (S-) Odor B. Mice were then trained on the discrimination of a second odor pair (C vs D), followed by a reversal learning task in which the S+ and S- were switched. Our preliminary results show a significant age effect but no differences due to genotype or sex in the number of trials required to reach a criterion of 85% correct. Older mice were more likely to fail to reach the learning criterion than young mice in both the initial odour pair discrimination and in reversal learning. Likewise, older mice took more trials to reach criterion in the first odour pair discrimination and in reversal learning. These results suggest that as mice age, they lose cognitive flexibility, indicating that this procedure may be used as a test for novel drugs which might increase cognitive function during aging.

P2-C-77 - Characterizing potassium-chloride co-transporter 2 (KCC2) protein interactions in Huntington's disease

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KCC2 is the neuron specific member of the K+-Cl- cotransporter family of proteins that maintains a low intracellular Cl- concentration required for fast synaptic inhibition mediated by gamma-aminobutyric acid (GABA) in the central nervous system. Several neurological disorders including Huntington's disease (HD) exhibit impaired synaptic inhibition and Cl- dysregulation due to decreased KCC2 expression and function. KCC2 expression and function are regulated by both post-translational modifications and protein-protein interactions (PPIs). Identification of the KCC2 interactome can thus provide substantial insight into the function of this cotransporter and serve as a valuable approach to characterize the pathological mechanisms underlying disease.   Emerging evidence suggests there is a neurodevelopmental component to HD prior to the onset of clinical symptoms. This may be a result of altered KCC2 PPIs during early postnatal development, however the KCC2 interactome has never been identified in a disease model.   To address this gap in our knowledge of KCC2 biology, we performed affinity purification-mass spectrometry in HD and wildtype mouse brains to characterize the KCC2 interactome during early postnatal development, and to identify disease specific alterations in KCC2 PPIs. We are also examining the biochemical and functional relationship of altered KCC2 PPIs to elucidate the neurodevelopmental mechanisms underlying Cl- dysregulation in HD.

P2-C-78 - Investigating early alterations in brain ensheathing pericytes and cerebral blood flow in 5xFAD mice

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Alzheimer's disease (AD) is characterized by cognitive decline and reduced cerebral blood flow (CBF), contributing to neuronal damage and disease progression. Pericytes, critical for regulating CBF and maintaining capillary integrity, remain poorly understood in AD, particularly regarding a pericyte subtype, ensheathing pericytes, located in the arteriole-capillary transition zone. To address this knowledge gap, we crossed the 5xFAD mouse model of AD with mice that express the genetically encoded Ca^{2+} indicator RCaMP1.07 in cells with alpha-smooth muscle actin, which include ensheathing pericytes. A chronic cranial window over the whisker barrel cortex allowed long-term observation by two-photon microscopy of pericyte Ca^{2+} activity. Additionally, fluorescent dextran was injected intravenously before each imaging session to track hemodynamic changes during AD progression. Preliminary findings reveal altered Ca^{2+} signaling in ensheathing pericytes, correlating with changes in blood vessel diameter and vasomotion. These effects are apparent early in disease and have important implications for blood delivery into downstream capillaries. This study provides the first analysis of ensheathing pericytes in a model of AD, offering critical insights into vascular stress and dysfunction that may occur in the prodromal phase of disease. This paves the way for future innovative therapeutic strategies.

P2-C-79 - Hippocampal Interneuron dysfunction in sensory encoding and working memory in a mouse model of schizophrenia

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Schizophrenia (SZ) is characterized by working memory (WM) deficits, believed to be underpinned by aberrant hippocampal (HPC) inhibitory signalling. Despite recent advances in SZ pathophysiology, physiological deficits of HPC interneurons and their microcircuits are not well understood in relation to WM paradigms. Notably, dysfunctional parvalbumin (PV) and somatostatin (SST) interneuron (IN) dynamics have been reported in Df(16)A+/- (Df16A) mouse, a model of the human 22q11.2 chromosomal deletion, which is a strong predictor of SZ. Therefore, during an olfactory WM task, we performed in-vivo voltage imaging in the CA1 hippocampal region on double transgenic PV-Cre and SST-Cre Df(16)A mice. We recorded spike and subthreshold activity of clusters of PVs and SSTs at a high temporal resolution (1000 frames/sec) with a large field of view. Our ongoing analyses compare spiking and subthreshold dynamics in Df16A versus littermate controls. Additionally, using in-vitro immunohistochemical staining, CA1 IN morphology and cell number are compared between Df16A and controls. Altered interneuronal activity and morphology in Df16A mice may underlie reduced stimulus-specific activation of CA1 pyramidal neurons, leading to disorganized representation of information, and culminating as WM deficits. These experiments will reveal how interneuron dysfunction leads to disruption of WM activation in SZ, yielding important implications for future therapeutic targets to HPC inhibitory circuits.

P2-C-80 - Application of an αSyn-binding aptamer to reduce protein aggregation in a cell model of Parkinson's disease

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Parkinson's disease (PD) is characterized by the pathogenic accumulation of misfolded alpha-synuclein (αSyn) proteins, which propagate between cells, precipitating the progressive loss of nigral dopamine neurons and the manifestation of motor impairments. Thus, the inhibition of αSyn aggregation represents a promising target for novel interventional treatments of PD. The current study examined the therapeutic impact of a DNA aptamer (a-syn-1), which binds to monomeric αSyn to inhibit fibrillization, in an in-vitro model of PD. Using differentiated SH-SY5Y cells treated with αSyn preformed fibrils (PFFs), we first examined the temporal evolution of αSyn aggregation before investigating the impact of the a-syn-1 aptamer following differing schedules of administration. Our results demonstrate the seeding capacity of PFFs, as evidenced by the time-dependent increase in αSyn aggregation. Further, co-administration of αSyn PFFs and the a-syn-1 aptamer resulted in a 20% reduction in aggregation levels, while an acute treatment of the a-syn-1 aptamer after PFF-mediated pathology had developed did not affect the intracellular aggregation of αSyn. These findings emphasize the potential benefits of utilizing the a-syn-1 aptamer to target αSyn pathogenesis to slow or prevent the progression of PD.

P2-C-81 - The impact of a murine coronavirus (mhv-jhm) upon alpha-synuclein and inflammatory factors in primary wild-type and lrrk2 g2019s mutant microglia and midbrain neuronal cultures

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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. A 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 gene, leucine rich repeat 2 (LRRK2), 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 microglia and neurons from wildtype 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-neuron 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 syncytia 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.

P2-C-82 - Differential responses to punishment: exploring individual variability in oral morphine consumption and foot shock sensitivity in rats

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Purpose: The present study investigates the individual differences that emerge as a result of contingent and non-contingent punishment on oral morphine (OM) intake. Methods: Male and female rats were randomly assigned to: 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 (SA) 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. Results: Male and female MCs are consuming similar amounts of drug in mg/kg, and this stabilizes over time. Contingent foot shock reduces drug intake in high and low consumers, but there appear to be strong individual differences between animals more resistant versus more sensitive to foot shock when it is linked with morphine seeking. High or low levels of non-contingent foot shock do not appear to decrease consumption in either high or low OM consumers. Notably, females show lower sensitivity to pain in the tail-flick test and both sexes show lower sensitivity at the end compared to baseline. Significance: 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.

P2-C-83 - Investigating cell types and pathways responsible for increased seizure generation in a model of neurofibromatosis type 1

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Rationale: Neurofibromatosis type 1 (NF1) is a neurocutaneous disorder in which individuals have increased prevalence of seizures and epilepsy compared to the general population. We previously showed increased seizure susceptibility and epileptogenesis in a mouse model of NF1, however the mechanism remains unknown. The mutation causing NF1 leads to decreased levels of the protein neurofibromin, resulting in RAS hyperactivation and increased signaling through downstream pathway intermediates AKT-mTOR and ERK, which may play a role in seizures. Methods: Mice with cell-specific deletions of the Nf1 gene in either excitatory or inhibitory neurons were generated, implanted with intracranial electrodes, and a subset were treated with the mTOR inhibitor Everolimus. Mice underwent continuous video-EEG monitoring to detect spontaneous epileptiform abnormalities. This was followed by challenge with kainic acid to determine differences in seizure susceptibility. Results: Mice with vGlut+ cell-specific Nf1 knockouts showed increased kainic acid-induced seizure susceptibility in comparison to WT mice, similar to the phenotype we observe in Nf1+/- mice. Testing of the effect of mTOR inhibition on seizure susceptibility in Nf1+/- mice is currently underway.Conclusions: Altered seizure susceptibility in NF1 seems to be driven by vGlut+ excitatory neurons. Understanding the role of mTOR signaling in NF1-associated seizures could help in developing new treatment strategies for this patient population.

P2-C-84 - Microglial gene signatures and therapeutic targets in a murine model of perinatal cerebellar injury

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Background: Perinatal cerebellar injuries are linked to long-term neurodevelopmental disorders. This study investigates microglial gene signatures in a murine model of perinatal cerebellar injury caused by hemorrhage and systemic inflammation, aiming to identify therapeutic targets to improve outcomes in affected infants.Methods: On postnatal day 2 (P2), cerebellar hemorrhage was induced by injecting collagenase (0.5 U/µl) into the right cerebellar hemisphere, followed by systemic inflammation simulated with intraperitoneal lipopolysaccharide (LPS, 300µg/kg). Two groups were submitted to single-cell RNA sequencing: control (Veh-Veh,n=5) and injury (Coll-LPS,n=5). Single-cell expression data for 1,647 microglial cells were analyzed using the Seurat R package. Hierarchical clustering of microglial cell expression data was assessed with the multiscale bootstrap resampling method.Results: Hierarchical clustering of significantly differentially expressed genes revealed six distinct subgroups of microglia based on their gene expression profiles. These subgroups were analyzed alongside Gene Ontology (GO) annotations and protein-protein interaction networks to identify functionally related genes. Notably, Gab2 and Numb (subgroup A) are implicated in neuronal development, while Skiv2l2 and Hist1h1c (subgroup F) are associated with energy metabolism and mitochondrial function.Conclusions: Perinatal insults significantly disrupt microglial gene expression. We identified four genes (Gab2, Numb, Skiv2l2, Hist1h1c) that represent promising microglial signatures for therapeutic targeting.

P2-C-85 - Dysregulated mRNA translation and schizophrenia-relevant behaviours in mice

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Schizophrenia (SCZ) is a heterogeneous neurodevelopmental disorder and a leading cause of disability worldwide. Several studies have implicated the mammalian target of rapamycin complex 1 (mTORC1) – eukaryotic initiation factor 4E binding protein (4E-BP) translation initiation pathway in the therapeutic effects of antipsychotics. Furthermore, a recent exome study identified a single nucleotide polymorphism in the EIF4EBP2 gene in a SCZ patient. Still, this pathway's causal role in this disorder's development has not been explored. Therefore, this study aims to examine how this mutation in the 4E-BP2 gene leads to a SCZ-like phenotype in mice. To test the effects of this mutation in vivo, mice bearing the single nucleotide mutation in 4E-BP2 were created using CRISPR-Cas9 (KI mice). These mice were compared to mice lacking the 4E-BP2 gene (KO mice). Mice from the wildtype and mutant strains were subjected to behavioural assays, including amphetamine-induced locomotion, pre-pulse inhibition of acoustic startle (PPI) and Y-maze. In the behavioural tasks, we found that adult male 4E-BP2 KI and 4E-BP2 KO mice had a greater locomotor response to amphetamine compared to WT controls. In contrast, female 4E-BP2 KI mice responded to amphetamine like wildtype mice. Male and female 4E-BP2 KO mice had deficits in the PPI task. In vitro analysis of mutant 4E-BP2 indicated that phosphorylation by mTORC1 was increased compared to WT controls, suggesting a greater rate of inactivation of this protein. Meanwhile, ex vivo analysis of KI P7 brains demonstrated decreased phosphorylation.

P2-C-86 - Accelerated intermittent theta burst stimulation targeted to M1 combined with balance training improves balance in an individual with corticobasal syndrome

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We used repetitive transcranial magnetic stimulation (rTMS) combined with balance motor training in a 77-year-old female living with corticobasal syndrome with associated alien-limb phenomena and dystonia. rTMS was delivered to the primary motor cortex (M1) as accelerated intermittent theta burst stimulation (aiTBS) followed by 10 minutes of balance training each day for 14 consecutive days. Balance training used a biofeedback-based application where the participant was trained to shift the center of pressure with the goal of improving the left/right, front/back and diagonal weight shifting ability. Following the intervention, the participant demonstrated clinically significant improvements in balance as measured by Limits of Stability and Balance and Fall Risk assessment. This is the first reported use of aiTBS in an individual with a Parkinson-plus disorder. This report provides insight into the potential clinical utility of aiTBS plus balance training to improve Parkinson-plus disorder symptoms.

P2-C-87 - Proteomic, metabolomic, and physiological profiling uncovers widespread dysfunction of energetic pathways in medial prefrontal cortex synapses during protracted abstinence from heroin intake

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Relapse makes substance use disorder extremely difficult to treat. Drug-induced changes to the medial prefrontal cortex (mPFC) are thought to produce a lasting "hypofrontality" that impairs top-town control over the reward circuit, thus disinhibiting drug-seeking behavior that leads to relapse. To identify a biological mechanism supporting this process, we integrated synaptic proteomics, metabolomics, and electrophysiology following extended abstinence from cocaine and heroin intake in rats. Rats underwent intravenous self-administration of saline (control), cocaine or heroin for 10 days, followed by 30 days of homecage forced abstinence – a period marked by heightened drug craving. Subsequently, the mPFC was dissected, and synaptoneurosomes were isolated for label-free quantitative mass spectrometry to survey the synaptic proteomic landscape during abstinence. Both cocaine and heroin abstinence resulted in differentially expressed proteins, with heroin causing more pronounced changes. Bioinformatic analysis revealed heroin-induced dysregulation of synaptic proteins involved in metabolism, with divergent effects on extra- and intra-mitochondrial processes. These findings were supported by metabolomic analysis of the whole mPFC. Additionally, whole-cell patch-clamp recordings of layer 5 mPFC pyramidal neurons in heroin-abstinent rats revealed blunted excitability and diminished spontaneous excitatory synaptic inputs. Together, these findings suggest that enduring metabolic changes in mPFC synapses may promote heroin relapse and highlight numerous candidates for intervention.

P2-C-88 - Repeated sub-concussive impact exposure compromises white matter integrity in collegiate football players

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Repetitive subconcussive impacts (SCIs) in contact sports may induce subtle yet cumulative brain changes, even without clinically observed concussions. We monitored 22 male collegiate football athletes across one season at three timepoints: pre-season (PRE), post–training camp (PTC), and post-season (POST). Helmet-mounted accelerometers tracked impact frequency for stratification into high (HE) and low-exposure (LE) groups. Using diffusion tensor imaging (DTI) and neurite orientation dispersion and density imaging (NODDI), we assessed white matter (WM) integrity in fibre tracts identified via probabilistic tractography. HE athletes showed decreased fractional anisotropy (FA) and increased mean diffusivity (MD) in several WM tracts at PRE compared to LE athletes[NC1] , suggesting persistent effects from prior seasons. FA declined further and MD rose in corticospinal tracts, thalamic radiations, and inferior fronto-occipital fasciculus for the HE group at POST. Short-term MD fluctuations, like transient decreases in the superior longitudinal fasciculus at PTC, suggest dynamic axonal responses to acute SCI loads. NODDI metrics—Viso (isotropic volume fraction), Vin (intracellular volume fraction), and ODI (orientation dispersion index)—revealed further disruptions: elevated Viso in HE at PRE [NC2] in the left superior thalamic radiation, lower Vin in HE at POST in the left anterior thalamic radiation, and reduced ODI at POST in multiple left-sided tracts for HE vs. LE. These findings underscore the risk of subclinical yet accumulating WM alterations from repeated head impacts.

P2-C-89 - Morphological and functional dysfunctions of mitochondria in the cerebellum of the Christianson syndrome mouse model

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Christianson Syndrome is a neurological disorder where ataxia, characterized by motor incoordination, is among the most debilitating symptoms. Currently, there is no cure available for this rare ataxia, despite its grave impact. Christianson Syndrome shows an intriguing cell death pattern where Purkinje cells, the primary output cells in the cerebellum, exhibit selective vulnerability. Purkinje cells in the anterior region are particularly susceptible to cell death, while those in the posterior region remain resilient, despite being exposed to the same genetic insult. Anterior Purkinje cells fire at higher frequencies and, consequently, require a higher energy demand than those in the posterior, suggesting energy-related issues may contribute to cell death in Christianson Syndrome. Using RNAseq and DESeq2 analysis, we identified several gene families regulating mitochondria as highly dysregulated in the vulnerable anterior cerebellum of Christianson Syndrome mice. Therefore, we hypothesize mitochondrial dysregulation in anterior Purkinje cells may contribute to their vulnerability. Using electron microscopy, we demonstrate a significantly increased number of damaged mitochondria and a significantly decreased number of mitochondria in anterior Purkinje cells of Christianson Syndrome mice. We also show changes in mitochondrial function by immunohistochemistry in both live and fixed cerebellar slices. Our findings suggest that mitochondrial dysfunctions may be a novel therapeutic target for Christianson Syndrome.

P2-C-90 - Opioidergic neurons in the anterior cingulate cortex are required for placebo analgesia in mice with chronic neuropathic pain

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Placebo analgesia is the best example of brain's potent endogenous pain modulatory systems and understanding its neural mechanisms may offer potential novel targets for chronic pain treatments. Animal models enable exploration of cellular and sub-cellular mechanisms unavailable in human studies. We therefore developed a mouse model of placebo analgesia in the context of chronic pain to identify brain regions critical for its production. Male CD1 mice with spared nerve injury underwent pharmacological conditioning, where morphine (10 mg/kg) was paired to context for four days. Placebo analgesia was elicited the next day by administering saline in the same context, with Von Frey testing confirming robust effects. C-Fos analysis revealed altered activity and connectivity in the anterior cingulate cortex (ACC), basomedial amygdala (BMA), and paraventricular thalamus (PVT). Using DREADDs, we then selectively activated (AAV5-CamKIIa-hM3Dq) or inhibited (AAV8-CamKIIa-hM4Di) these regions. ACC activation abolished placebo analgesia ($p<0.0001$), while inhibition had no effect. Ablation of opioid-receptor containing neurons with Dermorphin-Saporin also abolished placebo, confirming the ACC's critical role. Activation or inhibition of the BMA or PVT did not affect placebo, suggesting a less pivotal role for these regions. These findings establish opioid activity in the ACC as a key component of placebo-induced pain modulation. Directly targeting the ACC may therefore offer a new therapeutic avenue for chronic pain, reducing opioid reliance and addressing the global chronic pain burden.

P2-C-91 - Emergence of spatiotemporal engrams in stem cell-derived neuronal networks: Effects of SHANK2 mutations

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Long-term memories are stored in the connectivity patterns within neuronal networks. Yet, when and how human brain networks gain the capacity to store memories remain unclear.Here we used the Ngn2 protocol to differentiate human stem cells into excitatory neurons that form interconnected neural networks and recorded their electrical activity using high density multielectrode arrays (MEAs). We found that as the network develops, neurons start firing in a Poisson-like fashion, and then transition to synchronous firing (network bursts) by days 24-27. We compared networks containing a mutation of the ASD linked SHANK2 gene against isogenic controls (CTRL). We found that SHANK2 networks developed initially slower than CTRLs, showing fewer spiking neurons. However, around day 26, SHANK2 networks surpassed CTRLs in the number of spiking neurons. Network bursts originated first in CTRLs around day 24 followed by SHANK2 mutants around day 27. Notably, network bursts showed a repetitive spatiotemporal pattern, with neurons firing in the same spatiotemporal order within most bursts (spatiotemporal engram), a form of network memory that resembles the neural activation sequences reported in the hippocampus and other brain areas during memory tasks. Finally, CTRL networks seem to maintain more regular spatiotemporal patterns than SHANK2 mutants. Our results suggest that the ability of neuronal networks to form spatiotemporal memory engrams emerges early during development, and that SHANK2 mutations affect this process at very early stages of development.

P2-C-92 - Role of p66shc in hippocampal neurogenesis and modulating NRf2 activity in an Alzheimer's disease mouse model

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Alzheimer's disease (AD) is characterized by memory loss and the accumulation of amyloid-beta (Aβ) plaques. However, therapies targeting Aβ have mostly failed. Aβ activates the adapter protein p66Shc, which exacerbates oxidative stress by increasing reactive oxygen species and suppressing Nrf2-driven antioxidant responses. While inhibiting p66Shc can restore antioxidant defenses, its long-term effect on Nrf2 and with chronic Aβ exposure remains unclear. This study examines p66Shc's influence on Nrf2 activity in an in vitro neural stem cell (NSC) model and investigates Aβ, Nrf2, and p66Shc levels in an AD (APP/PS1) mouse model. We hypothesize that Aβ activates p66Shc in AD brains and that p66Shc knockout (KO) enhances Nrf2 nuclear localization in NSCs compared to wild-type cells after Aβ exposure.Methods: Nrf2 nuclear localization was analyzed in WT and p66Shc KO NSCs exposed to Aβ for 24 hours. Phosphorylated p66Shc, Nrf2, and Aβ levels were measured in hippocampal neurons in WT and AD mouse brains across various ages using microscopy and Western blot techniques.Results: 1. p66Shc KO NSCs show higher Nrf2 nuclear localization than WT NSCs 2. Elevated neuronal p66Shc correlates with reduced Nrf2 nuclear accumulation in old AD mice 3. Aβ plaque deposition is higher in old AD females compared to males 4. Activated p66Shc levels are higher within/around plaques in female AD mice.Conclusion: These findings position p66Shc as a promising therapeutic target to mitigate neurodegeneration and enhance neurogenesis, with particular relevance to the higher incidence of AD in women.

P2-C-93 - Progression of olfactory function and alpha-synucleinopathy in Parkinson's disease fibril model

Ruth Tran [1], Juliet Arsenault [1], Zihe Chen [1], Jun Chul Kim [1]

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Parkinson's disease (PD) is characterized by motor deficits which typically appear in stage 3/4 when approximately 50% of substantia nigral neurons are lost. Olfactory dysfunction occurs in 90% of patients and emerges at least 4 years before the motor symptom onset. Alpha-synuclein (a-syn) pathology accumulates in the olfactory system in the first stage of PD, particularly in the anterior olfactory nucleus (AON). The AON, a critical hub for olfactory processing, integrates bottom-up sensory input from the olfactory bulb (OB) and contextual input from the hippocampus, facilitating odour detection, discrimination and memory. Although hyposmia is prevalent in PD, the underlying mechanisms remain unclear. To address this gap, we used A53T mice injected with a-syn fibrils into the AON to model PD. Mice performed a go/no-go task to assess olfactory sensitivity, discrimination and memory. Histology was performed longitudinally to evaluate the progression of a-synucleinopathy. Olfactory detection and discrimination function remain intact in PD mice, possibly due to sparse labelling of a-syn in the OB. Preliminary findings indicate that contextual odour memory appears to be impaired at 1 month post-injection (mpi) but recovers over time, coinciding with dense a-syn pathology in the AON at 1mpi, followed by a reduction in a-syn at 4mpi, potentially reflecting clearing mechanisms. The outcomes of the current study will enhance our understanding of olfactory deficits in PD and aid in developing a diagnostic tool for identifying PD patients at the earliest possible stage.

P2-C-94 - Reserve or repair? Using a repeated injury model to challenge the regenerative capacity of radial glial cells in the zebrafish forebrain

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

P2-C-95 - Chloride regulation and GABAergic transmission during the developmental GABA switch in the striatum of Huntington's disease

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Huntington's disease (HD) is an inherited neurodegenerative disease characterized by striatal degeneration. It results in dysfunctional neuronal chloride regulation, which impacts gamma-aminobutyric acid (GABA) mediated inhibition of the striatum at symptomatic stages. The hyperpolarizing function of GABA is determined by the balance of cotransporters, KCC2 and NKCC1, which regulate intracellular chloride. During the first two postnatal weeks, GABAergic signalling transitions from excitatory to inhibitory due to an increase in KCC2 expression, thus reducing intracellular chloride levels. This developmental transition termed the "GABA switch" establishes GABA mediated inhibition in the mature brain. Emerging evidence suggests disruptions in neurodevelopmental processes in HD prior to the appearance of clinical symptoms. Given the critical role of the GABA switch for the establishment of inhibition in the CNS, it remains unknown if potential delays or alterations in this process are contributing to the inhibitory synaptic dysfunction observed in HD. To determine whether a delay in the GABA switch underlies inhibitory synaptic dysfunction in HD, we investigated the expression and function of KCC2 and NKCC1 during the first two postnatal weeks using biochemical and electrophysiological assays. We discovered reductions in KCC2 and NKCC1 during the first two postnatal weeks, and depolarized GABAergic signalling in the second week in HD. Our findings reveal a fundamental role of GABA maturation during the neurodevelopment of HD.

P2-C-96 - Effect of L-Theanine on Cerebellar Granule Cell Migration related to cognitive disorders

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Introduction: Cerebellar granule cell migration plays a crucial role in cerebellum development, and any abnormalities in CGC migration can lead to significant neurological disorders such as anxiety, a common psychological disorder that impacts a person's emotional, physical, and social health.L-theanine, an amino acid found in green tea, demonstrates neuroprotective properties and regulates the release of neurotransmitters by stimulating CGC migration. This study investigated the impact of L-theanine on CGC migration related to cognitive disorders.Methods: ddY male mice were treated with a single oral dose of L-theanine at different concentrations (0.05 mg/ml, 0.5 mg/ml, and 5 mg/ml), and their anxiety was tested via a maze test, where the average completion time taken by mice has been considered an indicator of cognitive performance. CGC microexplants were isolated from newly born C57BL/N6 mice and treated with a series of increasing concentrations of L-theanine. The migration distance of the CGC under the different L-theanine concentrations was assessed after 24, 48, and 72 h post-treatment using phase-contrast microscopy and image analysis software.Results and conclusion: Mice's anxiety symptoms improved based on their performance on the maze test after treatment with L-theanine at 5 mg/ml. Compared to other concentrations, L-theanine at 1 μM yielded the longest migration distance for CGC in vitro. These findings indicate that L-theanine may serve as a potential therapeutic agent in supporting cerebellar development and enhancing cognitive skills. Further investigation is required to fully elucidate the molecular mechanisms and therapeutic potential of L-theanine in neurodevelopmental disorders.

P2-C-97 - Stearoyl-CoA desaturase inhibition leads to fatty acids normalization and improved dendritic spine density in the hippocampus of the 5xFAD mouse model

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Alterations in brain lipids are a central feature of Alzheimer's disease (AD), nevertheless therapeutic strategies targeting brain lipid metabolism are still lacking. Our lab recently reported that a pharmacological inhibitor of the fatty acid enzyme, stearoyl-CoA desaturase (SCD), led to recovery of hippocampal synapses with associated improvements in learning and memory in the slow-progressing 3xTg-AD mouse model. Here, we used the 5xFAD rapidly progressing AD model to further delve into lipid metabolism disruptions in AD, and into the effect of the SCD inhibitor (SCDi) on fatty acid (FA) alterations and synapse loss. Hippocampi, cortex and plasma from 5xFAD and non-carrier control mice were collected for FA profiling and for disease hallmarks. SCDi or vehicle was infused via intracerebroventricular osmotic pumps for 28 days in 5 months old (5MO) 5xFAD and NC mice, and their hippocampi were processed for FA profiling and dendritic spine quantification. FA alterations were apparent in female hippocampus at 5 MO (together with plaque pathology and gliosis) and worsened by the age at 8 MO, while males first showed FA alterations at 8MO. The C16:1/C16:0 desaturation index, parameter associated to SCD enzymatic activity, showed a significant increase in 5xFAD mice at 8 MO, but starting at 5MO in females. Treating 5xFAD mice with SCDi improved dendritic spine density and normalized FA levels. These data demonstrate that SCDi treatment in the more aggressive 5xFAD model has beneficial effects on FA alterations and hippocampal dendritic spines and they add to accumulating data supporting SCDi as a promising novel therapeutic target for AD.

P2-C-98 - A novel intravenous antisense oligonucleotide therapy approach in a mouse model of Dravet syndrome

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Dravet Syndrome (DS) is a severe developmental epileptic encephalopathy caused by SCN1A mutations, with debilitating seizures, developmental delays, high morbidity and a high incidence of premature Sudden Death in Epilepsy (SUDEP). Current treatments provide only partial symptom relief but fail to address the underlying genetic abnormality, highlighting the need for effective and curative therapies. To address this, we evaluate a novel intravenous CNS-penetrating nanoparticle system of the therapeutic candidate, C-TERP-SCN1A Antisense Oligonucleotide (ASO), developed by QurCan Inc., in a 129SvTec/C57 SCN1A mutant mouse model. CTERP-ASO penetrates the blood brain barrier without hepatotoxicity and is de-risked for clinical applications. In preclinical models, it preferentially accumulates in neurons in the brain and is shown to silence target genes (>80%). This approach offers superior safety, efficacy, and extrahepatic targeting over other viral, non-viral, and lipid-nanoparticle delivery platforms. In this study, at postnatal day 8, SCN1A mutant mice injected via the facial vein at postnatal days 1-2 with CTERP-ASO had a higher fold change in mRNA and higher SCN1A protein expression, as compared to wildtype littermates and saline controls. SCN1A mutant DS mice injected with CTERP-ASO also had a higher survival rate (lower SUDEP-mortality rate) as compared to the no injection controls, as observed in P40 animals. Overall, this study serves as proof of principle of a novel non-viral intravenous ASO therapy targeting the brain and could be a promising treatment for DS.

P2-C-99 - Non-invasive delivery of AAV9.SIRT3-myc via MR-guided-focused ultrasound as a disease-modifying therapy in a rat model of Parkinson's disease

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Mitochondrial dysfunction is central to the pathology of Parkinson's disease (PD). SIRT3 is the major deacetylase in the mitochondria, regulating metabolic processes, oxidative stress, mitochondrial dynamics, proteolysis, and inflammation. In pre-clinical rat models of PD, we have previously shown that intranigral infusion of AAV1.SIRT3-myc has both neuroprotective and neurorestorative effects. This suggests that increasing SIRT3 expression may provide disease-modifying effects in PD. However, as PD mostly occurs in the elderly, treatment requiring brain surgery can result in complications. Thus, we assessed whether non-invasive MR-guided-focused ultrasound (MRgFUS)-mediated delivery of AAV9.SIRT3-myc into PD affected brain regions provides disease-modification in the AAV-transduced mutant A53T α-synuclein rat model of PD. Following IV infusion of AAV9.SIRT3-myc, MRgFUS temporarily opened the BBB of the targeted striatum, hippocampus, and SNc, resulting in a 2.8-, 2.5-, and 1.5-fold increase in SIRT3 expression respectively. In parkinsonian rats, elevation of SIRT3 prevented motor dysfunction in the forelimb asymmetry test (A53T+Control:43.0±8.7% vs. A53T+SIRT3:18.2±6.1%). In parkinsonian rats, stereology showed a 56.4±15.7% decrease in

dopaminergic cells compared to non-parkinsonian rats. This loss of dopaminergic neurons was prevented by MRgFUS-mediated delivery of AAV9.SIRT3-myc, degeneration of dopaminergic cells was reduced. These findings support the further development of MRgFUS-mediated delivery of AAV9.SIRT3-myc as a potential disease-modifying agent for PD.

P2-C-100 - Decoding alternative polyadenylation mechanisms in ALS and FTLD through a single-nucleus transcriptome atlas of the orbitofrontal cortex

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Background: The pathogenic mechanisms underlying amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) remain poorly understood, particularly at the cell type level.Objective: To decipher cell type-specific gene expression and alternative polyadenylation (APA) mechanisms in the orbitofrontal cortex (OFC) of ALS and FTLD cases.Methods: We generated a single-nucleus transcriptomic atlas of 103k cells from ALS/FTLD-affected OFC. Using 3' end sequencing, we identified APA changes with stringent thresholds and developed APA-Net, a deep learning model, to predict APA shifts using RNA sequences and RNA-binding protein (RBP) profiles.Results: Differential expression analyses highlighted cell type-specific disruptions, with upper-layer excitatory neurons most impacted. Genes involved in autophagy and glutamatergic signaling were upregulated, while those regulating chromatin remodeling and RNA metabolism, including RBPs, were downregulated. Integration with snRNA data from prefrontal and motor cortices revealed both region-specific and shared ALS transcriptomic signatures. APA analyses uncovered a global switch toward distal and intronic PA site usage, independent of gene expression, across neuronal and glial cells. APA-Net identified RBP interactions, including TDP-43 with HNRNPA1, MBNL1, and others, as key regulators.Conclusion: This study reveals overlapping and distinct transcriptomic profiles and regulatory processes influencing APA in ALS/FTLD.

P2-C-101 - The role of docosahexaenoic acid, an omega-3 fatty acid, as an acute post-stroke treatment

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Introduction:Stroke is the second leading cause of death and the primary cause of long-term disability. Dietary fats affect the risk of stroke. High fat diets (HFD) lead to obesity and systemic inflammation which elevates the risk of stroke and worsens recovery outcomes. In contrast, diets rich in omega-3 polyunsaturated fatty acids like Docosahexaenoic acid (DHA) are neuroprotective and anti-inflammatory. DHA is a major lipid component of the blood-brain barrier (BBB) and neuronal membranes, highlighting its essential role in brain function. I hypothesize that acute post-stroke DHA application will attenuate BBB breakdown and inflammation to rescue injured neurons from delayed cell death and improve post-stroke outcomes.Methods:Examine whether post-stroke DHA application improves stroke outcomes, in lean (Chow-fed) and obese (HFD-fed), male and female mice will receive daily DHA i.p. injections for two weeks post-stroke. Functional recovery, infarct volume and inflammation will be assessed. To test whether DHA has a direct effect on neuron survival post-stroke, DHA will be applied to mouse cortical neurons in culture following oxygen-glucose deprivation and cell survival will be analyzed.Results:Preliminary results have demonstrated a reduction in BBB

breakdown in lean and obese male mice at 24 h post-stroke. Analysis of functional recovery and infarct volumes in male and female mice are in progress.Conclusions:We hope to demonstrate that post-stroke DHA application can reduce inflammation, restore BBB integrity and improve stroke outcomes.

P2-C-102 - Long-term neuronal network disruptions in the retrosplenial cortex-hippocampus circuit underlie neonatal hypoxic-ischemic brain injury

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Perinatal and neonatal hypoxic-ischemia (HI) can result in hypoxic-ischemic encephalopathy (HIE), affecting 1–3 per 1000 newborns and constituting a leading cause of neonatal mortality. Survivors often experience persistent neurodevelopmental deficits and motor impairments, including impaired motor learning, which depends on the retrosplenial cortex (RSC) and hippocampus. However, the neuronal network alterations underlying HI-induced motor learning deficits remain unclear. This study examined the long-term effects of neonatal HI on neuronal activity in the RSC and hippocampus using a mouse model of neonatal HI brain injury. Simultaneous electrophysiological recordings of neuronal firing and local field potentials were conducted in freely moving adult mice subjected to sham or HI surgery at the neonatal stage. Following HI, a significant reduction in neurons recorded per tetrode was observed, with remaining pyramidal neurons displaying abnormal bursting and synchronized firing patterns in the ipsilateral RSC and hippocampus. Pathological spike-field synchrony was evident, with many pyramidal neurons phase-locked in the theta-to-alpha oscillatory band. Neonatal HI mice also exhibited impaired motor learning and reduced engagement of ipsilateral pyramidal neurons during behavior. This study reveals, for the first time, that disruptions in firing patterns and RSC-hippocampal communication contribute to long-term motor learning deficits following neonatal HI brain injury.

P2-C-103 - High 2-AG potentiates amphetamine-induced dopamine levels; Prolonging dopamine time-course and increasing peak levels

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Serious, debilitating, and lifelong conditions, including psychosis in schizophrenia, mania in bipolar disorder, and attention deficit hyperactivity disorder, are due to high dopamine (DA) signalling. We have found, through behavioural pharmacology, molecular, lipidomic, and PET studies, there is profound brain-region specific remodeling of 2-arachidonoylglycerol (2-AG) in high DA pathologies, highlighting harmful effects of 2-AG increase in DA pathologies. However, it is unknown how increasing 2-AG potentiates DA dynamics in pharmacological models of high DA. We assessed DA dynamics using fiber photometry, increasing both DA and 2-AG pharmacologically in two brain regions, dorsal lateral striatum (DLS) and nucleus accumbens (NAc) in mice.Acutely increasing 2-AG potentiated amphetamine-induced DA release, increasing area under the curve (AUC), max peak in time-course of DA signal; DLS (AUC: $p<0.0001$; peak: $p=0.0126$) and NAc (AUC: $p<0.0001$; peak: $p=0.0012$). We see influences of sex on potentiation magnitude.We confirm that modulation of DA release occurs with pharmacological increase of

2-AG in vivo; increased 2-AG potentiates high DA states. This confirms our hypothesis: increased 2-AG is detrimental to high DA pathologies. This provides mechanistic insight into our previous work where increased 2-AG in high DA states potentiates hyperactivity, increases reward association, dysregulates habituation to acoustic startle, dysregulates lipidomics, and compensates via downregulation of CB1 globally, suggesting a contraindication for increasing 2-AG in high DA states.

P2-C-104 - Purinergic dysregulation in the Fmr1-knockout mouse hippocampus

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Fragile-X Syndrome is the most common form of intellectual disability among children and is often comorbid with autism spectrum disorder. It occurs when the Fmr1 gene on the X chromosome is silenced, thereby preventing the production of Fragile X messenger ribonucleoprotein (FMRP). Since FMRP is an mRNA regulator mainly expressed by neural cells, protein homeostasis in these cells is primarily affected. Several pathways affected by this protein dysregulation have been identified, but viable treatments have yet to be developed. Using the Fmr1 knockout mouse model, our lab has focused on the dysregulation of the purinergic signalling pathway, where ATP, UTP and their metabolites are used for cell-cell signalling via purinergic receptors. Preliminary clinical trials with broad purinergic inhibitors have improved Fragile-X symptoms; however, a range of side effects from these general inhibitors make it necessary to find more specific inhibitors. As such, my work focuses on characterizing purinergic signalling in the Fmr1 knockout mouse hippocampus, a region heavily impacted in Fragile-X. Using this model, I found that the expression of the P2X7 and P2Y2 receptors was altered during the period of hippocampal synaptic refinement. The expression patterns of P2X7 suggested a neuroinflammatory state, as its expression was reduced on neurons yet increased on microglia. At the same time, an upregulation of P2Y2 was specific to astrocytes, affecting their activity profile. These results suggest that the dysregulation of purinergic signalling in the Fmr1 Knockout hippocampus involves several hippocampal cell types and may be involved in chronic neuroinflammation during hippocampal maturation.

P2-C-105 - Cytokine expression changes in the striatum of the AAV-aSyn rat model of Parkinson's Disease

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of dopaminergic neurons and aggregation of alpha-synuclein (aSyn). PD progression is amplified by inflammatory processes, such as pro-inflammatory cytokine release. We examined cytokine changes in a widely used rat PD model based on a unilateral injection of adeno-associated virus1/2 (AAV1/2)-containing a human A53T aSyn gene in the substantia nigra. The AAV-aSyn model was induced in female Sprague-Dawley. At 6 weeks post-injection, forelimb asymmetry measured by the cylinder test indicated a trend toward mild aSyn-induced motor deficit. Striatal dopamine, measured by liquid chromatography and mass spectrometry, was significantly reduced by 33% in AAV-aSyn injected animals compared to those receiving empty vector (EV). To assess changes in striatal cytokine levels, we analyzed 19 cytokines using a multiplex membrane-based antibody array. In striatal samples, Cytokine-Induced Neutrophil

Chemoattractant-2 and -3 (CINC-2, -3), Ciliary Neurotrophic Factor (CNTF), Interleukin-6 and -10 (IL-6, -10), fractalkine (CX3CL1) and C-X-C motif chemokine 5 (LIX/ CXCL5) exhibited a significant difference in expression between AAV-aSyn and control groups. At the same time, no differences in any of the 19 cytokines were detected in plasma samples, implying cytokine expression changes restricted to the nigro-striatal tract. The results of this study are valuable for understanding the cytokine changes occurring in an established preclinical PD model, supporting its utility for PD research and drug development.

P2-C-106 - Treatment with a pH modulator partially rescues glutamate-aspartate transporter (GLAST) mistrafficking in a Christianson syndrome mouse model

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Ataxia is one of the main characteristics of the neurological disorder Christianson syndrome (CS) for which there is no treatment yet. CS is caused by loss-of-function mutations in the SLC9A6 gene encoding the endosomal sodium-proton exchanger 6 (NHE6). NHE6 plays an important role in controlling the pH of endosomes to allow proper trafficking of cargo, such as receptors, for normal cell function. NHE6 loss-of-function leads to endosomal overacification causing cargo mistrafficking. In our CS mouse model, we found that the primary cells of the cerebellum, Purkinje cells (PC), are vulnerable to cell death in the anterior but resilient in the posterior cerebellum. In post-mortem brain tissue of CS patients and our mice, elevated glutamate levels were found, possibly causing excitotoxic cell death. To prevent excitotoxicity, excitatory amino acid transporters (EAATs) are important to remove excess glutamate. A large amount of glutamate is removed by EAAT1 (GLAST), found in Bergmann Glia. We hypothesized that GLAST is mistrafficked in the anterior cerebellum resulting in lower glutamate reuptake and PC death. Prior to PC death, we found lower GLAST levels in CS mice, more in the anterior than posterior cerebellum and GLAST was mislocalized more to the lysosomes, supporting the hypothesis that mislocalized GLAST leads to higher glutamate possibly causing cell death. Treatment with a pH modulator in vitro partially rescued GLAST mistrafficking. Future studies administering a pH modulator in vivo would reveal whether it could prevent cell death and improve motor coordination.

P2-C-107 - The impact of blood-brain barrier modulation by focused ultrasound on oligodendrogenesis for Alzheimer's disease

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Background: Myelin loss has been reported in the early stages of Alzheimer's disease (AD); however, strategies to support myelin-producing oligodendrocytes remain limited. Focused ultrasound (FUS) combined with microbubbles (MB) increases the permeability of the blood-brain-barrier (BBB) in a non-invasive and controlled manner. FUS promotes elements of brain regeneration, including hippocampal neurogenesis. To further explore the regenerative effects of FUS in AD, we evaluated its impact on oligodendrogenesis. Methods: TgCRND8 mice were used as a model of amyloidosis. FUS was targeted unilaterally to the hippocampus. Coinciding with FUS application, mice received an intravenous injection of MB; MB absorb ultrasound energy and

oscillate, transiently increasing the permeability of the BBB. We quantified the proliferation of OPCs and evaluated their maturation. Results: In the presence of amyloid pathology, FUS led to a 1.4-fold increase in mature oligodendrocytes in FUS treated compared to untreated hippocampi at 30 days post-FUS (n=4, 2M, 2F). At 7 days post-FUS, a trending increase in newborn OPCs was observed in FUS-treated compared to untreated hippocampi (n=4, 1M, 3F). A power analysis suggests a sample size of n=9 is required to reach significance (d=0.5, α=0.05). Significance: This first-of-a-kind study demonstrates that FUS stimulates oligodendrogenesis in the context of AD. Exploring the process of FUS-induced oligodendrogenesis and gaining mechanistic insights will advance the understanding of FUS as a novel therapeutic modality for AD and other white matter diseases.

P2-C-108 - Targeting anaplastic lymphoma kinase (ALK) ameliorates synaptic dysfunction linked to C9orf72 haploinsufficiency

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Hexanucleotide repeat expansions in C9orf72 are the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). These expansions lead to C9orf72 haploinsufficiency and reduced protein expression, disrupting synaptic function. This causes altered neuronal morphology, glutamatergic imbalances, and dysregulated pre- and post-synaptic proteins, linked with aberrant actin dynamics. Such disruptions are central to ALS/FTD pathogenesis. Lorlatinib, an ALK inhibitor used in cancer therapy, regulates actin dynamics via the PI3K-LIMK-cofilin pathway. We investigated lorlatinib's therapeutic potential in rescuing neuronal phenotypes caused by C9orf72 haploinsufficiency.Using embryonic primary cortical neurons from C9orf72+/- mouse, we assessed dendritic arborization and glutamatergic neurotransmitter systems via immunocytochemistry and immunoblots. C9orf72-haploinsufficient neurons showed reduced dendritic branching and heightened vulnerability to excitotoxicity, linked to increased calcium-permeable AMPAR subunit GluA1 at post-synaptic sites. These synaptic dysfunctions arise from dysregulated PI3K/Akt and LIMK1/cofilin pathways. Crucially, lorlatinib rescued dendritic complexity and improved neuronal resilience to glutamate-induced damage by normalizing these pathways.Our results underscore the role of synaptic pathology in ALS/FTD and highlight lorlatinib's potential as a therapeutic strategy targeting C9orf72 haploinsufficiency-induced synaptic deficits.

P2-C-109 - Temporal characterization of alpha-synuclein pathology in a bilateral model of Parkinson's disease

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One common feature of Parkinson's Disease (PD) is the presence of pathological forms of a-synuclein (aSyn) throughout the central nervous systems of patients . The established AAV-aSyn rat model of PD which utilizes viral vectors to mediate the overexpression of aSyn, produces neurodegeneration within the injection site with limited spread to other brain regions. In this study, we aimed to assess the temporal characteristics of pathological aSyn in a bilateral rat model of PD to better understand its advantages and limitations in recapitulating the human disease. The nigra of female Sprague-Dawley rats were bilaterally injected with AAV1/2 expressing hA53T-aSyn or empty vector. A battery of behavioural assessments were performed

at 3-week and 6-week post injection to assess development of motor and non-motor deficits (including Open Field Assay (OFA)). Tissue was collected at 3- and 6-weeks and used to quantify total transgenic and aggregated aSyn in the nigrostriatal pathway via ELISA and to evaluate the level of neurodegeneration. This bilateral rodent model of PD exhibited signs of motor dysfunction as indicated by a reduction in the time animals were mobile in the OFA test. In addition, while levels of transgenic and aggregated aSyn continued to rise over the 6-week period in the substantia nigra (injection site), levels of pathological aSyn plateaued within the striatum at 3-weeks. This work helps define the development of synucleinopathy and the emergence of motor and non-motor symptoms in this rodent model of PD.

P2-C-110 - Acute effects of functional electrical stimulation during visual feedback balance training on corticospinal and spinal excitability in individuals with incomplete spinal cord injury: a case-control study

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Functional electrical stimulation (FES) is widely used for gait and upper limb rehabilitation but less commonly applied to improve standing balance, with its neural mechanisms still not fully understood. This study aimed to investigate the immediate effects of FES on the excitability of neural pathways. Sixteen neurologically intact individuals and eleven individuals with chronic motor incomplete spinal cord injury (iSCI) participated in this study. They underwent two sessions of visual feedback balance training (VFBT), one with FES (FES+VFBT) and one without FES (VFBT), with at least 48 hours between sessions. Motor evoked potentials (MEPs) were assessed using transcranial magnetic stimulation targeting the soleus and tibialis anterior muscles to evaluate corticospinal excitability. Soleus F-wave was measured to quantify spinal excitability. Results showed a significant increase in soleus MEP amplitudes following FES+VFBT compared to VFBT alone in both groups (36.1 ± 41.5% vs. 0.461 ± 26.2%, $p < 0.001$). Tibialis anterior MEPs showed no significant changes post-training in both conditions across groups (55.2 ± 94.3% vs. 13.1 ± 47.0%, $p = 0.077$). In the iSCI group, soleus F-wave amplitudes were significantly higher post-FES+VFBT compared to VFBT (48.4 ± 77.6% vs. -9.69 ± 44.8%, $p = 0.003$). In conclusion, FES therapy may enhance corticospinal excitability of the plantarflexors, potentially influenced by increased spinal excitability.

P2-C-111 - The role of beta-parvin in the regulation of blood flow dynamics in Ischemic stroke

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Ischemic stroke is a vascular event resulting from an obstruction in a cerebral blood vessel. This in turn leads to a gradient of hypoxia not only impacting the initial infarct site, but also the surrounding tissue. In response to ischemia, the vascular endothelium upregulates expression of signalling pathways that regulate cell adhesion, angiogenesis, inflammation and phagocytic activity. This leads to the recruitment of immune cells, poor microcirculation, degradation of the blood-brain barrier (BBB) and angiogenesis. Recent transcriptomic work from our lab indicated that beta-parvin is upregulated in disease conditions associated with poor microcirculation and

neuroinflammation. Beta Parvin is expressed primarily in endothelial cells and is known to regulate cell migration and adhesion. In order to understand what role Beta Parvin might play in disease conditions such as stroke, we first examined the expression of this protein at different time points after stroke. Immunohistochemistry assays showed that beta-parvin expression was upregulated in the peri-infarct region after 24 hours, corresponding to the acute phase of tissue damage and inflammation. However, this expression subsided over the subsequent days, in which the immune response and repair mechanisms become activated. Ongoing experiments are focused on understanding the role that beta-parvin plays in regulating blood flow dynamics (e.g. capillary stalling and BBB permeability), vessel repair, and angiogenesis after ischemic stroke.

P2-C-112 - Habenula-targeted DBS as a therapy for emotional and sensory dysfunctions in fragile X-ASD mice

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Fragile X syndrome (FXS), the leading inherited cause of intellectual disability and autism spectrum disorder (ASD), results from mutations in the Fmr1 gene leading to a lack of the corresponding protein (FMRP). This protein deficiency disrupts neural functions, contributing to anxiety, altered sensory sensitivity, and cognitive impairments. The Fmr1 knockout (Fmr1-KO) mouse model exhibits these abnormalities, making it valuable for translational research. The habenula (Hb), a regulator of social and emotional behaviours within the fronto-limbic-striatal network, is a promising target for intervention. This study investigated the effects of deep brain stimulation (DBS) of the Hb on brain and behavioural deficits in Fmr1-KO mice. DBS-treated mice received daily Hb stimulations (0.3V, 100Hz, 60µs) for 3 hours over six days. Behavioural assessments included sociability, anxiety, thermal sensitivity, and circadian rhythms. Post-mortem MRI was used to analyze brain structures. Baseline Fmr1-KO mice exhibited heightened anxiety, reduced sociability, impaired thermal sensitivity, and disrupted circadian rhythms. Hb-DBS significantly improved anxiety (p<0.01), sociability (p<0.05), and sensory function (p<0.05) but did not alter circadian rhythms. Structural MRI revealed Hb-DBS-related changes in the orbitofrontal cortex and amygdala, regions critical for emotional regulation. These findings demonstrate that Hb-DBS mitigates emotional and sensory dysfunctions in FXS, supporting its potential as a therapeutic intervention for neurodevelopmental disorders.

P2-C-113 - Characterizing the effects on resting-state MRI, cognitive flexibility, and daily activity of preformed fibrils (PFF) injection in marmosets' striatum

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The proportion of Canadians at risk of Parkinson's disease (PD) has been increasing due to an aging population, but despite years of research, there is no effective cure. Abundant rodent research has been applied for drug development and testing but often failing to translate to humans. As such, we have opted to utilize the common marmoset (Callithrix jacchus) as an animal model of PD. Previous studies on marmosets showed that PFF injection led to an aggregation of alpha-synuclein (alpha-syn) (i.e. synucleinopathy), but its behavioral and cognitive effects have not been explored yet. To identify the relationship between neural PFF progression and its effect on cognition, the present study utilizes awake resting-state magnetic

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resonance imaging (MRI), structural MRI, touchscreen tasks and behavioral measures on PFF and saline-injected adult marmosets. Our first cohort consists of 3 marmosets (2 M, 1 F). After the PFF injection in the left striatum, MRI, touchscreen (pairwise visual discrimination task), and activity data are collected every two months, aiming to identify early biomarkers of PD pathology. Marmosets' behaviors are monitored using actigraphy watches and pose estimation software: DeepLabCut (DLC) and You Only Look Once (YOLO). Here, I will present the method, and the preliminary acquired data (baseline, and 2 months after injection). Due to PFF-induced loss of dopaminergic fibers, we expect a progressive decrease in functional connectivity around the injection sites, lower motor activity and task performance in PFF-injected marmosets over time.

P2-C-114 - Discovery of a novel chronic schizophrenia subtype and its association with vascular brain pathology on autopsy

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Cognitive impairment affects up to 98% of patients with chronic schizophrenia, but its biological basis remains unclear, leaving the debilitating symptom untreated. This impairment is not fully explained by established autopsy findings such as Alzheimer's or vascular disease, necessitating exploration of disease-specific mechanisms. This study analyzed clinical and autopsy data for 55 elderly patients with chronic schizophrenia, including neurocognitive assessments for a subset. Standard autopsies evaluated Alzheimer's and vascular pathologies, and clustering analysis was performed. Only half of patients had pathology severe enough to explain cognitive symptoms. While Alzheimer's prevalence in this population was similar to rates in healthy aging, vascular disease was more frequent and independent from typical risk factors like smoking or antipsychotic use, suggesting an inherent vascular or metabolic etiology. Composite pathology burden was consistent across three patient clusters, but worse cognitive function was only associated with more severe vascular findings in one group. This suggests that vascular features may drive cognitive decline in a specific patient subtype, highlighting schizophrenia's heterogeneity. This underscores the value of stratifying patients by neurocognitive profile to understand the disease and guide targeted interventions. Patients in this vascular subgroup, for example, may benefit most from blood pressure or glucose control. Future larger studies will help reinforce the clinical and biological characteristics of these subtypes at a finer scale.

P2-C-115 - Unlocking the impact of THC on sleep and breathing: A new frontier in sleep therapy

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Background: Sleep is a complex process controlled by the brain. Adequate sleep is crucial for maintaining good health and avoiding disorders such as immune system dysfunction, cardiovascular and psychiatric disorders. During sleep, breathing disturbances may occur, presenting as respiratory interruptions (apneas) and consequent O2 desaturation. With the legalization of cannabis, its active components are increasingly used for conditions including sleep disorders, pain, and anxiety. Among them, Δ9-tetrahydrocannabinol (THC) has dose-dependent effects: sedation at low doses and stimulation at higher doses. While cannabis

products are used for sleep disorders, studies demonstrating its efficacy and safety remain limited. Recently, the use of cannabinoids has been proposed for the treatment of obstructive sleep apnea. The project evaluates effects of acute THC administration on sleep and breathing in adult male rats. Methods: Rats were implanted with EEG and EMG electrodes to monitor sleep-wake cycles and respiration. Rats were recorded inside whole-body plethysmographs to measure breathing and metabolism after vehicle or THC (1-5 mg/kg, i.p.) administration. Results: THC increased NREM sleep bouts duration; THC altered REM sleep by increasing neocortical and decreasing hippocampal power. THC also increased tidal volume and reduced respiratory frequency during NREM without impacting the minute ventilation or apnea frequency. Conclusion: THC improves sleep consolidation and has modest effects on breathing in NREM and REM stages. It also modulates EEG power during sleep.

P2-C-116 - Anxiodepressive-like behaviour changes and dendritic structural plasticity in the ACC of mice with chronic neuropathic pain

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Chronic pain is comorbid with the development of anxiety and depression. These changes are likely governed by neuroplastic mechanisms across multiple brain regions associated with chronic pain, one such region being the anterior cingulate cortex (ACC). The ACC is necessary for perpetuating a state of chronic pain, acting as a hub of this network and is responsible for aspects of pain hypersensitivity and emotion-related changes. However, exactly how these neuroplastic changes manifest in the ACC across male and female mice is yet to be fully understood. In this work, adult male and female C57 mice were exposed to a spared nerve injury (SNI)-induced neuropathic pain model, or a SHAM surgery. The presence of a pain phenotype denoted by allodynia was found to be present in the SNI mice, and absent in healthy SHAM controls via the Von Frey test. The mice were then assessed for changes in anxiety-like and depression-like behaviour in a behaviour battery leading up to a time point of two months post-injury. Once reaching this time point, mice from each group were assessed for structural neuroplastic-related changes via Golgi-cox staining, where dendritic spine density on both apical and basal dendrites of pyramidal neurons in layers II/III of the ACC were assessed across groups. These data highlight the multiple avenues in which chronic pain can be quantified at the behavioral and histological levels, and the possible sex differences present to further elucidate associated neuropathic pain disorders.

P2-C-117 - Somatostatin receptor 1 promotes neuritogenesis and stabilizes Microtubule-associated proteins in Alzheimer's disease models

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Somatostatin (SST), a growth hormone inhibitory neuropeptide, is known to impede the aggregation of amyloid beta (Aβ). The spatial alignment of Aβ and SST within Alzheimer's disease (AD)-relevant brain regions suggests an interplay between SST biology and AD pathology. SST acts via five receptor subtypes (SSTR1-5), key members of the G-protein-coupled receptor superfamily. Despite the selective expression of SSTR1 in AD-associated brain regions, its role in AD pathophysiology remains unclear. To address this, we examined SSTR1 expression and function in AD models. We unveil SSTR1 downregulated in the cortex and hippocampus of 5xFAD

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mice compared to age-matched controls. Additionally, we noted reduced expression and immunoreactivity of SSTR1 in SH-SY5Y and MC-65 cells. To elucidate the role of SSTR1 in neuritogenesis and Microtubule-associated proteins (MAPs) stability, we overexpressed SSTR1 in SH-SY5Y cells induced differentiation using retinoic acid. Differentiated wild-type and SSTR1-overexpressing cells were treated with/without the SSTR1 agonist (L-797591) and Aβ for 24 hrs. Aβ caused retraction of neurites whereas, SSTR1 agonist suppressed Aβ effect and promoted neuritogenesis. SSTR1 treatment reinstated the perturbed distribution of MAPs in the apical and distal ends of neurites. These effects are linked to SSTR1's inhibition of Aβ-induced cAMP elevation. Our results demonstrate SSTR1-mediated neuritogenesis and MAPs stabilization, attesting to SSTR1 as a potential therapeutic intervention for restoring neuronal circuits and cognition in AD.

P2-C-118 - Role of Dickkopf-1 in thrombo-inflammation associated with cerebrovascular diseases

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Stroke represents a major cause of death and invalidity of adults in Canada. Ischemia and reperfusion jointly induce brain endothelial cell damage, leading to platelets' recruitment and subsequent invasion of immune cells, including neutrophils that drive netosis (neutrophil extracellular traps (NETs)). This cascade of events induces micro-thrombi formation and inflammation of distal microvasculature, a pathological process called thrombo-inflammation. We have recently demonstrated that DKK1 secreted by bone marrow-derived cells exacerbates stroke severity by impairing immune-vascular responses after stroke. Some reports have shown that DKK1 increases the expression of adhesion molecules in endothelial cells. Herein, we posit that DKK1 worsens brain injury by exacerbating thrombo-inflammation.Using a transgenic mouse model that enables conditional induction of DKK1 subjected to experimental stroke via transient middle cerebral artery occlusion (MCAo), we evaluated the impact of its elevated levels on the process of thrombo-inflammation early in the acute phase. Our preliminary results indicate that DKK1 elevated levels in plasma is associated with an increased expression of thrombotic and inflammatory markers, as well as to a high frequency of neutrophils and association with platelets. Moreover, DKK1 induction aggravates netosis and oxidative stress in the injured brain and disturbs fibrinolysis activity via inhibition of tPA. Our findings suggest that circulating DKK1 plays an important role in modulating the process of thrombo-inflammation after stroke.

P2-C-119 - A multimodal approach to studying STXBP1 disorders in zebrafish

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STXBP1 mutations are linked to severe neurodevelopmental disorders, including intractable epilepsy and movement deficits, affecting 1 in 30,000 individuals. To investigate underlying mechanisms and explore potential therapies, we utilized CRISPR-generated zebrafish models (stxbp1a−/− and stxbp1b−/−) replicating movement and epilepsy phenotypes, respectively. Our studies integrate electrophysiology, artificial intelligence (AI), behavior, metabolomics, and large-scale drug screening. A severe movement deficit in stxbp1a−/− larvae was confirmed using

high-resolution locomotion tracking coupled to AI-based movement analysis. Metabolomic profiling revealed significant alterations in small-molecule metabolites that could explain stxbp1a−/− movement deficits. Simultaneous local field potential recording (for monitoring of electrical brain activity) and fiber photometry (for detection of neuronal calcium signal) confirmed spontaneous seizures in stxbp1b−/−larvae. Two complimentary approaches were used for large-scale phenotypic drug discovery: (i) unbiased screening of commercially available libraries and (ii) AI-based screening using 4-phenylbutyrate (4-PBA). Movement or epileptic phenotypes, in stxbp1a−/− and stxbp1b−/− larvae, were not rescued by 4-PBA or 18 AI-identified analogs. Additional candidates identified in unbiased phenotypic screening are currently under further evaluation. These findings highlight the utility of zebrafish STXBP1 models as a powerful platform to advance our understanding of rare neurodevelopmental disorders and facilitate large-scale drug discovery.

P2-C-120 - Prophylactic effect of physical exercise and/or agmatine against lipopolysaccharide-induced depressive-like behavior and inflammatory-related parameters in male mice

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Depression is the psychiatric disorder with the highest prevalence worldwide, affecting approximately 5% of the population and contributing significantly to the global burden of disease. The current therapy approach has certain limitations that require novel strategies, including prophylaxis of this disorder. This study investigates the prophylactic effect of physical exercise (PE) and/or agmatine (AGM) on lipopolysaccharide (LPS)-induced depressive-like behavior and inflammatory-related parameters. Swiss male mice were subjected to 4-week PE, followed by AGM administration. After a 7-day washout period, LPS was administered, and behavioral tests and tissue collection occurred after 24 hours. LPS induced depressive-like behavior in the tail suspension test and anhedonic-like behavior in splash test, an effect prevented by PE, AGM, and PE+AGM. LPS-treated mice spent less time in the center of the open field apparatus (anxiety-related behavior), regardless of PE or AGM. Furthermore, LPS administration resulted in an increase in pro-inflammatory cytokines in the hippocampus (HC) and colon. This increase was mitigated, in part, by PE, AGM and PE+AGM. In addition, LPS increased permeability in the HC and colon, an effect prevented by PE, AGM, and PE+AGM. These results suggest that PE and/or AGM exerted a protective effect against LPS-induced gut and blood-brain barrier permeability, an effect associated with their prophylactic antidepressant effect under LPS-induced inflammatory challenge.

P2-C-121 - Characterization of a novel subpopulation of PDGFRβ-derived perivascular glial cells in the adult brain

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Homeostatic interactions between vascular, neuronal, and glial cells regulate critical neurovascular functions such as cerebral blood flow (CBF) regulation, blood-brain barrier (BBB) function, vascular stability, and immune modulation. Brain perivascular cells expressing

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PDGFRβ play key roles in integrating neighboring signals to maintain these functions. In the adult brain, PDGFRβ is primarily expressed in mural cells, but its expression has been reported in neuronal stem cells (NSCs) as well as a subpopulation of glial cells, the role of which remains unexplored. Using viral approaches combined to mouse genetic tools, we have recently identified a new subpopulation of adult glial-like cells derived from resident perivascular PDGFRβ+ progenitors. Herein, we aim to characterize the role of PDGFRβ-derived perivascular glial cells in regulating neurovascular functions under physiological and pathological conditions, particularly in cerebrovascular diseases.Our preliminary results show that PDGFRβ-derived cells are abundant in the adult cortex and exclusively associated with the vasculature. They closely interact with brain endothelial cells via dense ramifications and respond differently from mural and other glial cells to cerebrovascular disease. Transcriptomic studies are underway to fully elucidate their molecular signature and subsequent functions.Our study reveals the presence of specialized PDGFRβ-derived perivascular glial cells at the neurovascular interface.

P2-C-122 - Behavioural and proteomic effects of nicotine versus tobacco exposure 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 behavioural 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 and that there would be distinct brain proteomic changes resulting from long-term exposure to CSE, NIC, or vehicle (VEH). Methods: Behaviour was 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 28 days with CSE, NIC, or VEH, and brains were excised for proteomic processing. Results: Subjects discriminate between NIC and VEH and between CSE and VEH; however, they are unable to discriminate between CSE and NIC after 72 sessions. In contrast, preliminary proteomic analyses suggests that there are distinct changes evoked by CSE compared with NIC exposure in the midbrain. Conclusions: Our results confirm that CSE is a successful occasion setter and adds to prior NIC literature. Interestingly, we demonstrate that CSE and NIC do not create distinct interoceptive environments under these training conditions and differences may be occurring at the cellular level instead. This has implications for ongoing discussions regarding nicotine as a proxy for tobacco in animal models.

P2-C-124 - Alterations in cerebrospinal fluid biomarkers in COVID-19 patients presenting neurological symptoms

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COVID-19 induces acute and persistent neurological symptoms. Neuroinflammation plays a significant role in both COVID-19 and Alzheimer's disease (AD). A better understanding of molecular alterations and possible links between COVID-19- and AD would lead to improved patient follow-up. Here, we retrospectively investigated 35 COVID-19 hospitalized patients presenting neurological alterations subject to clinically indicated cerebrospinal fluid (CSF) sampling. Clinical and neurological investigation, brain imaging, and CSF molecular analyses were conducted. We compared CSF early AD biomarkers in controls (n=36), amnestic mild cognitive impairment (aMCI, n=19), and AD patients (n=20).Comparisons were corrected by sex, age, and comorbidities effects. The Brazilian Ministry of Health and IDOR approved the study protocol. We found that COVID-19 patients presented heterogeneous neurological symptoms dissociated from lung burden. Patients presented systemic inflammation and CSF proteomics alterations related to innate immunity and hemostasis. Severe COVID-19 patients presented elevated biomarkers of neurodegeneration (CSF Tau) but no changes in biomarkers specific to AD pathology (CSF Ab42/40, pTau-181/Ab42, or Tau/Ab42 ratios). In COVID-19 patients, systemic inflammatory index (SII), disease severity, and neuroinflammation (CSF IL6) correlated with disease severity, pronounced neuroimaging alterations, and AD biomarkers. Given that inflammation may persist post-COVID, our findings urge assessing possible AD-related biomarker changes in COVID-19 survivors.

P2-C-125 - A follow-up investigation: In vitro effects of kefir-derived biomolecules on β-amyloid aggregation

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Kefir is a probiotic-rich fermented milk beverage containing a symbiotic consortium of bacteria and yeasts. Evidence suggests its neuroprotective potential, including its metabolites and fractions, in mitigating β-amyloid (Aβ42)-induced neurotoxicity in neuronal cells and Drosophila melanogaster models of Alzheimer's disease (AD). Building on these findings, we investigated the in vitro effects of kefir-derived fractions and synthetic peptides on Aβ42 aggregation and disaggregation. We tested two kefir fractions (Ethyl Acetate and <10kDa) and two kefir-derived peptides (KDPs). In the preventive assay, Aβ42 (10 μM) was co-incubated with kefir fractions (0.25 mg/mL) or KDPs (1, 10, 100 μM) for 24h, with hourly Thioflavin T fluorescence readings. In the treatment assay, Aβ42 was pre-aggregated for 48h before adding the test compounds, followed by another 48h incubation. Experiments were performed in 96-well plates (quintuplicate). One-way ANOVA showed all treatments significantly reduced Aβ42 aggregation in the preventive assay (p<0.0001). In the late treatment assay, KDP-1 (p=0.0055) and KDP-2 (p<0.0001) significantly disrupted Aβ42 aggregates. These findings highlight the potential of kefir fractions and peptides to prevent and disrupt Aβ42 aggregation in vitro, supporting their therapeutic promise in neurodegeneration. Further studies should explore their mechanisms and in vivo efficacy.

P2-C-126 - Anti amyloid-beta aggregation activity of kefir-derived peptides

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Kefir is a fermented beverage rich in beneficial probiotics, and its water-soluble <10kDa fraction has demonstrated antioxidant activity, acetylcholinesterase inhibition, and neuroprotection in Drosophila melanogaster Alzheimer's disease (AD) models. Using in silico mutagenesis, we designed mutated versions of two kefir-derived peptides (KDPs) to enhance their binding affinity to amyloid-beta (Aβ) and their potential to cross the blood-brain barrier (BBB). We then evaluated their effectiveness in preventing or disrupting Aβ plaque formation in vitro. KDPs were mutated using ToxinPred. ExPASy PeptideCutter yielded digested KDPs (dKDPs). Bioactivity and BBB permeability were predicted with PeptideRanker and BBPpred. Mutated KDPs (mKDPs) and dKDPs were docked with Aβ monomers using ClusPro. Top mKDPs (1, 2, 3) and dKDP were synthesized and tested in a thioflavin T aggregation assay. For early treatment, peptides (1, 10 and 100 μM) were added with Aβ, and fluorescence was measured hourly for 24h. For late treatment, peptides (10 μM) were added after 48h of the addition of Aβ, with a reading at 96h. Statistical analysis used repeated measures one-way ANOVA. In early treatment, mKDP1 reduced Aβ aggregation by 23%, mKDP2 by 56%, mKDP3 by 16%, and dKDP by 57% after 24 hours (p<0.0001 for all comparisons). In late treatment, mKDP1 and mKDP2 reduced Aβ aggregation by approximately 45% (p=0.0002 and p=0.0001, respectively), while mKDP3 and dKDP showed no significant effects. All peptides showed anti-Aβ aggregation effects in early administration, and mKDP1 and mKDP2 in late stages. Further in vivo studies are needed to validate these findings.

D - SENSORY AND MOTOR SYSTEMS

P2-D-127 - Estradiol control of neural firing and auditory perception in mice and humans

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Female mammals experience cyclical physiological and behavioral changes due to hormonal fluctuations, affecting sensory processing. However, whether and how the estrous cycle affects different aspects of auditory sensory processing and the firing of distinct neuronal populations is unclear. We investigated brain responses to auditory stimulation in mice and humans across reproductive phases. On the first day of the ovarian cycle (low estradiol), females of both species showed increased baseline gamma activity, auditory entrainment, as well as altered habituation to repetitive auditory stimuli, with no difference in deviant detection. To probe estradiol's link to these phenotypes, we injected estradiol or saline solution in female mice in metestrus. Baseline gamma power, habituation, and auditory entrainment were rescued within 45 minutes of estradiol, but not vehicle administration, suggesting a causal relationship between estradiol levels and auditory brain responses. In mice, EEG recordings allowed the identification of regular firing (RS) and fast spiking (FS; putative PV+ inhibitory) neurons. Our data show that FS and RS neurons´ firing rates are significantly are reduced during metestrus, which could be reversed by a single dose of estradiol. Our findings suggest that neuronal excitability and firing dynamics driving EEG patterns underlying auditory functions depend on sex and hormonal concentration, emphasizing the need to consider hormonal changes in psychiatric research.

P2-D-128 - Axon-keratinocyte skin interactions and protocadherin γ

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The determinants of successful skin reinnervation by regenerating sensory axons have not been elucidated. Protocadherin-gamma (Pcdhγ), one of the three subfamilies of clustered protocadherin are homophilic cell adhesion proteins that impact dendritic arborization and neurite self-avoidance in the brain. The role of Pcdhγ in the peripheral nervous system (PNS) remains largely unexplored. Here we make the case for a major role of Pcdhγ in axon-keratinocyte interactions based on newly published (Long et al, J Neurosc 2023) and unpublished work. We have encountered Pcdhγ expression in adult sensory neurons but also selectively in skin keratinocytes. Pcdhγ knockdown (KD) alters the behaviour of adult sensory neurons in vitro, with greater outgrowth, plasticity and loss of inter-neurite repulsive growth. In vivo, regenerating axons into the skin of mouse hindpaws have greater extension, more branching and denser patterning after KD. In new work, through the development of adult neuron-keratinocyte cocultures, we show a preferential interaction and contact of neurites with differentiated, rather than undifferentiated keratinocytes. This may correlate with axon navigation past less differentiated stratum basale keratinocytes to later branch and ramify. Within intact skin, where ongoing axon plasticity is essential given keratinocyte turnover, Pcdhγ KD is associated with heightened hindpaw sensitivity. Taken together, our early findings suggest a major role for Pcdhγ in influencing axon-keratinocyte interactions and in the patterning of skin innervation.

P2-D-129 - Modeling long-term plasticity for optimizing functional electrical stimulation-therapy

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Functional Electrical Stimulation (FES)-therapy is a rehabilitation technology that restores motor function and improves quality of life in individuals with neurological impairments, by applying FES to target muscles during voluntary contraction. Tuning FES parameters significantly impacts clinical outcomes; however, traditional Hebbian plasticity frameworks fail to capture the nuanced and heterogeneous spiking dynamics of FES-therapy, limiting their utility in modeling and optimization. To address this, we developed a computational model of activity-dependent plasticity tailored to FES-therapy, incorporating voltage-dependent synaptic dynamics and high-order spiking interactions to simulate long-term potentiation (LTP) and long-term depression (LTD) during FES. Unlike previous models, ours replicates complex spiking protocols.Using this model, we evaluated synaptic weight distributions and plasticity outcomes under varied stimulation protocols, to show how parameters—such as FES frequency, heterogeneity, and timing—affect the balance of LTP and LTD.Our model integrates stimulation heterogeneity, demonstrating a relationship between interspike interval (ISI) variability and plasticity outcomes. Our findings show that ISI heterogeneity significantly influences synaptic weight distributions and plasticity. Reducing ISI heterogeneity biases the system towards LTD, suggesting a novel path for targeted synaptic weakening in rehabilitation protocols.Our work provides a platform to study plasticity in FES-therapy, paving the way for improved FES design and clinical translation.

P2-D-130 - Structure of spontaneous activity in mouse visual cortex

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Introduction: Spontaneous activity in visual cortex is high-dimensional and state-dependent, yet its organizing principles are unclear. We combine large-scale imaging, modelling, and theory to ask:Does spontaneous activity share the structure of evoked activity?Is spontaneous activity organized spatially?Methods: We used a Light Beads Microscope and developed an open-source volumetric cell extraction pipeline. These methods enabled simultaneous Ca2+ imaging of 30,000+ cells from awake mice.Results: Cells with similar visual preferences did not have higher spontaneous correlations, showing that evoked and spontaneous activity do not share the same structure. Spontaneous activity exhibited a weakly spatial structure, with pairwise correlations decaying over ~1mm. We found that the magnitude and dimensionality of shared activity decayed with distance between populations. Cortical correlations could thus be characterized by a few, strong, global dimensions and many weaker local dimensions. We modelled spontaneous activity using an analytically tractable linear RNN with distance-dependent connectivity driven by low-dimensional inputs, which was sufficient to produce high-dimensional activity similar to neural data. Conclusions: Spontaneous activity does not share its structure with evoked activity. It follows a spatial organization with a few global and many local modes. The global modes may correspond to a low-dimensional arousal state, while remaining dimensions may be "reverberations" predicted by our model.

P2-D-131 - The role of Pannexin-2 in vision and adaptive behavior: Insights from a zebrafish model

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Pannexin-2 (Panx2) is a unique ion channel localized to ER-mitochondria contact sites. These specialized intracellular domains are abundant in neurons and glia and essential for calcium homeostasis, inflammation, and apoptosis. How intracellular contacts contribute to neuronal function remains unclear. To investigate Panx2's role in neuronal communication, we generated a Panx2 knockout zebrafish model (Panx2Δ11) using TALEN technology. HCR-FISH demonstrated panx2 mRNA expression in visual centers, like the optic tectum and thalamus. Panx2 protein expression was observed in the retina and optic tract arborization fields, in 6 days post-fertilization TL larvae (Panx2+/+). RNA-seq profiling of Panx2Δ11 larvae revealed downregulation of genes involved in visual and sensory perception and lens development. Consistent with these molecular findings, behavioral assays demonstrated that Panx2 loss impairs visual information processing. Panx2 mRNA is also expressed in other brain regions, including the ventral and dorsal habenula. The adaptive behaviour of Panx2Δ11 larvae, assessed by visual habituation, a form of non-associative learning, demonstrated a decreased response 24 hours post-training. This result suggests a role for Panx2 in memory consolidation and adaptive responses, potentially modulated by the habenula. Given Panx2's localization at ER-mitochondria junctions, organelles critical for neuronal bioenergetics, these findings suggest a potential novel link between Panx2, cellular metabolism, and memory consolidation processes. Funded by NRSC DG RGPIN-2019-06378 (GRZ).

P2-D-132 - Visual Cortex and the Superior Colliculus make concurrent contributions to visual contrast perception in mice

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The primary visual cortex (V1) and the superior colliculus (SC) occupy early nodes in visual processing. Traditionally, V1 is thought to contribute to feature perception while the SC mediates both overt and covert orienting behaviors to sensory input. Recent work from us and others has challenged these divisions. SC neurons encode many of the same visual features as their V1 counterparts and manipulating different SC neurons in behaving subjects disrupts feature detection as well as orienting responses. Our most recent work shows that inhibition of V1 or the SC disrupts visual detection only during the very earliest moments after the onset of a visual stimulus, which suggests that these areas make concurrent contributions to visual detection. Our poster will discuss our recent experiments where mice performed a visual detection task. On some trials, we optogenetically inhibited either V1 or the SC. On other trials, both structures were inhibited concurrently. The behavioral impairment produced by dual V1+SC inhibition was greater than the effects of inhibiting either structure alone, suggesting concurrent contributions to perception. A simple model showed that our behavioral results were more strongly aligned with a model where visual signals arising in V1 and SC are combined into a common signal somewhere downstream compared to a model in which V1 and SC signals are readout independently. The work adds to a growing literature highlighting the role of the SC is visual feature processing and yields new insight into how brains decode visual information for guiding behavior.

P2-D-133 - Distinct temporal dynamics of anterior olfactory nucleus activity in odor-context memory

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The anterior olfactory nucleus (AON) serves a central role in early olfactory processing, integrating bottom-up inputs from 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 population AON activity in Thy1-GCaMP6 mice during the development and expression of odor memories. We found that distinct AON subdivisions showed distinct temporal dynamics of activity, which varies with task complexity. Notably, the dorsal AON fires preemptively in two-chamber but not single-chamber go/no-go tasks, and this preemptive activity is strongest when context-dependent odor memory is required. This supports our hypothesis that the AON stores odor-context memories. Future experiments will leverage one-photon calcium imaging to characterize how AON activity encodes odor memories. This study provides novel insight into the fundamental role of AON circuits in odor-context memory, which has significant implications for understanding how the brain processes sensory elements of episodic memory.

P2-D-134 - Plasticity of short-term and persistent somatosensory adaptation in wild-type and Rett syndrome model mice

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We studied a form of activity-dependent plasticity in Mecp2 mutant (Rett) mice to better understand the loss of MeCP2 function in neuronal circuit and sensory processing. Rett syndrome is a neurodevelopmental disorder displaying symptoms within the autism spectrum. Responses to tactile stimuli were assessed by Intrinsic Optical Signaling (IOS) imaging in primary hindlimb (HL) somatosensory cortex before, during, and after one hour of repeated HL stimulation in wild-type (WT), symptomatic and pre-symptomatic male and female Rett mice. Repetitive stimulation depresses cortical IOS to test stimuli. This "habituation" persists in WT for at least one hour in the absence of further repeated stimulation. In contrast, Rett mice show depression of IOS equivalent to WT, with test responses recovering within 30 min. or less. Using IOS to locate the primary HL cortex, we record cortical LFP in layer 4 from 7 test stimuli with 100 msec ISI. The resulting train of responses show a characteristic short-term adaptation (STA) from pulse 1 to 7. Repetitive stimulation results in depression of the summed 7 stimulus response due to an increase in the STA with little change in the response to stimulus 1. Increased STA persists in WT but reverses rapidly in Rett mice with time-course the same as for IOS. Our current studies investigate the neural substrate of this STA, the basis for its persistence and lack thereof in Rett mice focusing on thalamo-cortical and cortico-cortical circuitry using 2-photon Ca2+ imaging, multi-unit recording and optogenetic stimulation of thalamo-cortical pathways.

P2-D-135 - Investigating the role of the vestibular system in updating spatial auditory attention using galvanic vestibular stimulation

Grace Otto [1]

[1] Western University

Spatial selective auditory attention (SSAA) allows us to attend to sound locations in space; however, it must be updated as we move our head. How is the auditory system informed about this self-motion? Results of prior studies of auditory attention and sound localization from our laboratory suggest that input from the vestibular system may be a key factor; this study tested the hypothesis that vestibular input is both necessary and sufficient for updating of SSAA during rotational self-motion. To study vestibular input in isolation from proprioceptive activation, galvanic vestibular stimulation (GVS) was used to mask or create sensations of self-motion. We measured the effectiveness of noisy GVS in preventing updating during head rotations and of sinusoidal GVS in eliciting shifts of SSAA in the absence of actual self-motion. To characterize SSAA updating in each condition, we used a behavioral listening task in which participants repeated a series of spoken digits presented from a target loudspeaker while ignoring same-voice distractor digits from flanking loudspeakers. To assess the necessity of vestibular information for SSAA updating, we looked for poorer performance under the application of noisy GVS during self-motion. To assess its sufficiency, we looked for poorer performance and whether rates of distractor mis-reporting were related to instantaneous GVS phase under sinusoidal GVS. Final results will be reported.

P2-D-136 - Parsing the electrophysiological signatures of spatially selective versus distributed attention in neuronal population data

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Attention efficiently allocates our limited cognitive resources by prioritizing specific sensory inputs for further processing to enhance performance and speed reaction times. These behavioural changes are coupled to concurrent changes in neurophysiological processes such as enhanced responses of visual neurons, reduced influence of distracting information, and increased arousal. While these processes covary, emerging literature shows that they arise from distinct neurobiological processes. Given this, parsing the diverse neural mechanisms that support attention is critical to develop new diagnostic tools and therapeutics for attention-related disorders, ultimately improving mental health outcomes.To address, we will analyze a rich dataset of 2,525 neurons recorded in visual area V4 of two macaque monkeys while they performed a challenging visual attention task. Attention was manipulated to engage either selective or distributed attentional mechanisms, allowing us to examine interactions between attentional states, arousal, neuronal spiking, and task performance. Machine learning techniques, including Tensor Decomposition, ISOMAP, and projections onto experimenter-defined subspaces, were employed to identify low-dimensional signatures linked to attentional states. By parsing how shifting between selective and distributed attention impacts neurophysiological and behavioural correlates of attention in neuronal populations, and linking these low-dimensional signatures to spiking activity in visual neurons, we aim to refine our understanding of fundamental cognitive functions.

P2-D-137 - Functional architecture of areas V1, V6 and the dorsolateral prefrontal cortex in the common marmoset

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The primate visual system is hierarchically organized with thalamic inputs reaching area V1 and propagating through different areas until reaching the lateral prefrontal cortex (LPFC) (Felleman & Essen, 1991). A classical view is that each area contains microcircuits composed of canonical motifs, and differences in function across areas are due to their distinctive intra- and inter-area connectivity. An alternative view is that cortical microcircuits vary in their structural and functional properties across the visual processing hierarchy, with variations of the canonical circuit motif impacting the function of individual neurons and population dynamics. To test this hypothesis, we used simultaneous neuropixel recordings in areas V1, V6, and LPFC (Area 8a/46) of common marmosets (Callithrix jacchus, n=2) in two conditions: resting state (gray screen) and passive viewing of static images and animated cartoon clips (5-20 s). We spike sorted the data and isolated single neurons along different cortical layers.We observed that:V1 and V6 had higher firing rates and spike train variability than LPFC cells (p < 0.05).We found two main discharge patterns: Bursting and regular spiking. Bursting cells fired in clusters with ISIs < 10 ms, while regular spiking cells could be modeled with an inhomogeneous Poisson process.There is a functional gradient where cortical units near the occipital pole exhibit a bursting discharge pattern, whereas cells LPFC tend to show regular discharge patterns.Using spike train and waveform parameters, we classified cells from these areas with > 80% accuracy.These results suggest that firing patterns differ across brain regions, accounting for the specific computations these areas perform.

P2-D-138 - The role of G9a in regulating the expression of a cyclic GMP-dependent protein kinase and injury-induced nociceptive sensitivity

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The Drosophila melanogaster foraging gene encodes a cGMP-dependent protein kinase G (PKG) that plays a role in regulating nociception. PKG expression is increased in nerve injury-induced nociceptive hypersensitivity mouse models. It is unknown whether foraging expression changes in response to injury in Drosophila. Furthermore, the mechanisms by which PKG expression are regulated in response to injury remains elusive. The histone methyltransferase G9a has previously been shown to regulate foraging mRNA expression, and higher foraging levels were previously found to cause nociceptive hypersensitivity, whereas loss of foraging reduced nociceptive sensitivity. Despite this, a direct connection between foraging and G9a in the context of nociception has not yet been established. We found that G9a null mutants exhibited nociceptive hypersensitivity compared to their genetic controls. Western blots revealed that FORAGING protein levels, specifically the FORAGING P1 isoform, were elevated in the absence of G9a. Additionally, examination of the G9a foraging null double mutants suggests that G9a acts upstream of foraging in regulating nociception. Overexpression of FORAGING in nociceptors phenocopied the nociceptive hypersensitivity seen in G9a null mutants. Furthermore, ultraviolet (UV) injury induced a nociceptive hypersensitive response in control genotypes. However, this injury-induced nociceptive hypersensitivity was blocked in the absence of foraging. Collectively, our findings demonstrate that G9a negatively regulates nociception and FORAGING P1 protein expression.

P2-D-139 - Molecular and functional profiling of photosensation in the pond snail lymnaea stagnalis

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diverse animal models have been developed to investigate photoreception, however, there remains a pressing need for more integrative models. Invertebrate visual systems of early-diverging species have advanced our understanding of photoreceptive behaviours and retinal processing, but models for invertebrate photoreception remain limited. Here, we established the pond snail Lymnaea stagnalis as a model for visual system research using anatomical, histological, behavioural, and proteomic analyses. Specifically, we characterized the retinal and dermal organization of L. stagnalis, identifying rhodopsin-positive photoreceptor cells in ocular and non-ocular tissues. Using DeepLabCut software, we developed a neurobehavioral test to study phototaxis, and demonstrated positive phototaxis in most snails, likely mediated by TRP channels. Through transcriptome analysis, we further revealed that L. stagnalis expresses genes encoding both vertebrate-like Gt-coupled and invertebrate-like Gq-coupled phototransduction pathways, indicating evolutionary divergence in visual modalities. Finally, proteomic profiling identified conserved signalling pathways in photosensory tissues, highlighting the molecular complexity of its visual system. This study highlights L. stagnalis as a valuable model for research into photosensory systems, providing insights into photoreception and phototaxis mechanisms, and laying the groundwork for exploring molecular and evolutionary aspects of visual function.

P2-D-140 - Attention gates feature dimensions during value-based decision-making

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Reward biases attention to valuable features. However, when value is associated with a conjunction of features, it's unknown whether this prioritization is applied equally across visual attributes, or alternatively, whether only certain attributes are prioritized. To examine this, we utilized a value-based decision-making task where value varied as a 2D Gaussian function over a color × orientation feature-space and subjects learned to associate conjunctions with reward. Subjects could choose between all possible feature conjunctions on each trial and button presses were used to indicate choices allowing us to dissociate economic choices from overt attention. We observed that as feature-reward associations strengthened, an attentional template guided eye movements to valuable features, coinciding with (1) saccades that were faster, shorter, more accurate, and less numerous and (2) a stronger autocorrelation of feature selections between successive saccades. However, feature guidance was primarily driven by color, with orientation matching occurring for only the last few saccades on a trial and color matching occurring for every saccade. After decomposing perceptual biases and salience into color and orientation components, we saw that when saccadic search prioritized one feature dimension, bias and salience of the orthogonal component was suppressed. These results suggest that attentional mechanisms utilize a dimensionality reduction strategy during value-based visual search, even when both feature dimensions provide equal amounts of value information.

P2-D-142 - Individual differences in the evolution of strategies in spatial problem solving

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People vary in their use of strategies to solve problems. Strategy use is, in part, determined by individual differences in cognitive abilities. For instance, some individuals are stronger spatial learners. These people not only solve spatial tasks faster but are also more likely to use spatial information to solve tasks even when other information is available. In addition, peoples' problem-solving strategies evolve over time. Although an array of studies has investigated varying spatial strategies, it remains unknown if performance on an initial spatial task can predict strategy use over time with repeated experience. We addressed this gap and examined the individual differences in the evolution of strategies in spatial problem solving. over time? Participants were tested on mnemonic similarity and object-in-place tasks. Performance in in these tasks was then compared with a virtual navigation task in which participants had to find a random subset of objects placed throughout a virtual maze from varying starting points. Participants were shown a set route to each object, although shorter paths (i.e., shortcuts) were possible. This testing was repeated for 13 testing sessions to assess strategy switching over time. We found that performance on our acute memory tasks predicted both overall performance (i.e., accuracy and latency) as well as the rate at which participants deviated from the demonstrated path and adopted shortcuts. repeated virtual navigation. These data suggest strategy use is largely determined by general spatial memory abilities.

P2-D-143 - Serotonergic neurons in the dorsal raphe regulate visual attention

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Visual attention enhances the neural representation of salient stimuli within the visual cortex. Although it is generally thought that this enhancement is driven by glutamatergic feedback from frontal cortical areas, cortically released neuromodulators are also believed to play a major uncharacterized role. Here we report the unexpected observation that dorsal raphe (DR) derived serotonin (5HT) controls visual attention. We adopted a behavioral model that captured how mice allocated attention to cued and uncued visual features. Simultaneous photometry recordings showed that DR activity decreased when mice deployed attention to the cued features, whereas high DR activity was observed when mice were less attentive. Optogenetic excitation of DR-5HT neurons impaired attention to the cue and degraded behavioral performance, whereas optogenetic suppression improved attention and performance. A genetically encoded sensor of 5HT release showed reduced 5HT levels in visual cortex when mice attend and detect stimuli. These results demonstrate that DR-5HT neurons are members of the brain's attentional circuit and suggest that 5HT is a novel biological carrier of visual attention.

P2-D-144 - Segregated localization of target-SNARE proteins within presynaptic terminals of Munc18-1 deficient photoreceptors

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Sec1/Munc18 family proteins are essential for SNARE-mediated vesicular exocytosis. However, the location of SNARE proteins in Munc18-1 deficient presynaptic terminals remains unclear due to the rapid degeneration of neurons lacking Munc18-1. Using Munc18-1 conditional knockout mice, we found that specific removal of Munc18-1 from photoreceptor cells did not result in major cellular loss until postnatal day 14, which allowed us to investigate the role of Munc18-1 in endogenous presynaptic terminals. In the absence of Munc18-1, even before major photoreceptor cell degeneration, functional impairments were present. While Munc18-1 was not required for the pre-synaptic enrichment of the t-SNARE proteins syntaxin-3 and SNAP-25, it played a critical role in their proper localization. In wild-type conditions, t-SNAREs are highly colocalized. However, in the absence of Munc18-1, their distribution becomes strikingly segregated. Immuno-electron microscopy revealed that without Munc18-1, syntaxin-3 is retained within various organelle membranes rather than being targeted to synaptic plasma membranes. These findings provide the first evidence that Munc18-1 is important to prevent segregation of syntaxin-3 and SNAP-25 within presynaptic terminals.

P2-D-145 - Mapping visual search errors to covert operations with frontal eye field neurophysiology and double factorial design

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Behavior is the outcome of covert perceptual, cognitive, and motor operations that can be described by mathematical models and are produced by brain circuitries. To resolve the processing stages underlying visual decision-making, we designed a task to independently

modify two critical operations — target localization ("Where is the informative item?") and response selection ("What does that item instruct me to do?"). Two macaque monkeys were trained to search for a color singleton among distractors. Target localizability was manipulated by varying the similarity of singleton and distractor colors. Response selection was manipulated by varying the discriminability of search array shape, signaling GO/NOGO response. The organization and termination rule of the two operations were determined using System Factorial Technology (SFT). However, the logic of SFT is confounded by errors, so we describe the neural origin of errors with single-units in Frontal Eye Field (FEF). Monkeys made two common errors: failure to locate the target on GO trials and failure to inhibit the saccade on NOGO trials. Localization and response inhibition errors arise through distinct neural processes. This shows that visual attention and decision making are distinct, which challenges the canonical accumulator model of decision-making.

E - HOMEOSTATIC AND NEUROENDOCRINE SYSTEMS

P2-E-146 - Mechanisms underlying estrogen's rapid facilitative effects on social recognition in the medial amygdala of female mice

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Social recognition (SR), the ability to remember and distinguish conspecifics, allows for social behaviours to be modulated appropriately. Estrogens are well-established to affect SR through rapid, non-genomic mechanisms. In ovariectomized (OVX) female mice, intra-medial amygdala and intrahippocampal administration of 17β-estradiol (E2) rapidly facilitates short-term SR memory. While previous research has shown that this estrogenic effect in the dorsal hippocampus requires the activation of the extracellular signal-regulated (ERK) pathway, whether this same cell signalling cascade is necessary in the medial amygdala (MeA) is still unknown. Thus, the present study aims to elucidate whether the ERK pathway in the MeA is required for SR and its rapid facilitation by E2 in OVX female mice. First, the role of ERK in SR within the MeA will be assessed with a dose-response study, and the highest dose of the ERK activity inhibitor U0126 that does not block SR when administered bilaterally into the MeA will be determined. Next, a behavioural paradigm where control mice do not show short-term SR will be used to assess whether this sub-optimal dose can block the rapid facilitative effects of E2. We predict that inhibiting the ERK pathway in the MeA will impair short-term SR memory, and that ERK activity in this region is necessary for E2's rapid facilitation of SR. This study will further our understanding of the non-genomic mechanisms through which estrogens rapidly modulate memory and cognition in the social brain.

P2-E-147 - Intranasal administration of 17β-estradiol using a novel vehicle for brain-targeted delivery in rats

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Estrogens, such as 17β-estradiol (E2), are steroids that regulate neuronal function and behaviour. Low circulating levels of E2 are associated with cognitive decline in neurological and psychiatric disorders, and E2 might be protective against neurodegenerative diseases, such as Alzheimer's disease. However, clinical use of E2 for brain disorders in both women and men is limited by adverse effects in the periphery (e.g., estrogen-sensitive breast cancer, gynecomastia). Intranasal (i.n.) administration provides a direct nose-to-brain route that greatly reduces exposure of peripheral organs. We developed an i.n. method with a novel vehicle to selectively deliver E2 to the brain in rats. Compared to other vehicles (e.g. saline), this starch nanoparticle (SNP) network hydrogel allows for a more controlled release. First, in an in vitro study, we observed a controlled release of E2 over 24 hr. Second, in an in vivo study, female and male adult Long Evans rats were administered i.n. E2 (0-4 mg/kg), and brain and blood were collected 6 or 24 hr later (n=3/sex/dose/timepoint). E2 will be measured in the blood and microdissected brain regions (olfactory bulb, medial prefrontal cortex, hypothalamus, and cerebellum) via a highly sensitive and specific liquid chromatography tandem mass spectrometry (LC-MS/MS) assay. The results of this study will allow us to select a sex-specific dose of E2 to be used in future studies. This study will provide a foundation for developing intranasal steroid treatments for steroid-sensitive brain diseases.

P2-E-148 - Friend or Foe: Role of CRH-PVN neurons in social threat detection

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Our understanding of how animals detect and respond to threat rely on a framework of predator-prey interactions. Threat, however, can also arise from conspecifics. Accurately discerning the potential threat posed by others is vital for building social relationships, yet the mechanisms underlying this process remain unclear. Using behavioral analysis and fiber photometry, we examined CRHPVN activity in resident mice during interactions with intruders. CRHPVN activity increased during approach behavior, with a significantly larger response to unfamiliar intruders compared to familiar ones. This was followed by elevated anogenital sniffing toward unfamiliar intruders. When intruders exhibited a negative affective state (via foot-shock), residents spent more time investigating stressed familiar intruders compared to naïve ones, accompanied by corresponding changes in CRHPVN activity. However, CRHPVN responses were similar for stressed and naïve unfamiliar intruders. These findings suggest CRHPVN neurons play a key role in social threat detection and safety assessment, modulating responses based on familiarity and affective state. Differences in CRHPVN activity associated with familiarity and affective state reflect its role in guiding appropriate social investigative behaviors. Overall, our study highlights CRHPVN neurons as critical mediators in identifying potential social threats and directing adaptive social responses.

P2-E-149 - Effects of early life stress and concurrent maternal dietary changes on circadian expression of metabolic and mitochondrial genes in the rat offspring

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Adverse experiences in early life can have a lasting impact, contributing to both mental and physical pathologies in adulthood, including metabolic disorders. However, how early adversity

disrupts circadian metabolic homeostasis remains unclear. Using the limited bedding (LB) model of early adversity, we found that circadian expression of clock genes was modified in the liver and adrenal of neonatal and juvenile rat offspring. Circadian variations in clock genes regulate key metabolic processes, while nutrient intake modifies circadian rhythms via metabolic sensors that govern mitochondrial function. Here, we tested whether fragmented maternal care and reduced energy intake caused by LB disturbs rhythmic clock-controlled gene expression via circadian changes in mitochondrial function and metabolic signals. To test if maternal diet can mitigate these effects, we compared offspring of LB-exposed mothers fed normal chow, high-fat diet (60% fat, HFD), or subjected to 6 hours of time-restricted feeding (TRF6). Tissues (brain, liver, adrenal, fat pads) were collected at circadian timepoints (CT2, 8, 14, 20) to measure metabolic and mitochondrial gene expression (e.g., Sirt1/3, Cat, Sod1, Fis1) by qPCR. These findings probe the relationship between maternal caloric intake and LB's impact on offspring circadian metabolism and will help uncover how early adversity shapes metabolic systems during development. As several metabolic effectors are also epigenetic modifiers, they have the potential to link early LB-induced circadian disruption to adult physiological disruptions.

P2-E-150 - Hypothalamic neurons expressing CRABP1: The missing link to understanding the regulation of energy homeostasis?

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Neurons of the arcuate nucleus of the hypothalamus (ARC) play critical roles in sensing and responding to metabolic signals such as leptin and glucagon-like peptide 1 (GLP-1). In the ARC, pro-opiomelanocortin (POMC) and agouti-related peptide (AgRP)-expressing neurons are key populations on which signals converge to regulate energy homeostasis. However, recent work suggests that unidentified GABAergic neurons of the ARC are essential for the control of energy balance. Based on RNAseq studies, we identified an uncharacterized GABAergic population of the ARC that express the Cellular retinoic acid binding protein 1 (Crabp1). We hypothesize that Crabp1ARC neurons are critical for the control of energy homeostasis.Using in situ hybridization, we found that Crabp1ARC neurons are distinct from POMC and AgRP neurons. Subsets of Crabp1ARC neurons co-express the leptin receptor (Lepr; 10.8%) and glucagon-like peptide 1 receptor (Glp1r; 35.1%). We then determined whether Crabp1ARC neurons are sensitive to fasting, leptin and GLP1R agonism by assessing cFOS immunoreactivity as a marker of neuronal activity. Interestingly, the activity of Crabp1ARC neurons increases in response to fasting but is reduced in fasted mice injected with liraglutide (a GLP1R agonist).Crabp1ARC neurons could represent a new GABAergic 'hub' of the ARC, towards which metabolic signals may converge to mediate some of their effects. Patch-clamp electrophysiology experiments using a Crabp1-eGFP mouse model will allow us to investigate the mechanisms underlying the effects of liraglutide on these neurons.

P2-E-151 - Brain expression and integrated physiological responses mediated by the endocannabinoid system and the metabolic ghrelin/ghsr axis

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Background: The growth hormone secretagogue receptor (GHSR) and cannabinoid receptor type 1 (CB1R) are highly expressed in the brain, regulating energy homeostasis, appetite, reward, and stress. While their individual roles are well characterized, the potential for co-expression in the same neuronal subsets and its functional implications remains unexplored.Methods: We conducted a neuroanatomical analysis using GHSR-eGFP mice, Fr-ghrelin labeling, and CB1R-specific immunostaining, complemented by transcriptomic datasets, to investigate their co-expression in the brain. To explore functional implications, we assessed the behavioral effects of co-administration of their agonists, ghrelin (15 pmol/g) and THC via voluntary oral administration (5 mg/kg THC) in 300 mg peanut butter in adolescent mice. Mice were fasted 2h prior to cannabis consumption. The average time to consume the cannabis/peanut butter mixture was 15 min.Results: We identified significant overlap between GHSR+ and CB1R+ neurons in the cerebral cortex, hippocampus, and amygdala. Transcriptomic data revealed distinct Ghsr+/Cnr1+ glutamatergic neurons in the hippocampus and amygdala, as well as diverse subtypes co-expressing these receptors in the midbrain, hypothalamus, pons, and medulla. These findings suggest that GHSR and CB1R may mediate region-specific physiological responses, such as feeding. Voluntary oral cannabis significantly increased food intake, and reduced body temperature. Future experiments will address the interaction of ghrelin on cannabis-induced food intake. Conclusion: These results highlight the neuroanatomical convergence of GHSR and CB1R, that may be influencing acute cannabis-induced appetite and may highlight novel therapeutic targets for cannabis addiction.

P2-E-152 - Effects of repeated injections of CB1 agonist on neuropeptide Y expression in the arcuate nucleus

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Drugs that stimulate the cannabinoid system have been associated with an increase in food intake, inducing hyperphagia and weight gain in rodents. Research suggests that this could be explained by a modulating effect of CB1 receptors on satiety signals in the central nervous system. CB1 receptors have been located in moderate quantity in key regions of the brain associated with satiety and food intake, like the nucleus accumbens, the lateral hypothalamus and the ventral tegmental area. However, the potential long-term effects of the stimulation of CB1 receptors on peptides associated with homeostasis and satiety, especially following central injections. In this study, adolescent male and female Wistar rats (aged 5 weeks) underwent stereotaxic surgery to place a guide cannula. One-week post-op, animals received the first injection of the CB1 agonist WIN 55, 212-2 in either the nucleus accumbens (N = 17) or the lateral hypothalamus (N = 16). In total, they received 5 injections every other day over a period of 10 days. Controls underwent the same surgery but only received the vehicle (N = 16 per injection site) at the same time. Two weeks after the last injection, animals were sacrificed and their brains collected for immunohistofluorescence of neuropeptide Y expression in the arcuate nucleus.

This region was chosen for its central role in homeostasis and its connections to both the lateral hypothalamus and the ventral tegmental area. Results suggest limited effects CB1 agonism on NPY expression in the ARC, indicating that either the adolescent brain is resilient to long term changes, or that CB1 receptors have a more local impact on satiety peptides.

P2-E-153 - Effects of repeated CB1 stimulation in the lateral hypothalamus on orexinergic neurons in the adolescent rat brain

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There is significant evidence to support that stimulation of cannabinoid 1 receptors (CB1R) stimulation exert important effects on ingestive behaviours. Research on rodents has shown that systemic CB1R agonism increases food intake, weight gain and fasting-induced hyperphagia shortly after the administration. CB1R are found throughout the satiety signals and may influence satiety through complex interactions with the orexin system. Therefore, this study aimed to identify the effects of repeated CB1R agonism in the lateral hypothalamus, a primary orexinergic nucleus where CB1R and orexin receptor type 1 (OX1) are colocalized, on the expression of OX1 in the lateral hypothalamus (LH), arcuate nucleus (ARC) and ventral tegmental area (VTA) in the developing brain. Adolescent male and female Wistar rats (aged 5 weeks) received 5 microinjections of either the CB1 agonist WIN 55, 212-2 (N = 16) or the vehicle (N = 17) every other day over a 10-day period. Their food intake and body weight were recorded daily. Two weeks after the final injection, the rats were euthanized and their brains were collected for immunohistofluorescence (IHF). It was found that repeated CB1R agonism had no significant effect on food intake and on OX1 expression between groups. A strong colocalization of CB1R and OX1 was observed in the VTA in all groups. These results suggest that repeated stimulation of CB1R in the LH alone has limited effects on orexinergic functions, specifically in the adolescent brain, but the interaction between endocannabinoids and the orexin system should still be further examined.

P2-E-154 - Neurometabolic remodelling in hypothalamic appetite neurons in obesity

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Obesity results from chronic excess energy intake through feeding, which is prevented by leptin's inhibition of hunger neurons in the hypothalamus. However, the brain can become leptin-resistant in overfeeding, driving weight gain. Preventing leptin resistance is a key target for anti-obesity therapeutics, but previous studies did not account for spatial changes in the obese brain that impair appetite circuit function. We therefore sought to uncover the transcriptomic changes driving leptin resistance in a spatial context, to understand how this impairs appetite circuits. We used spatial transcriptomics in brains from obese mice to test the hypothesis that several appetite-associated brain areas contribute to central leptin resistance in obesity. Hippocampus and hypothalamus, but not basolateral amygdala, had reduced expression of genes associated with synaptogenesis (Notch1 and Bdnf), while all three regions expressed reduced neurometabolic receptors (Insr and Lepr). Obese mice also had reduced expression of glucagon-like peptide receptors, particularly in appetite neurons near circumventricular organs. Interestingly, this pattern is hypothesized to reflect hormonal gradients in the hypothalamus. These data suggest that neurons most exposed to changes in leptin have reduced endocrine

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receptivity in obesity, which may impair downstream satiety circuitry to increase appetite, drive weight gain, and manifest comorbidities. Our findings shed light on obesity pathology, which we hope will help inform new therapeutic strategies for the management of obesity and diabetes.

P2-E-155 - Mapping cellular and molecular plasticity in the maternal and postpartum brain

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The maternal brain undergoes profound adaptations during pregnancy, yet the underlying molecular and cellular mechanisms remain incompletely understood. Here, we applied two complementary spatial transcriptomics approaches, Slide-tags and Multiplexed Error-Robust Fluorescence In Situ Hybridization (MERFISH), at three timepoints (nulliparous, pregnant, and postpartum) to map pregnancy-related changes across the mouse brain. Slide-tags provided genome-wide coverage of approximately 100,000 cells, revealing extensive transcriptional remodeling in hypothalamic, cortical, and limbic circuits. In parallel, MERFISH measured 500 targeted genes in over one million cells, offering subcellular resolution to validate key findings and uncover dynamic cell-cell interactions.Our analyses revealed marked shifts in cell-type composition, including the expansion of specific vascular and hypothalamic neuronal populations that may underpin maternal behavioral adaptations. Differential gene expression analysis pinpointed hormone receptors, immune mediators, and neuroplasticity-related genes as key contributors to maternal state transitions. Notably, the integrated Slide-tags and MERFISH datasets highlighted significant changes in neuropeptide signaling pathways, suggesting coordinated molecular programs that promote caregiving behaviors and mood regulation. Taken together, these results provide the first spatially resolved, single-cell atlas of the pregnant and postpartum rodent brain, offering novel insights into how peripartum neuroplasticity supports maternal adaptations.

F - COGNITION, EMOTION AND MOTIVATION

P2-F-156 - The role of retrosplenial cortex parvalbumin interneurons in regulating memory encoding

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We have previously observed impaired activity of parvalbumin interneurons (PV-INs) in the RSC during the early stage of Alzheimer's Disease. Here, we performed a series of experiments to better understand the implications of these findings to the encoding of memories at the cellular and population level. We hypothesized that RSC PV-INs play a central role in modulating the encoding of spatial and contextual memories in local circuits. First, using head-mounted miniscopes, we confirmed the disruption of RSC PV-INs in vivo in a mouse model of AD and, we observed decreased PV-IN GCaMP activity. Next, we examined the implications of impaired PV-IN survival on memory encoding in normal WT mice. Using fiber photometry, we established that selective deletion of RSC PV-INs resulted in increased population activity and impaired spatial recognition memory. This suggests that PV-INs restrict the RSC population activity during

encoding.   To further examine this idea, we performed a Compartment Analysis of Temporal activity by Fluorescence In Situ Hybridization (catFISH) experiment. Mice explored two contexts and, the activity of RSC neurons in each context was analyzed. We observed an increase in the number of RSC neurons that were active while exploring both contexts in the mice with deleted PV-INs suggestive of impaired pattern separation. Finally, we examined the impact of impaired PV-IN activity on place cell activity in the RSC. Impaired PV-IN activity increased the size of place fields indicating a less specific spatial representation.   Together, the results presented here demonstrate that PV-INs modulate the specificity of RSC memory encoding.

P2-F-157 - The impacts of non-aversive handling methods on background stress in mouse withdrawal behavioural experiments

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Handling mice by the tail has been a common practice for decades of mouse research. Recent studies demonstrate the stressful impacts of tail handling. Consequently, mouse behavioural assays can be impacted by background stress created by tail handling. Alternatives to tail handling including cup handling and hand cupping significantly minimize stress and anxiety like behaviours in mice. Our lab tested the impact of cup handling versus tail handling on an opioid withdrawal scoring behaviour scale in both morphine and saline treated mice. Mice received ascending morphine treatment or saline followed by naloxone induced withdrawal. Behavioural assessment included scoring for precipitated behaviours like grooming, headshakes, jumping, wet-dog shakes, among others. These behaviours are heightened during withdrawal but some can also be impacted by stress. Controlling experimental variables that could contribute to stress and anxiety in mice is crucial for the quality of withdrawal data. We found that cup handling causes a significant decrease in the background stress associated with the experimental design, especially in the saline-treated control animals. The implementation of a one-week handling regimen on mice adapts them to the experimenter and experimental design. Mice are exposed to scheduled cup handling, scruffing and behavioural set-up habituations. These results demonstrate the importance of decreasing animal stress through alternative handling techniques and the impacts of handling choice on withdrawal assessments. Furthermore, mouse handling has provided a significant reduction in data variability providing further validity to withdrawal models. Beyond withdrawal behaviours, these findings have future implications for handling and safety of animals in other types of behavioural experiments, such as pain behaviours.

P2-F-158 - Microglia regulate memory formation by modulating engram-neuron activity and their synaptic connection

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Beyond their canonical role as the brain's resident immune cells, microglia are also known to actively interact with neurons and remodel synaptic connections in the healthy brain. However, whether these interactions and remodeling can influence cognitive functions such as memory

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and learning remains unclear. Here, we propose that microglia play a crucial role in regulating memory formation by modulating the activity of memory cells (i.e., engrams). Using contextual fear conditioning as the behavioral paradigm to study fear memory in mice, we found that microglia depletion at training impaired memory recall, indicating that microglia are important for memory formation. Engram-labeling techniques further revealed that, under microglia depletion, cells encoding the memory became less active when memory was recalled, suggesting that the cellular basis of memory formation is disrupted. Additionally, 1-photon in vivo calcium imaging across multiple time points showed that microglia depletion disrupted the reactivation of engram neurons during the consolidation phase and, by assessing functional connectivity, impaired the strengthening of their connections after training, indicating the potential function of microglia in promoting memory consolidation via synaptic connections. These results highlight a novel function of microglia in shaping memory formation through modulating learning-induced neuronal activation and synaptic connection.

P2-F-159 - Investigating the impact of animal source and conditioned stimulus variations on morphine positive-feature occasion setting in rats

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Introduction: Our research group has developed a stable effect of morphine occasion setting in a Pavlovian drug discrimination task, wherein a positive-feature occasion setter (OS), the drug stimulus, is trained to disambiguate when an exteroceptive white noise (WN) conditioned stimulus (CS) is signaling sucrose availability. Male and female Sprague Dawley rats can reliably distinguish between morphine and saline under these conditions. Recently, some unknown change had resulted in a loss of effect. Previous literature has proposed an effect of animal source on other drug-related behavioural mechanisms, specifically in place conditioning. Experiments evaluated the impact of animal source, CS manipulation, and environmental factors on morphine OS training. Methods: In experiment 1, male and female Sprague Dawley rats were ordered from 2 sources, Charles River and Envigo. Rats received daily, intermixed injections of 3.2mg/kg morphine or saline before training sessions. Each session consisted of 8 presentations of a WN stimulus, followed by 15s access to sucrose on morphine, but not saline, sessions. The CS was then shifted to a light stimulus. A second cohort of Charles River rats were trained with the same conditions as experiment 1, but housed in static, rather than dynamic, housing. Results: Animal source was not a driving factor in the loss of effect. Both sources performed the task equally under WN conditions. Light as the CS resulted in increased learning for both sources. Conclusion: The constant noise in dynamic housing likely resulted in a WN CS-preexposure effect.

P2-F-160 - Exploring the impacts of adolescent nicotine vaping exposure on mood and risk-taking pathophysiology in the amygdala-striatal network

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Adolescence is a critical period of neurodevelopment, vulnerable to disruptions from neurotoxins like nicotine. Preclinical research has linked adolescent nicotine exposure to

increased anxiety and depressive-like behaviours, altered decision-making, impulsivity, and risk-taking behaviour. Given the rise in adolescent nicotine vaping, understanding the neurological and behavioural consequences is crucial. Evidence suggests disruptions within neuronal activity patterns and dysregulations in molecular signalling pathways. Therefore, this study aims to address existing limitations by using an OpenVape nicotine inhalation model to closely mimic human nicotine consumption. Adolescent male and female Sprague-Dawley rats were exposed to either 0% nicotine vapour (vehicle) or 40% nicotine vapour for 10 minutes 3x per day for 10 consecutive days from postnatal day (PD) 35-44. Behavioural tests conducted in adulthood (PD>75) indexed emotional behaviour, such as anxiety, depression, and risk-taking behaviours. In-vivo electrophysiology and molecular analyses targeted emotional regulation regions, including the nucleus accumbens (NAc) and basolateral amygdala (BLA).Adult behavioural tests demonstrated a significant reduction in anxiety-related behaviours, potentially displaying elevated risk-taking and impulsivity behaviours. In-vivo electrophysiology and Western Blot analyses are ongoing. The findings of the current study could provide valuable insight into the harms associated with adolescent nicotine exposure, aiming to improve the construct validity of vape models.

P2-F-161 - The role of the marmoset dorsolateral prefrontal cortex in memory-guided navigation

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Episodic memory—often known as the ability to recollect features such as the time and location of specific past events—is integral to advanced cognition and is particularly vulnerable in Alzheimer's disease. To explore this capacity, we trained a marmoset on a memory-guided navigation task and wirelessly recorded (1) the animal's location (OptiTrack) and (2) single-unit activity from the dorsolateral prefrontal cortex (Blackrock Microsystems). The animal was trained to navigate a maze with four reward spouts, requiring strict adherence to spatiotemporal sequences to obtain rewards. In the simpler (2 spouts) condition, the animal learned to collect rewards by licking two specific spouts in sequence and achieved 93% performance accuracy, while in the more complex condition, it navigated a sequence of 3 spouts and performance dropped to 70%. Behavioral analysis revealed a nearly sevenfold increase in the average number of misses before licking the correct spout during the 3-spout relative to the 2-spout condition. The latter suggests a memory capacity limitation.Dorsolateral prefrontal recordings revealed heightened activity at decision points, i.e., when the animal paused to evaluate choices complying with the correct sequence experienced in the past, implicating this region's role in remembering back in time to past experiences. While the subjective "mental time travel" central to human episodic memory remains unconfirmed in marmosets, these findings demonstrate their ability to encode and retrieve spatiotemporal sequences in a manner consistent with episodic-like memory.

P2-F-162 - Investigating hippocampal representations of spatial and abstract variables using a dynamic cue-directed t-maze task

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Hippocampal neurons can encode both spatial and non-spatial abstract variables, forming a task-relevant cognitive map. We seek to examine whether hippocampal neurons can encode both types of variables simultaneously, and how the generalized task structure is encoded by the hippocampal-cortical network. I have designed a Dynamic Cue-Directed T-Maze (DCT) task, where rats make a binary choice based on a visual cue they receive at the onset of each trial. Rats perform the task in a dynamic rodent maze apparatus that we designed and constructed in the lab. The maze is a 3x3 grid of interconnected octagonal compartments with walls that can go up and down to have dynamically changing configurations. These spatial configurations can switch across trials without experimenter intervention while maintaining the same task structure. We developed an effective training protocol for rats to learn the complicated DCT task. If the cognitive map is purely spatial, the activity of place cells will form a classic Euclidean representation. If based solely on abstract task-related variables, the place cells will form a graph-like representation, responding similarly to the cued vs. uncued goal locations. We hypothesize that the hippocampus will represent a combination of the two structures, with the balance dictated by formation of a generalized task structure in cortical structures. Our goal is to simultaneously record hippocampal and prefrontal neurons to examine how they differ in their formation of generalized non-spatial representations, and how these representations evolve during learning.

P2-F-163 - Investigating the bidirectional impact of ulcerative colitis and chronic stress on blood-brain barrier and gut barrier permeability

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Ulcerative colitis (UC) is a chronic inflammatory bowel disease affecting the large intestine, with a global prevalence of 5 million individuals. Symptoms include abdominal pain, bloody diarrhea, fatigue, and anemia. While its exact cause is unknown, UC involves gut barrier dysfunction, allowing luminal pathogens and toxins to penetrate the intestinal epithelium and trigger inflammation. UC is often comorbid with stress and mood disorders. Chronic intestinal inflammation leads to increased circulating levels of inflammatory mediators, suggesting that gut barrier disruption may contribute to the neuroimmune mechanisms of depression by fragilizing the blood-brain barrier (BBB), the ultimate frontier between the blood and the brain. Epithelial and endothelial barriers are central to the gut-brain axis, the bidirectional communication between the gastrointestinal tract and the brain, yet mechanistic interactions remain poorly understood. We hypothesize that UC alters both gut barrier and BBB permeability and exposure to chronic stress exacerbates colitis-induced barrier disruptions. Using the dextran sodium sulfate (DSS)-induced UC model in mice, we observed clinical and histological features akin to human UC, along with depressive-like behaviors. Ongoing molecular and morphological analyses will elucidate how UC affects barrier permeability. In parallel, DSS-treated mice are subjected to chronic social defeat stress, a mouse model of depression. Together, these studies will decipher how UC-driven inflammation disrupts gut barrier and BBB function and its interplay with stress.

P2-F-164 - Dopaminergic activity in basolateral amygdala mediated by conditioning and optogenetic stimulation

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Background: Dopamine neurons of the ventral tegmental area (VTA) are crucial for reward-based learning. Dopamine release in the basolateral amygdala (BLA) encodes associative memories. This study examined dopamine signaling in the BLA during contextual food conditioning and identifies the contribution of lateral hypothalamic (LH) projections co-expressing the neuropeptides orexin and dynorphin to the VTA to dopamine release in the BLA. Methods: In mice expressing GRABDA, a dopamine sensor, in the BLA, we used fiber photometry to monitor dopaminergic activity during conditioning of a contextual preference for food. In separate mice, the cre-dependent opsin, Chrismon, was bilaterally injected into the LH orexin/dynorphin containing neurons of orexin-cre mice and GRABDA was unilaterally injected into the BLA. We then tested the effects of optical stimulation of LH orexin/dynorphin inputs to the VTA (30 Hz, 30 s, 5 ms pulse width) on dopamine release in the BLA during 30-minute home-cage sessions.Results: Mice increased time spent in the food-paired context after conditioning (p < 0.05). This was associated with increased dopamine concentration in the BLA. Increased BLA dopamine transients were associated with increased entries and time within the conditioned compartment. Additionally, optical stimulation of LH orexin/dynorphin inputs in to the VTA induced dopamine release in the BLA, which decayed with repeated stimulations. An inactive laser wavelength did not evoke significant BLA dopamine responses.Conclusions: Associative conditioning drives dopamine release in the BLA during preference expression. Optogenetic stimulation of LH-VTA projections modulates BLA dopaminergic activity. These findings provide insights into reward processing and appetitive associative learning.

P2-F-165 - Examining the generalization of the stimulus effects of morphine to other opioids in rats trained on opposing learning contingencies

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Background: Interoceptive effects elicited by opioids can guide behaviours through Pavlovian associations with drug-related stimuli. Following acquisition of appropriate conditioned sucrose-seeking guided by a morphine (M) stimulus, stimulus specificity can be determined by comparing behaviour elicited by different opioid receptor agonists to baseline discrimination (DS) behaviour. Methods: Male and female rats received daily injections of either M or saline (S) before training sessions, which consisted of 8 presentations of a white noise (WN) conditioned stimulus. WN was followed by sucrose delivery on M, but not on intermixed S sessions for rats trained on a feature positive (FP) learning contingency. The opposite was true for rats trained on a feature negative (FN) contingency. Following stable DS, rats completed generalization cycles with two qualification (Q) sessions identical to training, followed by a test session if DS between Q sessions was demonstrated. On tests, responding to a single WN presentation was recorded after pre-treatment 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: All rats acquired DS. Preliminary results for FP rats indicate that generalization curves for M, O, H, and F appear to follow an inverted-U pattern. Curves for FN rats appear to follow a U pattern. N blocks agonist-appropriate responding. Conclusions: Our findings demonstrate that the stimulus effects of M can generalize to other opioid agonists, and the antagonist blocks the effect of M.

P2-F-166 - Impulsivity and delay discounting in gambling: Sex-specific cognitive strategies and the impact of atomoxetine

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Introduction:Gambling disorder is characterized by impulsive decision-making and delay discounting (DD) - the tendency to prefer immediate rewards over larger delayed ones. We explored how DD affects gambling behavior in males and females using the cued rodent Gambling Task (crGT). We presented risky options sooner (worsening phenotype) or optimal options sooner (ideal phenotype), to assess differences in how males and females employ cognitive flexibility to adjust their strategies. We tested whether atomoxetine, a norepinephrine reuptake inhibitor, improves maladaptive decision-making in the DD-crGT.Methods:In a pilot study, 32 Long Evans rats (16M, 16F) were trained on the crGT. When stable, rats were exposed to worsening or ideal DD-crGT. In a follow-up study, 25 female rats were trained on the crGT. Rats were dosed daily with atomoxetine (1.0mg/kg) or vehicle prior to DD-crGT testing.Results:Risky females persevered in maladaptive strategies, choosing high-risk options even when optimal choices were available sooner. In contrast, risky males switched to favoring optimal strategies. When replicated, vehicle-treated risky females continued to prefer risky choices, while atomoxetine-treated risky females significantly improved ($t(112) = 2.453$, p=0.016).Conclusion:Females with risky gambling tendencies showed greater perseverance in suboptimal decision-making, while males displayed more flexibility. Atomoxetine improved optimal strategy. These sex differences highlight the need for tailored interventions to address maladaptive behaviors in gambling disorder.

P2-F-167 - Investigating the role of a cadm2 recursive splice site in ADHD-related behaviours: Insights from rodent neuroimaging and behaviour

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Attention-Deficit/Hyperactivity Disorder (ADHD) is a prevalent neurodevelopmental disorder with a well-established genetic basis. Prior studies have linked the human Cell Adhesion Molecule 2 (CADM2) gene to externalizing traits, which have in turn been associated with sign-tracking (ST) behaviours (placing incentive value on cues). Recent research suggests that recursive splicing variations in CADM2 may increase ADHD risk, but their behavioural effects are unknown. In this study, we explore the effects of a novel mutation in CADM2's recursive splice site (RS1) on rodent brain connectivity and behaviour. Cadm2 RS1-/-, RS1+/-, and RS1+/+ rats (n = 6/sex/genotype) underwent functional and structural MRI (9.4T). ST behaviours were assessed using a Pavlovian Conditioned Approach task, where rats were shown two retractable levers – each one response-independently associated with either a reward (CS+) or no reward (CS-). We find a main effect of sex on several ST metrics. Notably, females showed a higher probability of pressing the CS+ during acquisition (p = 0.016), but this reverses during extinction, where males show higher probability instead (p = 0.032). No main effects of genotype or sex-genotype interaction were found, possibly due to a low number of animals per sex per genotype in the first cohort. This indicates that reward-related learning processes differ between sexes. A lack of genotype effects suggests that CADM2 RS1 variation may not play a significant role in Pavlovian learning. Additional paradigms should be sought to clarify CADM2's role in ADHD-related behaviours.

P2-F-168 - Human time cells during working memory maintenance

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Working memory, the active retention of information for short time periods, is a basic cognitive process. But the neural mechanisms underlying working memory maintenance in the human brain remain unclear. Sequences of spiking neurons after a cue is presented, encoding both the memory of the cue and time since its presentation, have recently emerged as a potential mechanism in rodent working memory, but their existence in the human brain is not established. Leveraging a dataset of 902 neurons recorded from 21 patients during the Sternberg working memory task (Kyzar et al., 2024), we analyzed single-neuron activity in the medial frontal and medial temporal lobes. During the memory maintenance period of each trial, we identified so-called 'time cells' with distinct activation time-points. Collectively, these cells exhibited sequential firing throughout memory maintenance, so that their population activity enabled more accurate decoding of time compared to broader neuronal populations. Notably, correct trial outcome was strongly correlated with both the reproducibility of the firing sequence and the precision of decoded time, linking their temporal dynamics to memory activation. These findings provide compelling evidence that temporally selective neuronal firing in the medial temporal and medial frontal lobes plays a crucial role in working memory maintenance, underscoring the importance of temporal coding as a neural mechanism for cognitive processes.

P2-F-169 - Effort discounting and exploration deficits drive apathy via distinct brain alterations after traumatic brain injury

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Amotivation in acquired and developmental brain disorders is common, and can have profound impacts on functional outcomes for patients and their families. Mild-to-moderate traumatic brain injury (mmTBI) is one patient group that often experiences 'apathy' (i.e., reduced motivation to initiate goal-directed behaviors). Yet, despite extensive clinical work on apathy in mmTBI, the neural mechanisms driving this symptom remain unclear. We used computational modeling and task-based functional MRI (fMRI) in healthy volunteers (N=20) and patients with chronic mmTBI (N=40) to examine the contributions of value and effort to apathy in mmTBI. Our results indicate that the ability to explore new value-based choices that could help to maximize rewards over the long-term is selectively decreased in mmTBI patients with clinical apathy relative to both non-apathetic mmTBI and controls. Notably, this mmTBI-specific behavioral correlate of apathy was driven by diminished feedback-locked encoding of reward prediction errors in the lateral frontopolar cortex. Error-based discounting of rewards was uniformly correlated with apathy across mmTBI and controls, but was selectively associated with effort-attenuated value signals in dorsal striatum in mmTBI patients with apathy. These results reveal novel evidence about the neurocomputational bases of motivational challenges in mmTBI patients. This work generates testable hypotheses that can guide the development of future circuit-based interventions aimed at alleviating apathy and improving daily functioning in individuals with chronic mmTBI.

P2-F-170 - Sex-dependent impact of rat tau ko on cognitive flexibility and spatial discrimination

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Tau is a ubiquitously expressed protein in the brain that plays a key role in stabilizing the cytoskeletal architecture of neurons and glia by interacting with microtubules. Beyond this classical role, tau is also involved with axonal transport, intracellular signaling and synaptic plasticity. Consequently, aberrant changes to tau functions during tauopathies, such as Alzheimer's disease, impact essential physiological processes that leads to learning and memory deficits, and cognitive decline. Since rats offer a richer behavioural repertoire in comparison to mice, we have generated a novel microtubule-associated protein tau homozygous knock-out (Mapt-/-) rat model to explore how the deletion of tau impacts behaviour. To accomplish this, we examined both sexes of young adult wild-type (WT) and Mapt-/- littermate rats in a battery of spontaneous and motivated behavioural tasks. Specifically, we examined object location memory (OLM) at 2-3-months old, followed by pairwise discrimination (PD) and reversal learning (RL) at 3-6 months old. We found tau deletion did not significantly impact OLM in young adults, regardless of sex. Furthermore, tau deletion did not affect PD or RL in male rats. Interestingly, however, female Mapt-/- rats took significantly longer to learn the touchscreen tasks, compared to WT controls. Taken together, findings suggest a sex-dependent role for tau in young adulthood, with female rats showing increased reliance on tau for some learning and memory tasks.

P2-F-171 - Contributions of the medial amygdala to social memory

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Sofiya Zbaranska1,2, Paul W. Frankland1,2,3, Sheena A. Josselyn1,2,31Program in Neuroscience & Mental Health, Hospital for Sick Children, 555 University Avenue,Toronto, ON M5G 1X8, Canada2Department of Physiology, University of Toronto, Toronto, ON M5S 1A8, Canada3Department of Psychology, University of Toronto, Toronto, ON M5S 3G3, Canada*Correspondence: sheena.josselyn@sickkids.ca Our ability to recognize and remember other individuals—known as social memory—isindispensable for everyday interactions. Disruptions in social memory are implicated inseveral neuropsychiatric disorders, such as autism spectrum disorder, schizophrenia orsocial anxiety. Generally, memories are thought to be stored in populations of neuronsactive during memory encoding and retrieval, termed engrams. Neurons supporting socialmemory were identified in the hippocampal regions CA2 and ventral CA1. However, little isknown about how other brain regions are involved in the social memory network. Wepropose the medial amygdala (MeA) as a candidate region essential for social memoryformation and retrieval. Our data show that disruption of oxytocin signalling followingtraining abolishes both short- and long-term social memory in mice. This effect isaccompanied by a reduction in cFos expression (i.e., activity) in the MeA. Furthermore, weobserved that the proportion of cFos+ neurons in the MeA positively scales with socialmemory performance tested one day after training. Next, we tagged the putative engramensemble supporting social memory in the MeA and found that the activity of the taggedneurons might exhibit higher selectivity to particular social subjects. Thus, we hypothesizethat the MeA stores

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engrams supporting social memories which are both necessary andsufficient to drive recognition of familiar individuals.

P2-F-172 - Cortical networks for egocentric vs. landmark-centered coding of remembered reach

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Introduction: Reach target locations can be encoded in egocentric (EGO) or allocentric (ALLO, landmark-centered) reference frames. The functional organization of EGO and ALLO brain networks is not well understood. We hypothesized that landmark-based reaching would involve more communication between the dorsal (EGO) and ventral (ALLO) visual streams, potentially influenced by trends in cortical activation. Methods: We performed a secondary analysis of an event-related fMRI task from Chen et al. (2014), examining brain networks involved in EGO and ALLO encoding of reach targets. The paradigm included EGO reach (remember target location) and ALLO reach (remember location relative to a landmark). We applied graph theory to time-series data from the memory delay period, comparing EGO and ALLO conditions to baseline, detrending the data, and analyzing network hubs, clustering, and efficiency. Results: The ALLO network showed greater functional interaction in inferior-occipito-dorsal-parietal regions than EGO. Modularity of superior-occipito-ventral-parietal regions was unique to EGO, while temporal regions were unique to ALLO. Both tasks shared temporofrontal and inferior-occipito-dorsal-parietal modules but differed in node and hub locations — more ventral in ALLO. Removing linear trends reduced interaction in the inferior-occipito-dorsal-parietal module in the ALLO network. Conclusion: Landmark-based reaching involves specialized interactions between early visual and dorsal parietal areas, influenced by the linear trend in the timeseries.

P2-F-173 - Examining neuronal allocation to an engram supporting an auditory threat memory throughout the brain

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Memory is thought to be encoded by widely distributed, sparse ensembles of neurons active during memory acquisition, known as engram neurons. In the lateral amygdala (LA), neurons with higher excitability compared to their neighbours at the time of learning become preferentially allocated to part of an engram, a process termed allocation. Whether competitive allocation occurs in other brain regions implicated in auditory fear memory is an open question. Using auditory threat conditioning combined with both excitatory and inhibitory optogenetic constructs expressed in the same neuron, we investigated if excitability-based allocation occurs in brain regions implicated in auditory fear memory in mice. By optogentically exciting a random, sparse population of excitatory neurons in specific regions, we tested whether these neurons were preferentially allocated to an engram. Subsequent optogenetic inhibition of these neurons either disrupted or did not affect fear memory, confirming whether allocation occurred in the targeted region. With this all-optical approach, our results show that interestingly, within the amygdala, allocation occurs in the LA but not in the basal amygdala, a downstream region. However, upstream of the LA, the medial geniculate nucleus of the thalamus showed allocation, narrowed down to its projections to the LA. These results suggest competitive excitability-based allocation

does not occur across all brain regions implicated in auditory threat memory. We hypothesize that regions with hard-coded circuitry lack the necessary tools for neuronal allocation.

P2-F-174 - The effects of parental experience on spatial memory and anxiety-like behaviour in female and male degus

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Parenthood is associated with prominent neural, physiological, and behavioural adaptations in humans and rodents. In rats, motherhood experience is associated with improved spatial learning and memory after the pups are weaned, while research is equivocal on anxiety. In biparental rodents, fathers show enhanced spatial learning in some studies but not others. To date, studies have focused on maternal experience in uniparental species and paternal experience in biparental species. The objective of this study is to compare the effects of parental experience on spatial cognition and anxiety-like behaviour in females and males of the same species, the degu. Degus are biparental rodents allowing us to examine parental experience in both sexes and the experience of single mothers when the male partner is removed. Adult degus were assigned to one of three groups: (1) breeding pairs (biparental group), (2) breeding with male partner removal (single mother group) or (3) non-breeding animals (naïve group). After weaning the pups, all adult degus were tested in the Barnes maze, open field, and elevated plus maze. Preliminary results suggest both groups of experienced mothers have enhanced spatial learning compared to naïve females, while fathers display impaired spatial memory and reduced anxiety-like behaviour compared to naïve males. Current work is investigating neural signatures of parental experience, such as the regulation of perineuronal nets and microglia. This work will help provide insight on how such an important life experience remodels the adult brain.

P2-F-175 - The role of CB1 receptors on lateral amygdala parvalbumin interneurons during stress and fear extinction learning

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The interplay between stress and memory has long been recognized as a key factor in post-traumatic stress disorder (PTSD), an anxiety-based disorder characterized by intrusive memories and impaired fear memory extinction. While exposure therapy, a form of extinction training, is widely used to treat PTSD, fear memories often resurface following treatment in both patients and animal models. Previous research implicates the lateral amygdala (LA) in auditory fear memory acquisition and highlights the importance of parvalbumin (PV+) interneurons in regulating these processes. Endogenous cannabinoids (eCBs) are key regulators of stress and memory, with evidence suggesting that alterations in eCB signaling can impair the extinction of fear memories. Stress-induced changes in eCB activity are thought to decrease PV+ inhibitory activity in the LA, potentially disrupting fear extinction. My preliminary findings suggest that cannabinoid 1 (CB1) receptors on PV+ interneurons in the LA may be pivotal players in extinction learning. This work aims to clarify how CB1 receptor activity modulates LA PV+ interneuron function in response to stress and fear extinction learning. Defining the precise role of eCB activity and PV+ interneurons is critical to develop our understanding of neural mechanisms

underlying PTSD. By investigating the role of eCB signaling in regulating fear extinction, this research may inform novel therapeutic strategies for memory disorders and other stress-related disorders.

P2-F-176 - The effects of early-life adversity on cognitive bias in mice

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Early life adversity is implicated in several enduring neurological disorders in humans. Maternal separation (MS) and limited-bedding and nesting (LBN) are two widely used modes of ELA in rodents whose long-term effects are poorly understood. Here, we investigated the effects of ELA on cognitive bias (decision-making under ambiguity) in adulthood using a Go-Go visual decision-making task. Separate cohorts of mice pups underwent early (PND2-10) or late (PND11-19) MS or LBN. During training, adult mice (2-3mo old) are presented with either a small or a large square on a touchscreen, and correct touches on the screen are reinforced with a small (SR) or large reward (LR) on 75% of the trials. On the probe days, the unrewarded trials are replaced with ambiguous squares, whose sizes fall between the reinforced squares. A cognitive bias index (CBI), (touch LR side - touch SR side)/(touch LR side + touch SR side), is calculated for each of the five ambiguous stimuli as well as an overall area under the CBI curve (AUC). Mice exposed to ELA, particularly those with MS, took longer (1-2 days) to reach 85% accuracy. ELA has sex, treatment, and period-dependent effects on AUC. Specifically, only female LBN and male MS in the early ELA groups show an elevated AUC, indicating optimism when facing an ambiguous stimulus. These findings suggest a potentially critical period for ELA's impact and highlight possible differences in how MS and LBN affect neural networks.

P2-F-177 - Symptomatic efficacy of α5-GABAA positive allosteric modulation

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Alzheimer's disease (AD) is characterized by cognitive impairments, which have been linked to dysregulated excitation/inhibition balance, mainly due to dysfunction of the somatostatin-positive inhibitory (SST) neuron. SST-neurons signal in part through the α5-containing GABAA receptor (α5-GABAAR). GL-II-73, a positive allosteric modulator at the α5-GABAAR, showed efficacy at reversing cognitive deficits and neuronal shrinkage in mouse models of stress, age, and amyloid deposition. However, the requirement of α5-GABAAR modulation to exert such effects remains to be demonstrated. We hypothesize that allosteric modulation at α5-GABAAR is necessary for GL-II-73 to improve cognitive functions. To test this hypothesis, we used a double transgenic mouse model with amyloid deposition (5xFAD) and point mutation in the α5-GABAAR binding pocket (α5KI), limiting drug binding and modulation. Double transgenic mice and their wild-type littermates (n=12/group, 50% female, 4-5 months old) underwent 3 weeks of drug treatment (GL-II-73, 30 mg/kg). Y-Maze alternation task and Morris Water Maze were conducted to assess working memory and spatial cognition. Drug-sensitive 5xFAD mice receiving GL-II-73 showed better cognitive performance than the control group in both Morris Water Maze and Y-Maze alternation task, while such facilitation was not observed in the α5KI mice. Our findings

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demonstrated that the effects of GL-II-73 are mediated by activity at α5-GABAARs, as expected, specifically at improving working memory and spatial cognition.

P2-F-178 - Age-related declines in brain network segregation and its heightened impact on cognitive performance in older adults

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Healthy aging brings changes in both cognition and brain network organization. Previous research on resting state scans has shown that functional brain networks become less segregated in older adults. Furthermore, these declines in segregation have been associated with reduced cognitive performance. However, it remains to be seen whether the effect of segregation on cognition is specific to older adults or if lower segregation is generally related to poor cognitive performance across all age groups. This study investigated the association between three cognitive metrics (semantic, executive and episodic) and system segregation (SS) in resting-state fMRI data from 179 young adults (18-29 years) and 117 older adults (60-89 years) in open-source data (Goal-Directed Cognition in Older and Younger Adults). Our findings confirmed an age-related decline in SS. Furthermore, we found that SS was negatively associated with semantic, episodic and executive performance in older, but not younger, adults. A mediation analysis confirmed that variability in SS mediated a negative effect of age on both semantic and executive performance but not on episodic performance. When examining segregation as a network specific measure, we find declines in segregation for the SMN, VAN, and FPN, and correlations with one or more cognitive metrics across each higher order/associative network. Altogether, these results highlight the importance of system segregation, both in its potential links to cognition, and in its maintenance for promoting healthy cognitive functioning specifically in older adults.

P2-F-179 - Expectation-driven modulation of pain: Neural and behavioral divergences between static and dynamic stimuli

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Most painful heat stimuli in daily life are dynamic, involving gradual temperature changes, while static stimuli, featuring brief heat spikes, are less common. This study examines whether dynamic stimuli elicit greater prediction errors than static stimuli. Prior research shows expectations influence pain perception for static stimuli, but it remains unclear if similar mechanisms apply to dynamic stimuli, which may engage distinct neural circuits. We assessed pain responses to uncued stimuli and those paired with expectancy cues that accurately predicted(matched) or violated (mismatched) stimulus intensity. Dynamic stimuli were rated as more painful than static ones. Both types showed expectation-driven modulation under matched conditions, but only static stimuli exhibited such effects in mismatched conditions, suggesting constant prediction errors in dynamic stimuli disrupt cognitive modulation. Static stimuli activated fronto-parietal regions associated with cognitive control, while dynamic stimuli engaged pain, fear, and reward circuits more robustly. These findings reveal distinct anticipatory mechanisms for dynamic pain.

P2-F-180 - Childhood cognitive profile as a predictor of academic performance in the adolescent brain cognitive development study

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Background: Evidence suggests that cognition is associated with academic achievement in children and adolescents. However, the role of childhood cognitive function on future academic performance is unclear. This study examines the temporal relationship between cognitive profiles and subsequent academic achievement among participants in the Adolescent Brain Cognitive Development Study.Methods: Adolescents were recruited from community settings, and cognitive profiles were identified using latent profile analysis across six cognitive domains (working memory, long-term memory, attention, executive function, processing speed, language) at baseline. Academic performance was assessed at 2-year follow-up, using parent-reported measures. The relationship between cognitive profile membership and academic performance was examined using multinomial logistic regression, controlling for covariates.Results: Participants (n=10,337) had a mean age of 9.9 ± 0.6 years, and 48% were female. Two cognitive profiles were identified, with the majority of participants (n= 9,589) showing higher cognitive function, while 748 children exhibited lower cognitive function across all six domains. Participants in the lower cognition group were more likely to exhibit academic performance below expectations (OR = 1.54; 95% CI: 1.27,1.88) and less likely to exceed academic expectations (OR = 0.54; 95% CI: 0.44, 0.68) at year 2, compared to participants in the higher cognition group. Conclusions: In this community-based sample of adolescents, lower cognitive function was associated with lower future academic performance. Identification and intervention for cognitive deficits in early childhood may support prevention of poor academic achievement in children at risk.

P2-F-181 - Microrna regulation of grp78 in somatostatin-positive GABAergic interneurons: Unveiling molecular mechanisms of age-related cognitive decline

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Cognitive decline is a hallmark of aging. Studies showed a reduction of function of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) in the aging brain, particularly related to somatostatin-positive (SST+) GABAergic interneurons. SST cells play a critical role in regulating neuronal circuits, and their dysfunction has been linked to cognitive deficits. Emerging evidence identifies endoplasmic reticulum (ER) stress and disrupted response to ER stress as central factors contributing to the decline of SST+ neurons. We hypothesize that aging causes a downregulation of Hspa5, a gene coding for a key ER chaperone, GRP78, involved in ER stress regulation and associated cognitive deficits. This project also aims to investigate the changes of microRNAs (miRNAs) regulating this pathway in SST neurons. A combination of bioinformatics analysis and experimental validation in a mouse model of aging was used to identify miRNA candidates affected by aging in SST+ neurons. 24 months old mice (N=12), and 2 months old counterparts (N=14) were tested in cognitive assays, and brain tissues were collected for downstream analyses. Behavioral assessment confirmed cognitive decline. Differentially Expressed Gene (DEG) analysis in aged mice revealed significant miRNA profile alterations in SST+ neurons. qPCR experiments confirmed the alteration of potential inhibitory miRNA candidates, consistent with Hspa5 downregulation.These findings shed light on the molecular

mechanisms driving SST+ interneuron vulnerability and provide a foundation for future therapeutic interventions.

P2-F-182 - The effect of different concentrations of trimethylthiazoline on defensive behaviours

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Trimethylthiazoline (TMT), a single molecule component of fox feces, is widely used in laboratory research to induce innate defensive behaviours such as freezing in rodents. Despite its prevalence as an environmental stress model, the literature lacks consistency in the concentrations of TMT reported across studies, if concentrations are reported at all. This variability complicates experimental conditions and contributes to inconsistent findings. Here, we characterized defensive behaviours in mice exposed to 3%, 10%, and 30% TMT, using butyric acid (BA) at similar concentrations and reverse osmosis (RO) water as controls. Our findings demonstrate that 10% TMT elicited the most robust freezing response, followed by 30% TMT, indicating a non-linear relationship between concentration and defensive behaviour. These results suggest that higher concentrations of TMT do not proportionally increase the amount defensive behaviours evoked. Standardizing TMT concentrations is crucial for ensuring consistency and reproducibility in future research.

P2-F-183 - Fear extinction requires PKMζ in the infralimbic cortex and AMPA receptor endocytosis in the prelimbic cortex

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IntroductionExtinction memory enables the suppression of fear responses to stimuli that no longer predict threat. The prelimbic cortex (PL) is central for fear memory expression, while the infralimbic cortex (IL) is key for its suppression. Protein kinase M zeta (PKMζ) maintains the storage of many memories by inhibiting the endocytosis of GluA2-containing AMPA receptors (GluA2-AMPARs) from synapses. Notwithstanding, whether PKMζ mediates extinction memory storage and the role of GluA2-AMPAR endocytosis in the IL and PL in extinction remain elusive. MethodsTo address this, we trained rats in an auditory fear conditioning paradigm, submitted them to a behavioral extinction protocol and pharmacologically manipulated PKMζ activity and GluA2-AMPAR endocytosis in the IL and in the PL in different epochs. ResultsWe discovered that inhibiting PKMζ in the IL during maintenance abolished extinction memory, but that concomitantly blocking GluA2-AMPAR endocytosis prevented this effect. Conversely, PKMζ inhibition in the PL did not affect extinction memory maintenance. Furthermore, extinction required endocytosis of GluA2-AMPARs in the PL, since blocking this process impaired both extinction learning and memory. On the other hand, the same manipulation in the IL enhanced extinction memory. ConclusionOur results demonstrate that PKMζ maintains fear extinction memory in the IL and that extinction regulates GluA2-AMPAR dynamics in opposite directions in the IL and the PL. These findings provide crucial insights into the molecular underpinnings of extinction memory.

P2-F-184 - Context fear discrimination learning modulates dopamine signaling in the medial prefrontal cortex

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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. The medial prefrontal cortex (mPFC) is a key structure for context-guided fear behaviour because a) it's engaged during fear expression b) its neural representation changes when the meaning of a context is altered and c) mesocortical dopamine guides adaptive actions. To further characterize the role of the mPFC during context threat uncertainty, we developed an apparatus to "teleport" mice between contexts to measure fear discrimination. We expressed the biosensor GRABDA in the mPFC to monitor dopamine signaling with fiber photometry during the task. We observed that stronger conditioning protocols produced prolonged dopamine transients. During the test day, mice discriminated threatening from neutral contexts and the "teleportations" were accompanied by increased dopamine signaling. Moreover, strong conditioning produced the greatest mPFC dopamine during context "teleportations". Males and females discriminated similarly, but differences in dopamine dynamics emerged. Optogenetic inhibition during context transitions is required to assess the necessity of mPFC dopamine in context fear discrimination. These results suggest that prefrontal dopamine dynamics are altered during context fear memory encoding and retrieval. Together our findings indicate prefrontal dopamine may be a target for therapeutics designed to reduce fear generalization which could benefit those with anxiety disorders.

P2-F-185 - Long-term fear memory formation in juvenile zebrafish

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Memories serve as a toolbox for adjusting experiences to be used in favor of the survival and adaptation of the organism. However, the brain cannot store all the experiences the organism goes through and selectively prioritizes certain ones over others. While repeated exposure to an event can consolidate memory, a single instance of a life-threatening event may suffice to create a lasting memory. Although both classical and operant conditioning have been extensively studied in zebrafish, most of those studies require training with multiple sessions lasting for hours to generate a memory trace and they do not last longer than 24 hours. Here we investigated long-term memory formation by using associative learning with a single conditioning event. We built an Arduino-controlled behavioral setup where we established fear conditioning by pairing a color with the shock stimulus. Fish were tested for fear memories after 24h, 1 week, and 2 weeks. Although the control and most of the test fish did not show a freezing response to the stimulus during the two-week assessment, a portion of the test fish displayed freezing behavior in response to the conditioned color during the test period, in both wild-type and the transgenic line (HUC: H2B-Gcamp6f). The next task is to perform whole-brain calcium imaging and observe the whole-brain dynamics for long-term fear memory retention by leveraging the small and transparent skull of juvenile fish. The findings of the study may shed light on differential memory retention mechanisms.

P2-F-186 - Defensive behaviour and periaqueductal correlates following contexual fear conditioning

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Generating context-appropriate defensive responses to threat is essential for survival. It is proposed that defensive behaviour is regulated by the periaqueductal grey (PAG) along a dorsal-ventral (DV) continuum in which the former (dPAG) regulates escape-like behaviour and the latter (vPAG) controls freezing (DeOca et al., 1998). The functional role of the lateral PAG (lPAG) is less characterized, but we hypothesize that it also controls expression of defensive responses. To test this, we used a learned fear test in mice that paired one context with shock and the other context with no shock. On test, mice could freely move between the shock-associated and neutral contexts. We first determined the ideal parameters to produce context avoidance as well as a range of defensive behavioural responses in male and female mice as they move between neutral and shock-associated contexts. We then used fiber photometry to record bulk calcium-evoked fluorescence in the lPAG to correlate its dynamic activity with these behaviours and context transitions. We used deep learning and recurrent neural network tools to define and analyze the expression pattern of defensive behaviour poses. Together these data reveal the dynamic brain-behaviour relationships during a range of defensive behaviours.

P2-F-187 - Brain network integration increases from rest to task and decreases with task load during n-back task: A fMRI study in healthy participants

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The working memory system is the interface where incoming sensory stimuli interact with prior expectations to form perception. Understanding the complexities of this system is vital for uncovering the relationship between working memory dysfunction and its clinical comorbidities. System segregation refers to the balance of within- and between-network connections and may be associated with working memory. Here we used functional MRI to observe how system segregation across five canonical resting-state networks (RSNs) changes across rest and varying levels of working memory task load in a healthy cohort, as well as the interrelationship between these brain measures and working memory. We report that greater system segregation at rest is predictive of overall working memory accuracy. Further, we report a decrease in system segregation during the entire task scan compared to the rest scan, but within the task scan we report an increase in system segregation as task load increases, and the degree of this change predicts performance on the high working memory task load. In addition, we report RSN changes in integration and segregation are not uniform across networks. Notably, as task load increases, the attention/executive network becomes more segregated (increase in within-network connections) and loses connectivity with the sensory network. These findings raise new insights on how the selected networks influence system segregation findings and that observing how specific networks change across task load provides useful information about the functional role of system segregation.

G - NOVEL METHODS AND TECHNOLOGY DEVELOPMENT

P2-G-188 - Developing a radiotracer to image the dopamine D1 receptor

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The dopamine D1 receptor (D1R) is the most populous D1-class GPCR, a receptor type associated with mood and movement. Although receptor abnormalities correlate with prevalent neurological conditions like Parkinson's disease, D1R remains underexplored in the dopaminergic system. D1R antagonists have been radiolabeled for PET imaging, but they show suboptimal target selectivity. However, positive allosteric modulators (PAMs) display non-orthosteric binding, reducing off-target signals. Among such compounds, ASP4345 was examined in preliminary clinical trials and showed activity at rodent and human receptors. Thus, we aim to evaluate [11C]ASP4345 as a positive allosteric modulator D1R radiotracer in a Parkinsonian context. We synthesized ASP4345 over six steps and assessed molecular identity via NMR and mass spectrometry, achieving an 86% yield in the final coupling step. Functional allostery was analyzed using dopamine and MLS1082, a known D1R PAM, in cAMP accumulation assays. In tandem, these experiments explored target selectivity by expressing the homologous dopamine D5 receptor (D5R). Schild analyses implied that ASP4345 binds to D1R/D5R and exhibits a combined agonist-PAM profile. Our next steps include producing a radiolabeling precursor and analyzing in vivo binding in rodents. Upon establishing kinetic and targeting parameters, we will determine [11C]ASP4345 uptake through PET imaging of Parkinsonian mouse models. Overall, these experiments will develop a PAM radiotracer workflow to study the dopaminergic system and neurological disease.

P2-G-189 - Simulating stress, depression, and schizophrenia in AI: A deep reinforcement learning approach

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The similarity between Deep Reinforcement Learning (DRL) models and dopamine-driven learning mechanisms in the brain makes DRL a useful abstract representation of biological learning. Additionally, DRL can also simulate various learning dysfunctions. We modelled Major Depressive Disorder (MDD) by replicating the dendritic spine loss seen in the prefrontal cortex of depressed animals, inducing depression-like behaviours such as anhedonia, greater temporal discounting, avoidance, and reduced exploration. After reversing the spine loss effect, the agent's behaviour normalized despite unchanged network patterns. Next, we linked spine loss to the agent's experiences in the environment, simulating a pathway connecting stress to neuronal health. Consequently, we see the agent develop depression-like symptoms after stress and sometimes remain permanently depressed if the stress is prolonged. We also used DRL to simulate schizophrenia. Previous research suggests that excessive pruning of excitatory connections during adolescence contributes to the disorder's pathology. We find that simulating this excitation/inhibition imbalance has little impact but makes the network vulnerable to noise. Introducing noise results in various schizophrenia-like behaviours and hallucinations. These DRL models provide insights and predictions regarding psychiatric and neurological disorders, serving as a potential method for preliminary hypothesis testing before employing animal models.

P2-G-190 - Precisiontrack: Reliable tracking of large groups of animals interacting in complex environments over extended periods

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Mice living in social groups within ethological environments exhibit a wide range of adaptive behaviors, including individual and group dynamics, often undetected in standard behavioral studies. To better understand the coping and adaptive strategies employed by each member of a social colony, it is necessary to develop tools that enable accurate, long-term monitoring of large groups of animals in a fully automated and unbiased manner. In this work, we introduce PrecisionTrack, as a solution to the multi-animal pose tracking problem. This solution enables accurate and reliable tracking of large groups of animals socially interacting within complex environments, even over prolonged periods. Our algorithm builds on a Transformer-CNN hybrid neural network for cross-species classification, detection, and pose estimation, leveraging pose-based matching AI-driven ArUco identification for fast and accurate re-identification. PrecisionTrack demonstrates superior performance in tracking accuracy and latency compared to current gold standards, while sustaining these capabilities over extended periods when tracking large groups of animals in complex environments. Furthermore, we trained PrecisionTrack to identify and monitor over 30 animal species. Overall, PrecisionTrack represents a reliable, accessible step toward adopting more ethological methodologies in behavioral research.

P2-G-191 - Guidelines for non-human primate chemogenetics

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Chemogenetics is a powerful method to control the activity of cells by targeting specific populations with a designer receptor activated by a designer drug. The use of chemogenetics in neuroscience has rapidly expanded in small animal models, and more research groups are now attempting experiments in non-human primates (NHP) with hopes for therapeutic use in humans. To speed up translational applications to patients and facilitate future basic science experiments with the technology, we have created a database listing all attempts, successful and unsuccessful, published and unpublished, of using chemogenetics in the primate brain. We received data from 39 laboratories in 7 countries who provided detailed methods and outcomes on more than 800 experiments, 500 of them never published before. We examine the success of those approaches as a function of viral vector, gene cassettes, brain regions, and 42 other experimental parameters. With this multi-center effort, we hope to provide guidelines for the research community using chemogenetics in primates and speed up the translation of this cell-type-specific neuromodulation technology for clinical applications in neurology and psychiatry.

P2-G-192 - Dendritic polyglycerol amine (dPGA) substrate coating enhances mixed glial culture viability

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Primary mixed glial cultures are key tools to isolate and study astrocytes, microglia and oligodendrocytes. Healthy cell-substrate adhesion is critical for survival and differentiation of

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neural cells. Cationic polymers like poly-D-lysine (PDL) have been widely used for decades to promote cell adhesion to cell culture substrates, however, PDL is not stable long-term, with cultures often detaching (peeling) after 2-3 weeks. Substrates coated with dendritic polyglycerol amine (dPGA), a synthetic polycationic non-protein macromolecular biomimetic of poly-lysine, promote neuronal adhesion and survival in long-term cell culture. Here we assessed dPGA as a substrate coating for mixed glial cultures. Oligodendrocyte precursor cells (OPCs) were isolated weekly from cultures grown in PDL or dPGA-coated flasks. Following two isolations, 80% of PDL-coated flasks fully detached. In contrast, dPGA-coated flasks consistently yielded ~0.5 million cells/flask per isolation for up to six weeks. Following isolation, at 2 DIV, dPGA-coated flasks produced more cells, a greater percentage of O4+ cells, and maintained similar fractions of OPCs and MBP+ cells as PDL. At 7DIV, the total number of cells and number of oligodendrocytes were comparable between groups. dPGA is a cytocompatible, functionally superior, easy to use, low cost and highly stable alternative to conventional cell substrate coatings. The enhanced long-term stability of mixed glial cultures grown on a dPGA substrate has the capacity to increase cellular yield and facilitate studies of fundamental glial cell biology.

P2-G-193 - Decoding neuronal diversity from extracellular recordings: A novel approach integrating neuropixels, optogenetics and intracellular recordings

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Single cell electrophysiological recordings are essential for understanding neuronal information encoding in behavioral contexts. Extracellular recordings (EC) have provided significant insights into cortical function but struggle to identify distinct cell types. Traditionally, neurons are classified as narrow or broad spiking based on action potential duration, often equating these types with inhibitory and excitatory neurons, respectively. However, this classification is problematic due to the existence of broad spiking interneurons and narrow spiking pyramidal neurons. Recent unsupervised clustering techniques for cell type classification in vivo have struggled to align identified clusters with a consensus cell type taxonomy.In this project, we use the "Visual Coding – Neuropixels" dataset from the Allen Institute for Brain Science, which includes optogenetic labeling of the following subpopulations: vasoactive intestinal polypeptide (VIP), somatostatin (SST), and parvalbumin (PV) expressing neurons. Additionally, we obtained a sparse label for excitatory cells (EXC) based on cross-correlograms. We leverage characteristic feature profiles of these non-overlapping subpopulations for supervised classification of EC units with high accuracy in mouse V1. Our goal is to apply this procedure across species, informed by single-cell in-vitro characterizations in marmoset. Prediction outcomes were evaluated by comparing predicitons with functional connectivity.Our results demonstrate that EC unit classification can be applied to species lacking transgenic tools, which is particularly useful for primate studies. We enhance our understanding of neuronal responses on the circuit level by offering a probabilistic readout during in vivo experiments. These techniques will be invaluable for systems neurophysiologists studying the cerebral cortex of behaving animals.

P2-G-194 - Capturing brain response patterns to subcallosal cingulate deep brain stimulation using fMRI

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Deep brain stimulation targeting the subcallosal cingulate (SCC-DBS) is a promising therapy for treatment-resistant depression. However, the lack of a consistent, rapid behavioural response to SCC-DBS complicates the selection of optimal stimulation settings following implantation, requiring a prolonged and burdensome trial-and-error process. Immediate biomarkers of effective stimulation could overcome this problem. In this proof-of-concept study, we scanned three SCC-DBS patients using 3T functional MRI (fMRI). A block-design paradigm was employed, wherein participants' DBS settings alternated between "on" and "off" states in 30-second cycles during a single acquisition sequence. Contrasting these settings enabled the identification of regional BOLD signal changes associated with effective DBS, revealing consistent hemodynamic changes in several brain regions during active stimulation. Specifically, the precuneus, posterior cingulate cortex, middle frontal gyrus, and frontal pole exhibited decreased BOLD responses during active DBS, while the occipital cortex, middle temporal gyrus, inferior parietal lobule, and superior frontal gyrus showed increased BOLD responses. Exploratory analyses further revealed a strong correlation between precuneus BOLD signal change during active stimulation and clinical improvement (R = -0.98). These findings highlight the utility of block-design fMRI with cycling DBS stimulation as a tool to identify objective, brain-based biomarkers of effective SCC-DBS, potentially expediting stimulation parameter selection and therapeutic optimization.

P2-G-195 - Insights into neural network firing patterns from chemical and optical interventions

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Neuronal cultures derived from primary or stem cells serve as vital platforms for predicting the effects of newly- developed drugs, particularly those with potential to modify neuronal excitability, before advancing to clinical research. Additionally, these platforms are valuable for modeling common neurological disorders, such as epilepsy, to uncover fundamental mechanisms underlying these conditions. Recent studies using electrophysiological techniques like multielectrode arrays (MEAs) revealed how metrics such as spike- rate change in response to known drugs, including 4-aminopyridine (4AP). These analyses, however, often fail to fully capture the complexity of drug effects, as some studies report increased activity with convulsant drugs, while others report decreased activity, indicating discrepancies between in-vitro and in-vivo results. To address these challenges, we analyzed the inter-spike intervals (ISI) probability distribution in primary neuronal cultures derived from E17/18 embryos of C57BL/6 mice exposed to 4AP for 30 minutes. Results showed that the firing patterns of neuronal cultures change compared to the baseline. These analyses gives additional insights on the spiking modality of the culture under convulsant drug exposure beyond the average spike-rate. To restore firing activity closer to baseline levels, we utilized light stimulation via patterned illumination in cultures expressing the inhibitory opsin ArchT. These findings may advance understanding of neuronal network behavior and inform future research efforts.

P2-G-196 - Next-generation electrophysiology for functional characterization of human neural organoids and assembloids

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Human-induced pluripotent stem cell (hiPSC)-derived 3D neural models (e.g. organoids, assembloids, etc.) are crucial tools for replicating human brain development and studying neurological disorders like Alzheimer's and Parkinson's disease. High-density microelectrode arrays (HD-MEAs) offer a label-free, non-invasive approach to real-time, high-resolution electrophysiological recordings from neural organoids, assembloids, and tissue explants. We used the MaxOne and MaxTwo HD-MEA platforms, each featuring 26.400 electrodes per well, to record extracellular action potentials from various 3D neural models across different scales, ranging from cell population networks down to single-cell and subcellular levels. We showcased the flexible selection of recording electrodes, enhancing the data's reproducibility and statistical power. Key parameters, including firing rate, spike amplitude, and network burst profile, were extrapolated. The AxonTracking Assay was employed to trace action potential propagation along axonal branches and analyze conduction velocity, latency, and axonal morphology. This breakthrough assay enables high-resolution analysis of disease models targeting axon initial segment, development, and conduction. The here presented HD-MEA platforms' capability for targeted electrode selection improves data consistency and enables more comprehensive statistical insights. Furthermore, automated data visualization and metric extraction make these systems a robust and user-friendly choice for in-vitro disease modeling and drug testing in both acute and longitudinal studies.

P2-G-197 - A novel machine-learning and bioinformatics pipeline for multiplex imaging in malformations of cortical development

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Tuberous sclerosis complex (TSC) is a genetic disorder characterized by hyperactivation of the mechanistic target of rapamycin (mTOR) pathway, leading to multisystem lesions, including cortical malformations (tubers) and epilepsy. Histological hallmarks of tubers include cortical disorganization, giant cells (balloon cells, BCs), gliosis, and dysmorphic neurons (DNs). Abnormal cortical structures termed micronodules/microtubers have also been observed in peri-tuberous areas and are hypothesized to contribute to epileptogenesis. Using multiplex imaging and machine-learning-based analysis, we developed a novel pipeline, including an algorithm that identifies micronodules based on vimentin (VIM) and pS6 expression, capable of quantifying multiple parameters and spatial features. Our findings reveal the complexity and heterogeneity of cell composition within micronodules and TSC-affected tissue. Specifically, we found that BCs, but not DNs, are key drivers of micronodule formation. Micronodules exhibit a high accumulation of immature astrogliosis (VIM+), with an increase in Lamp5+ glia at their periphery, and distinct neuronal subtype profiles compared to adjacent normal-appearing regions. By advancing methodologies for TSC pathology, our work enhances diagnostic precision and opens avenues to better understand disease mechanisms, ultimately aiming to improve patient outcomes.

P2-G-198 - Improving cross-species validity in attention studies by modifying a touchscreen continuous performance test

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Attention deficits are a hallmark symptom of numerous neuropsychiatric and neurodegenerative disorders. To understand and treat attentional changes in these conditions, we need preclinical models and paradigms that can translate to human research. However, preclinical methods for assessing attention often fail to translate to clinical settings, hindering the development of effective therapeutics. The rodent Continuous Performance Test (rCPT) is a highly translatable touchscreen-based version of the human CPT task for assessing attention in clinical and preclinical research. The CPT requires mice to respond to a target stimulus while ignoring non-target stimuli. Humans often require complex visual stimuli for the CPT to detect attentional deficits, whereas mice use simple line stimuli, creating challenges for cross-species comparability and limiting the CPT's face validity. To address this disconnect, we modified the rCPT to use "Fribble" stimuli, which are complex enough for humans while distinct enough to be visually discriminated by mice. 15 C57BL6/J mice were trained to perform the Fribble CPT, differentiating a target Fribble from four non-target Fribbles. The target stimulus hit rate, the non-target stimuli false alarm rate, and discrimination sensitivity—a metric that quantifies the ability to distinguish between target and non-target stimuli —were measured to assess attention. After training, performance metrics indicated the Fribble CPT accurately assessed mouse attention. Thus, the Fribble rCPT may serve as a valuable tool for bridging preclinical and clinical research on attention.

P2-G-199 - NC4 touch: An open-source platform for rodent behavioral training

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In recent years, rodent touchscreen-based paradigms have gained considerable popularity as a method for assessing cognitive function due to their flexibility in task design, improved standardization across studies, and automatic data collection that minimizes experimenter intervention and contributes to more replicable and reliable research. However, the high costs associated with commercially available systems present a financial barrier for smaller labs or researchers with limited resources.To address this issue, we have developed a custom-built, modular testing setup called the "NC4 Touch". It features three independent touchscreens and a feeding port, allowing for the training of rodents in tasks such as pairwise discrimination or delayed match-to-sample paradigms. The system includes a user-friendly graphical interface that offers real-time control, task customization, video recording, and data management.This work highlights the importance of open-source innovations in expanding the scope of scientific research, allowing researchers to overcome traditional financial barriers and creating a more inclusive, collaborative community.

P2-G-200 - Evoked activity from deep brain stimulation as biomarker of inhibitory effects

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Deep brain stimulation (DBS) of the thalamic ventral intermediate nucleus (Vim) has been a standard therapy for reducing essential tremor symptoms. Vim-DBS of high frequency (≥100Hz) has been clinically effective; yet, the underlying neuronal circuit mechanisms require further explorations. Neuronal biomarkers – e.g., firing rate and local field potential – have been used in previous studies to investigate the dynamics of DBS-targeted neurons, which are often surrounded by modulatory inhibitory neurons. Yet, there lacks a biomarker specific of these inhibitory effects, which are critical in stabilizing the underlying circuits. In this work, we discovered a biomarker of the hyperpolarizing inhibitory effects evoked by Vim-DBS that was present in 17.4% of single unit extracellular recordings. We developed a novel algorithm to remove the DBS artifacts and preserve and detect these evoked activities in human single-unit recordings of Vim neurons. We further modeled and analyzed these DBS evoked activities demonstrating different temporal characteristics. We found, in all cases, these evoked activities were significantly negatively correlated with firing rate and their presence and temporal characteristics demonstrated slow oscillatory patterns at 1-2 Hz frequency. We hypothesized that the evoked activity by high-frequency Vim-DBS is a biomarker of the spiking of inhibitory neurons, which stabilize the Vim activities through the excitation (Vim) – inhibition recurrent network.

P2-G-201 - Liquid biopsy of cell-specific extracellular vesicles to identify molecular signatures of Alzheimer's disease

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Alzheimer's disease (AD) is characterized by neuroinflammation and cognitive decline, with traditional diagnostics lacking early detection specificity. Advances in liquid biopsy have brought extracellular vesicles (EVs) to the forefront as biomarkers reflecting brain cell origins in the brain. Once isolated and analyzed, EVs can reveal molecular signatures of AD pathology and potential treatments.Methods: One ml of plasma from patients with AD and healthy controls was obtained to extract EVs. Fractions of 100 ul were incubated with antibodies to isolate populations of neuronal (NCAM), astrocytic (EAAT1), microglial (TMEM119), and oligodendrocyte-derived (MOG) EVs. Magnetic sorting was performed to pull down the cell-specific EVs. Samples were characterized through nanoscale flow cytometry, and levels of CD9 and CD63 were used to determine EVs populations. Concentrations of inflammatory-related proteins (TNF-α and IL-10) were determined by ELISA.Results: In AD samples compared to controls, TNF-α levels were higher in neuron-derived EVs (235 vs. 80 pg/ml) and oligodendrocyte-derived EVs (91 vs. 26 pg/ml). In AD, IL-10 levels in oligodendrocyte-derived EVs were higher (4.5 pg/ml vs. 1.2 pg/ml) and lower in microglia-derived EVs (0.23 pg/ml vs. 0.87 pg/ml) compared to controls. Conclusion: Our innovative liquid biopsy approach reveals distinct cytokine patterns in patients with AD, highlighting cell-type-specific inflammation, and offering insights into AD pathology to pave the way for minimally invasive diagnostics and personalized therapies targeting neuroinflammation.

P2-G-202 - A novel mrna-lipid nanoparticle platform to rapidly generate functional forebrain neurons from human pluripotent stem cells using ngn2

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Lentiviral delivery of neurogenin-2 (NGN2) has been widely adopted to rapidly convert human pluripotent stem cells (hPSCs) into neurons, driving advances in disease modeling and drug discovery. However, lentiviral based protocols are labor-intensive, pose genomic integration risks, and have viral safety requirements. To overcome these challenges, we developed the STEMdiff™-TF Forebrain Induced Neuron Differentiation Kit, a next-generation system delivering rapid, efficient, and reproducible NGN2-driven neural differentiation. This integration-free approach uses mRNA-Lipid nanoparticle (LNP) delivery of NGN2 to generate neurons from hPSCs within six days. The kit was evaluated across four hPSC lines:H9, H1, WLS-1C and SCTi003-A. On day 6, the system achieved 96% ± 3% TUJ1-positive cells and yielded 7.6 × 10^5 ± 3 × 10^3 neurons per kit. After a further, two weeks of maturation using BrainPhys™ hPSC Neuron Kit, 94% ± 2.6% of neurons expressed MAP2, and 90% ± 4.9% expressed the synaptic vesicle marker synapsin I (n = 8, 4 cell lines). Transcriptomic profiling confirmed a forebrain identity with high expression of FOXG1, TBR1, and SLC17A7. Electrophysiological analysis revealed spontaneous neuronal activity as early as day 8 (mean firing rate 0.11 ± 0.03 Hz), peaking at day 21 (1.5 ± 0.5 Hz), and persisting beyond 48 days, demonstrating sustained functionality. STEMdiff™-TF Forebrain Induced Neuron Differentiation Kit eliminates bottlenecks and offers a robust, integration-free solution to efficiently generate functional neurons and accelerate neurobiological discoveries.

P2-G-203 - Monitoring orofacial kinematics using an MEG-compatible tracking system during word and non-word repetition tasks: A pilot study

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Recording speech movements simultaneously with brain activity has traditionally posed significant technical challenges. This study employed the Magneto-articulography for the Assessment of Speech Kinematics (MASK) system to investigate the neuromotor control of word/non-word repetition and non-speech tasks. We recorded concurrent magnetoencephalographic (MEG) data and three-dimensional kinematic measurements of orofacial movements during speech production and tongue movement tasks. Our participant (one healthy adult) performed three tasks: tongue movement (left/right), non-word repetition (/ipa/, /api/, /ita/, /ati/), and word repetition (three-word sequences). Each task consisted of 8-second trials with self-paced repetitions. The system tracked upper and lower lip movements for bilabial closure (/p/), along with tongue body and tip positions for vowel and consonant (/t/ and /g/) formation. Speech-related brain activity was observed in regions of the precentral gyrus and middle/inferior frontal gyrus, with stronger left hemisphere lateralization compared to the bilateral tongue movement task. The kinematic profiles obtained through MASK demonstrated reliable characterization of movement coordination parameters, providing insights into speech motor control patterns. This approach allows for precise synchronization of articulatory movements with brain activity measurements, advancing our understanding of the neural mechanisms underlying speech production and offering new possibilities for investigating speech motor disorders.

P2-G-204 - Exploration of the robustness of neural decoding architectures for brain-computer interfaces

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Brain-computer interfaces (BCI) represent a promising advance to assist paralyzed individuals, yet their widespread application is hindered by significant challenges such as fluctuations in neural signals over time and the adaptability of models to different behavioral contexts. This study explores the robustness of various neural signal decoding architectures to identify those with the greatest potential for enhancing the generalizability of BCIs.Our approach relies on the dimensionality reduction of neural signals obtained via electrophysiology, which facilitates a global analysis of activity at the level of neuronal population rather than relying directly on individual neurons. To achieve this, we employ dimensionality reduction techniques such as Principal Component Analysis (PCA) and Uniform Manifold Approximation and Projection (UMAP), thereby fostering a better understanding of global dynamics while mitigating the effects of variations specific to individual neuronal units. The experiments utilize neural data collected during the execution of precise motor tasks involving forelimb force application in monkeys. Subsequently, we assess the performance of various decoding algorithms, including a linear method and two recurrent neural networks (LSTM and GRU). This experimental framework provides a unique comparative perspective for evaluating the robustness of decoders across time and behavioral contexts.

P2-G-205 - Investigating the association between P2Y12 receptor, SV2A, and tau expression in postmortem AD and control brains using in vitro autoradiography

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Background: Synaptic vesicle glycoprotein 2A (SV2A), a biomarker for synaptic density; purinergic receptor P2Y12R, a marker of microglia activation; and hyperphosphorylated tau have been extensively studied in Alzheimer's disease (AD), with growing interest in how these factors affect each other and enhance their impact. In vitro autoradiography (ARG) studies were carried out to evaluate the expression and distribution of P2Y12R, SV2A, and tau using [3H]PSB-0413, [3H]UCB-J and [18F]MK-6240 respectively, in postmortem brain tissues from AD versus cognitively normal health controls (CN) . Methods: [18F]MK-6240 was synthesized in-house, whereas [3H]PSB-0413 and [3H]UCB-J were custom synthesized (Novandi AB, Sweden). Thin section ARG binding assays were performed on post-mortem hippocampal/BA28 and entorhinal cortex tissues from CN (n = 3) and AD patients (n = 5). Non-specific binding was determined through homologous blockage with 10 μM of Suramin, Levetiracetam, and cold MK-6240, known ligands for P2Y12R, SV2A and tau, respectively. Results: [3H]PSB-0413 ARG revealed significant decreased binding in the subiculum (SUB) and entorhinal cortex (EC) of AD compared to CN. [3H]UCB-J ARG showed significant decrease in the dentate gyrus (DG), CA1, and EC of AD compared to CN. [18F]MK-6420 showed strong binding to tau aggregates throughout the hippocampus of AD, with a significant increased in binding observed in AD patients in CA1 and SUB compared to CN. When comparing binding in subregions in AD patients, [3H]UCB-J ($R2=0.57$, $p<0.0001$) and [3H]MK-6240 ($R2=0.57$, $p<0.0001$) have a significant correlation with [3H]PSB-0413. Conclusions: This study demonstrates that SV2A levels decrease while tau accumulations increase in AD brains with [3H]UCB-J and [18F]MK-6420 respectively. We also showed that P2Y12R is decreased in AD brains with [3H]PSB-0413.

P2-G-206 - Neurovascular coupling during periods of neuronal and vascular co-fluctuations and arousal in awake mice

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fMRI is widely used to assess brain activity and connectivity in humans. However, since fMRI relies on blood oxygenation levels as a proxy for neuronal activity, it cannot disentangle the vascular and neuronal networks using fMRI data alone. Mouse brains, unlike humans, allow invasive optical imaging for direct vascular and neuronal measurements. This approach allows exploration of factors thought to influence neurovascular coupling, such as arousal level, brain activity, and neuromodulators. Using widefield imaging of calcium and intrinsic optical signals, we measured spontaneous cortical neuronal and vascular dynamics simultaneously along with face motion and pupillometry in awake mice. The resulting data was split into moments of high and low co-fluctuation events, as well as moments of high and low arousal states, and these segments were used to compute the correlation between the vascular and neuronal measurements (neurovascular coupling). We found that the moments of high neuronal co-fluctuations were the strongest indicators of high neurovascular coupling, followed by moments of high vascular co-fluctuations. The optimal lag values at which correlations were highest indicate that the neuronal signal precedes the vascular signal, as expected. We aim to clarify how neurovascular coupling depends on time, brain activity, and arousal. We plan to extend our study by including more mice, improving temporal resolution, analyzing functional connectivity, employing a region-based approach, and including additional metrics to refine time point selection.

P2-G-207 - Accelerating our understanding of childhood brain development from infancy to adolescence: The Canadian pediatric imaging platform (c-pip) neuroimaging protocol

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Disruptions to typical brain development during childhood are important risk factors for a variety of neurodevelopmental and psychiatric conditions. Magnetic-resonance imaging (MRI) can help chart normative brain development and identify critical windows where disruptions might influence adverse developmental outcomes. However, characterizing neurodevelopmental trajectories remains challenging due to significant heterogeneity in data acquisition and limited availability of longitudinal data across childhood and adolescence. To address these challenges, the Canadian Pediatric Imaging Platform (C-PIP) was launched at three of Canada's largest pediatric hospitals: Alberta Children's Hospital, Centre Hospitalier Universitaire Sainte-Justine and Hospital for Sick Children. A large representative sample of youth aged 0–18 years (N=820) will be recruited using an accelerated three-wave longitudinal design. C-PIP will encourage innovative scientific research through high-precision, multimodal imaging sequences and standardized processing workflows to maximize data validity and harmonization. Advanced sequences include high-resolution anatomical and diffusion MRI, multi-echo functional MRI, MR spectroscopy, and inhomogeneous magnetization transfer imaging. The protocol and quality-assessment metrics will be presented to demonstrate the functionality of the platform. Together, CPIP will offer unprecedented opportunities for advancing scientific knowledge and

identification of intervention targets to mitigate the behavioural sequalae of disrupted pediatric neurodevelopment.

P2-G-208 - A multimodal micro-optrode enabling single-cell electrophysiology and optogenetics to establish spike inference algorithms from calcium measurements in vivo across cell types

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Genetically encoded calcium indicators (GECIs) are widely used for monitoring neuronal activity due to their cell-type specificity and high signal-to-noise ratio. However, calcium indicators do not directly capture action potentials, the primary information-carrying events. Current spike inference algorithms based on machine learning approaches are limited by the scarcity of ground truth datasets, particularly from diverse neuronal subtypes and deep brain regions such as the thalamus. This project addresses this gap by characterizing the relationship between spiking and calcium dynamics in excitatory (VGluT2) and inhibitory (GAD2 and PV) neurons, specifically in their encoding of sensory modalities (visual, thermal, mechanical) in different regions of the cortex (S1 and V1) and the thalamus (VPL). Using a minimally invasive microprobe combining optical and electrical recording capabilities at single-neuron scale (<10 µm tip) we investigated how calcium transients correlate with spiking across diverse firing patterns and response amplitudes under spontaneous and stimulus-evoked conditions in anesthetized, head-fixed mice. Additionally, we compared the deconvolution performance of GCaMP6s and jGCaMP8s, considering their distinct characteristics (dynamic range and kinetics). By characterizing these indicators in different contexts, we assessed differences in calcium signaling relative to electrophysiological activity. Establishing these benchmarks will enhance spike inference algorithms, improving both the accuracy and interpretability of calcium imaging techniques.

P2-G-209 - cAMP-dependent CA1 neuronal population activity underlies murine hippocampus-dependent learning & memory

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The spatiotemporal organization of neuronal activity is critical for learning & memory. However, the molecular pathways underlying the coordination of neuron activity and their task-related dynamics remain unclear. Here, we investigate the role of cAMP (adenosine 3',5'-cyclic monophosphate) signaling to regulate CA1 neuronal population activity during hippocampus-dependent short-term memory (STM). cAMP is a ubiquitous intracellular messenger that serves for certain types of synaptic plasticity. We previously reported a rapid cAMP-dependent enhancement of synaptic strength and neuronal depolarization in murine hippocampal slices, suggesting that cAMP may regulate neuronal activity during STM. To characterize the role of cAMP to regulate neuronal activity for STM, we recorded the CA1 neuronal response profiles during the novel object (NORT) and displaced object location (OLT) tests by in vivo calcium imaging in adult mice and examined the effect of cAMP suppression by co-expression of constitutively active phosphodiesterase 4 mutant. We found similar frequency and duration of neuronal activity between the control and cAMP suppression groups, however, the organization of latent neuronal population states was perturbed by cAMP suppression following a significant reduction in object recognition memory, suggesting the critical role of cAMP in population activity

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for STM. We will further discuss our approach to optically interrogate cAMP-dependent neuronal activity specific to memory formation or recall by combination of optogenetic cAMP manipulation & in vivo calcium imaging.

P2-G-210 - BIDS-FLUX: a flexible data management workflow for continuous production of high-quality FAIR neuroimaging data within the Canadian Pediatric Imaging Platform

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Multi-site neuroimaging studies are essential for boosting statistical power and enhancing generalizability. A critical challenge in scaling multi-site studies is reducing the interval between data production and curation; automating data operations is essential for rapidly processing data and conducting quality control with standardized tools. The Canadian Pediatric Imaging Platform (C-PIP) has developed a federated, automated framework —BIDS-FLUX— for continuous data processing and quality control. Raw imaging data are securely stored at each site, where local processing environments handle de-identification, conversion to the standard BIDS format, and automated preprocessing using the open-source NiPreps ecosystem. To ensure reproducibility, workflows are containerized and version-controlled, thereby maintaining uniformity across sites. A structured framework orchestrates preprocessing, data tracking, and monitoring to ensure seamless integration as data collection progresses. Every step of the data management process is logged using DataLad, ensuring detailed provenance. Processed datasets are made accessible to the community via a queryable repository on the Canadian Digital Research Alliance cloud, facilitating rapid review, analysis, and cross-site collaboration. Beyond neuroimaging, C-PIP integrates multimodal data—including demographics, genetics, and cognitive assessments—into a validated BIDS dataset, streamlining analyses. BIDS-FLUX enables the rapid production of FAIR (findable, accessible, interoperable, and reusable) neuroimaging data, laying a scalable foundation for collaborative neuroscience.

P2-G-211 - Peripheral optogenetic stimulation for muscle activation in rats

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Background: Functional electrical stimulation (FES) may partially restore motor function after nerve or spinal injuries but has limitations, including non-physiological motor unit recruitment, leading to coarse movement and increased fatigue. Functional optogenetic stimulation (FOS) is a potential alternative, using viral constructs to express opsins in muscles or nerves, enabling light-induced depolarization and contractions. FOS may allow more natural motor unit recruitment, improving motor control.Aim: To compare optogenetic and electrical stimulation on rat muscle responses, focusing on fatigue and motor unit recruitment.Hypothesis: Optogenetic stimulation will enable finer movements and reduced fatigue compared to electrical stimulation.Method: An iterative approach is being used to identify a viral construct allowing efficient expression of opsins directly into the injected rat's wrist flexor muscles or into the corresponding motor neurons. Opsin expression and muscle responses are assessed using electromyography (EMG) and immunohistology. Various opsins are tested and characterized by

muscle recruitment curves in terms of force and evoked electromyographic activity. These results are then compared to those from electrical stimulation to evaluate differences in force resolution and fatigue between the two methods.Conclusion: This study may guide future research and contribute to developing technologies to mitigate neuromuscular lesions in humans.

P2-G-212 - A cutting-edge mitochondrial purification approach to investigate cell-autonomous and non-cell autonomous mechanisms of mitochondrial axon support

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[1] The Ohio State University

Mitochondrial abnormalities are a central pathological driver of numerous neurological conditions, including various forms of neurodegeneration. While neuronal mitochondria—particularly in axons—have been a major focus of this perspective, recent studies show that mitochondrial metabolism in glial cells is also essential for axon survival. Accordingly, we discovered that genetically induced mitochondrial dysfunction in Schwann cells or oligodendrocytes triggers spontaneous axon degeneration through an unknown mechanism. To distinguish the specific contributions of mitochondrial abnormalities in axons and oligodendrocytes to neurodegeneration, here we present an innovative magnetic immunocapture strategy. This method remarkably enables the rapid, cell-type-specific isolation of intact mitochondria ex vivo. It is efficient enough to extract the minimally abundant mitochondria in peripheral axons—a feat never previously achieved. Proteomic analysis demonstrated that our purity level rivals the few similar approaches, despite our focus upon lower-abundance cell types. Downstream, the strategy facilitates comprehensive ex vivo organelle profiling, including extracellular flux analysis and multi-omic characterization. Our implementation of this strategy will offer unique insight into the glia-mediated mechanisms of neurodegeneration—important for understanding diseases such as Alzheimer's, Parkinson's, and diabetic neuropathy. Such findings would inform the development of axon-protective therapeutics, potentially identifying molecular targets located outside the axon itself.

P2-G-213 - Accuracy of the qBOLD model to quantify oxygen extraction fraction: a Monte Carlo study in mice angiograms

Aurélie Beaudoin [1], Laura Beltran [1], Andrew Forester [1], Jordan Charest [1], Élie Genois [2], Michèle Desjardins [1], Louis Gagnon [1]

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Brain oxygenation levels inform us about its function and vascularization. Such information can be used to access cerebral tumours, as abnormalities in vascularization are indications of tumour type and grade. This information can help in treatment, especially in the case of hypoxic tumours, which are resistant to conventional cancer treatments. Quantitative BOLD (qBOLD) uses a biophysical model to recover Oxygen Extraction Fraction (OEF) from T2* measurements at rest using asymmetric spin echo (ASE) sequence. We implemented a Monte Carlo method to test the accuracy of qBOLD MRI to recover OEF. Simulations were performed over 5 mice cortical angiograms measured with two-photon microscopy and over homogenous randomly oriented cylinders. We populated the angiogram vessels with oxygen concentrations according to specified OEF values between 0.20 and 0.60 with increments of 0.05, and generated synthetic BOLD signals following an ASE sequence. Calculated OEF was averaged across all five

angiograms and was underestimated by 50% in real mice cortical angiograms. Analysis revealed that part of the underestimation comes from the heterogeneous oxygen saturation in the vessels and the geometry of the vasculature, as simulation performed over homogeneous cylinders decrease the underestimation to 25%. Our result suggests to revisit the qBOLD model to obtain reliable OEF measurements, as OEF is underestimated by half in realistic cortical angiograms. Future work will optimize the analytical qBOLD model by adjusting some intrinsic parameters relating dR2 to OEF from the simulations.

P2-G-214 - Automated machine learning pipeline to measure negative affect facial expressions following chronic social defeat stress states in freely moving male and female mice

Andre Telfer [1], Brenna Macaulay [1], Andrea Smith [1], Alfonso Abizaid [1]

[1] Carleton University

In preclinical rodent work, it is difficult to assess effects of stress on emotional states due to human bias, time constraints, and labor-intensity of manual behavioral screens. Recently, machine learning algorithms have been used to accurately classify facial expressions triggered by emotionally salient stimuli in tethered mice. This project aims to build an automated system to quantify negative affect using facial expressions in freely moving mice. To do this, we created a negative affect facial expression scale using a machine learning model that detects changes in facial expression following different doses of lipopolysaccharide (LPS). To increase scalability, we created a preprocessing pipeline that extracts frames with clear side profiles and optimal lighting from large quantities of video data. Current results show that the model ca detect changes in facial expressions after LPS treatment in a dose-dependent gradient. We also showed 71.4% precision in identifying frames from stressed mice vs non-stressed mice without using the scale. The next steps involve the comparison between frames from males and female mice that were stressed with those of controls and determine where they fall in the scale constructed with the LPS frames at different doses. Ultimately, the use of this graded pipeline will provide with a calibrated measure of negative affect. This will reduce human variability and labor, and advance preclinical stress research that includes sex as a variable to enhance translational relevance of findings.

POSTER SESSION 3

A-DEVELOPMENT

P3-A-01 - Unveiling the role of PTEN in Müller glial development

Alissa Pak [1], Yacine Touahri [2], Yaroslav Ilnytskyy [3], Luke Ajay David [4], Igor Kovalchuk [3], Robert Screaton [5], Carol Schuurmans [2]

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PTEN suppresses PI3K-Akt signaling, regulating cell survival, growth, proliferation, metabolism, and migration. Our lab previously showed that Pten conditional knock-out (cKO) in mouse retinal progenitor cells (RPCs), leads to precocious neuronal differentiation, depleting the RPC pool and reducing amacrine cells and rod photoreceptors. Pten also controls starburst amacrine cell radial and tangential positioning and neurite arborization. Despite extensive research on Pten function in the retina, little is known about its role in Müller glia, the major glial cell type in the retina. Using Pten RPC-cKO mice, we found that RPC-derived Müller glia fail to halt interkinetic nuclear migration during the transition of RPCs to Müller glia. In Pten RPC-cKOs, SOX9 immunofluorescence revealed disorganized Müller glial nuclei in the inner nuclear layer, with some nuclei delaminating into the outer nuclear layer, where photoreceptors reside. Despite this developmental defect, mature Müller glia markers (Glul, Slc1a3, Rlbp1) are expressed in differentiating Müller glia. Transcriptomic analysis showed downregulated differentiation inhibitors (Id1/2), upregulated Igf1, Fgf2, and elevated Gfap and Slc1a2, suggesting that the developmental defects result in reactive gliosis, a normal response to injury. These findings provide crucial insight into Müller glial development, essential for designing gene therapies targeting Müller glia for neuronal reprogramming in retinal neurodegenerative diseases.

P3-B-02 - Regulation of excitatory synaptic transmission and plasticity in postnatal hippocampal neurons by semaphorin 3A.

Harshit Thakare [1], Zoe Gagnon [1], Madeleine King [1], Stephen Glasgow [1]

[1] Brock University

Memory formation requires rapid modifications to synapses, key sites of electrical and chemical transmission between brain cells, however the molecular mechanisms underlying these structural and functional changes remain unclear. We have recently shown that guidance cues, membrane-bound and secreted proteins originally described in the developing nervous system that help guide axonal extension through directing cytoskeletal rearrangement, continue to play key roles functional and structural synaptic plasticity underlying memory consolidation. Semaphorin 3A (Sema3A) is a secreted guidance cue originally described as a chemorepellent protein to trigger axonal growth cone collapse and retraction, but has also been linked to paradoxical dendritic outgrowth. Here, we show that the Sema3A co-receptor, neuropilin-1

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(Nrp1), is expressed throughout postnatal development, and changes in Sema3A signaling are associated with spatial learning and memory consolidation. Moreover, we report that bath application of Sema3A (100 ng/ml; 1-2h) can modulate excitatory synaptic transmission in postnatal hippocampal neurons, likely through recruitment of Nrp1 to PSD95-positive synapses. Together, these findings indicate that Sema3A can contribute to on-going modification of excitatory neural circuits underlying learning and memory formation.

P3-A-03 - Maturation of intrinsic and synaptic properties of the mouse dorsal peduncular cortex

Anna Canella [1], Abdessattar Khlaifia [2], Francesca Violi [1], Justin Botterill [3], Maithe Arruda Carvalho [2]

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The dorsal peduncular cortex (DP), the most ventral subregion of the rodent medial prefrontal cortex (mPFC), plays an important role in modulating anxiety-like behavior, stress responsivity, sympathetic activation and fear learning. Deficits in mPFC neuronal and synaptic maturation have been linked to several neurodevelopmental and mental disorders. Yet, how early life changes in DP synaptic maturation and connectivity may affect anxiety, emotional learning and stress responsivity in adulthood is unknown. Here we examined the development of synaptic and intrinsic properties of DP pyramidal neurons in both superficial (layer 2/3) and deeper layers (layer 5) of C57BL/6J mice throughout the first postnatal month using whole cell patch clamp recordings. We found that within the second postnatal week there is a shift into a mature cellular profile, marked by the resting membrane potential and action potential threshold hyperpolarization, decreased input resistance, increased rheobase and decreased after-hyperpolarization amplitude across layers. Excitatory spontaneous responses of DP layer 2/3 and 5 pyramidal neurons plateau during the second and third weeks of age, respectively, whereas spontaneous inhibitory responses show a gradual increase, plateauing by the fourth postnatal week. Overall, our data show similar maturation of intrinsic and synaptic properties of pyramidal neurons in both DP layers. Ongoing experiments aim to characterize the downstream connectivity of DP pyramidal neurons with the hypothalamus. This work will provide a blueprint for the developmental trajectory of the DP, highlighting potential sensitive periods of synaptic maturation when external factors such as stress may potentiate anxiety-like and fear behavior.

P3-A-04 - Phenotypic characterization from infancy to adolescence: Canadian pediatric imaging platform phenotyping protocol

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Background: Understanding neurodevelopmental trajectories and the impact of disruptors to brain development requires integrating neuroimaging with robust phenotyping assessments. This study outlines a comprehensive assessment protocol designed to complement MRI imaging data in a longitudinal multi-site Canadian cohort spanning infancy to adolescence (0-16 years at baseline).Methods: Assessments were selected based on 1) alignment with existing literature on

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relevant and developmentally appropriate constructs, 2) applicability across different developmental stages with parallel versions, when necessary, 3) validation in both English and French, and 4) sensitivity to year-to-year changes in cognitive and psychosocial functioning. Observational measures and online questionnaires assess domains critical to neurodevelopmental research, including cognitive functioning, psychopathology, personality and temperament, behavioural risks, and physical health and development.Results: The strategic administration across multiple time points ensures a thorough, longitudinal understanding of neurodevelopment. This approach facilitates cross-sectional and longitudinal analyses of the interplay between brain development, cognition, mental health, and environmental influences.Conclusion: By integrating phenotypic and neuroimaging data, this study will contribute to a deeper understanding of individual differences in neurodevelopment, cognition, and behaviour. This protocol also facilitates harmonization across pediatric studies.

P3-A-05 - Regulation of inhibitory interneuron survival by the clustered Protocadherins and PTEN/PI3K signaling

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Mammalian nervous system development requires balancing excitatory and inhibitory interneurons (INs), with disruptions linked to neurodevelopmental and psychiatric disorders. A process that refines neuronal populations is developmentally programmed cell death, whereby ~35% of inhibitory (GABAergic) neurons undergo apoptosis in early postnatal days. The molecular mechanisms determining their final numbers remain unknown. Our lab and others identified the clustered protocadherin gammas (Pcdhγs) as regulators of inhibitory IN survival in the cortex, cerebellum, and retina, with their loss reducing GABAergic populations. Among these, PcdhγC4 is being particularly indispensable. We explored Pcdhγ-mediated signaling pathways and found the PTEN/PI3K-Akt pathway to be crucial for inhibitory IN survival. Pten deletion increases IN numbers and p-Akt levels, opposing the effects of Pcdhγs. Additionally, we showed that PTEN acts upstream of and inhibits Pcdhγs. To further analyze PcdhγC4's role in survival, I generated mice that conditionally express PcdhγC4-GFP or PcdhγC3-Cherry in inhibitory INs in early development and performed immunohistochemistry experiments. To determine whether PTEN regulates Pcdhγ protein levels, I conducted western blotting on Pten-deficient retinas. My results reveal differential localization and expression patterns of Pcdhγ isoforms in the retina. PcdhγC3 is highly expressed in inhibitory INs, while PcdhγC4 is mainly enriched in Muller glia, with lower expression in GABAergic INs. Interestingly, PcdhγC4 expression is temporal, peaking at P7 and diminishing by P28. Preliminary analysis suggests that PcdhγC4 overexpression slightly increases IN survival in wild-type retinas, which is not seen for PcdhγC3. Finally, western blotting reveals slightly increased Pcdhγ protein levels in Pten knockouts.

P3-A-06 - Tracking morphological and functional changes in the developing Xenopus retinotectal system

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Early development in the Xenopus retinotectal system involves extensive cell proliferation and expansion of both the retina and the optic tectum. Much remains unknown regarding what

changes occur in the retinotectal circuit during this process, and how visual processing functions are maintained in this system with constantly changing components. Here we performed longitudinal imaging in the optic tectum of Xenopus tadpoles expressing GCaMP6s, in which individual or a small cluster of tectal cells were labelled with Alexa 594-dextran dye. We imaged animals over a 2-3 day period at developmental stage 45-46 and at stage 48, quantifying changes in the morphology and functional retinotopic representations of the dextran-labelled tectal cells, and compared them with morphometric and functional changes in the whole tectum. We observed matched expansion of the dendritic arborizations of tectal neurons and the whole tectal neuropil, such that their relative volumes did not change over time. Although the overall retinotopic map was surprisingly labile during this period, the inputs to individual tectal neurons remained highly convergent, suggesting that the early lability of the maps is unlikely to be driven by postsynaptic structural fine-tuning or by constraining access to functional inputs. Our results grant a deeper insight into how developmental changes in the retinotectal system occur at the individual neuron and at the whole circuit level, and how morphological changes relate to functional reorganization.

P3-A-07 - Distinct receptor tyrosine kinase phosphorylation profiles of neural stem cell derived neurons and astrocytes

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Background: Receptor tyrosine kinases (RTKs) play a vital role in cell growth, survival, and differentiation. In neurons, key RTKs such as IGF-1R and TrkB regulate processes such as dendritic growth, long-term potentiation, and neurodevelopment. While in astrocytes RTKs such as EGFR and VEGFR can regulate blood brain barrier (BBB) interactions, reactive gliosis, and astrocyte proliferation. Despite their vital role, the baseline phosphorylation status of RTKs in neural stem cell (NSC)-derived neurons and astrocytes remains poorly characterized. Understanding these signalling differences may provide insight into neural development and distinguishing disease related changes from normal cellular function. Results: We differentiated NSCs into neurons and astrocytes, confirming their phenotypes using western blotting and immunocytochemistry for VGLUT1 (glutamatergic neurons), GAD1 (inhibitory neurons), and GFAP (astrocytes). Using phospho-proteomics, we evaluated the phosphorylation state of 49 RTKs in both models, revealing distinct baseline phosphorylation patterns in both cell types. Key receptors such as ErbB4, IGF-1R, Insulin R, TrkB, EphA6, and EphA5 showed increased basal phosphorylation in neurons, while HGFR, PDGFRβ, VEGFR2, and EphA7 exhibited increased basal phosphorylation in astrocytes. Conclusion: This study reveals distinct RTK phosphorylation patterns in NSC-derived neurons and astrocytes, offering insights into cell type specific signalling and a foundation for understanding neurodevelopment and disease progression.

P3-A-08 - Combinatorial expression of the proneural genes Ascl1 and Neurog2 restricts retinal progenitor cell fate

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The retina is comprised of one glial and six neuronal cell types, all derived from a multipotent pool of retinal progenitor cells (RPCs). To generate this diverse array of cell types, RPCs undergo temporal identity transitions so that the correct numbers of neuronal and glial cells are generated in a precise order. Expression-profiling studies have revealed that RPCs are molecularly heterogeneous, but how different genes combine to specify unique cellular phenotypes remains poorly understood. Here, we report that the proneural transcription factors Ascl1 and Neurog2 act in a combinatorial fashion to specify the molecular identity of a precise population of retinal amacrine cells. First, using Neurog2-mCherry and Ascl1-GFP transgenic lines, we sorted negative, Neurog2 and Ascl1 negative, single+ and double+ RPCs and performed transcriptional analyses. We identified 7145 differentially expressed genes (DEGs) distinguishing the four RPCs pools, and revealed that genes involved with an amacrine cell fate were specifically enriched in double+ RPCs. To examine the fate of Ascl1+/Neurog2+ RPCs, we employed a split-Cre lineage tracing system in which the N-terminus of Cre was knocked into the Neurog2 locus and the C-terminus of Cre was knocked into the Ascl1 locus. Strikingly, double+ RPCs give rise to a precise population of non-GABAergic/non-glycinergic (nGnG) amacrine cells. We functionally validated this fate bias by selectively ablating double+ RPCs, leading to a significant loss of nGnG amacrine cells by P0. Taken together, Ascl1 and Neurog2 combine to specify a unique nGnG amacrine cell fate, revealing that cellular heterogeneity is specified by a combinatorial transcription factor code.

P3-A-09 - Shared vertebrate photoreceptor developmental network is positively selected in snakes and lizards

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Among vertebrates, snakes and lizards are unusual in having repeatedly evolved simplex retinas through secondary losses of either one of two of the distinct and highly specialized photoreceptor classes: rods and cones. Though the complementary functions of rods and cones allow for vision across a wide range of visual environments, the loss of one type can be advantageous if the visual environment suits only one type. Over 50 years ago Gordon Walls hypothesized that presentation of cone- or rod-only retinas in snakes and lizards occurred not by the outright loss of one type, but by photoreceptor "transmutation", or the evolutionary transition between rod and cone identity whereby one photoreceptor class adapts to evolve features of the other. Transmutation in adult photoreceptors was identified first by morphology, and more recently from our group by molecular markers. However, it remains unresolved how these unique retinal compositions develop. Photoreceptor development is tightly controlled by a network of genes largely conserved across vertebrates and so we hypothesized that patterns of evolutionary sequence constraint occurring in these genes in reptiles support the diversity seen in photoreceptor composition. To address this, we use a comparative computational approach to examine the evolutionary patterns of sequence constraint and selection on photoreceptor development genes combined with an examination of morphological and molecular markers in retinal tissues. Preliminary results reveal that reptile orthologs of many photoreceptor differentiation factors are present, and the evolutionary rates of these genes point to a divergence of selection pressures across a wide sampling of taxa. This is the first evidence for a mechanism underlying transmutation in snakes and lizards.

P3-A-10 - Experience dependent modulation of adult hippocampal neurogenesis in female mice

Jacqueline Boon [1], Linda Le [1], Michael Chrusch [1], Simon Spanswick [1], Jo Anne Stratton [2], Prajay Shah [1], Haley Vecchiarelli [3], Payal Patel [1], Jeff Biernaskie [1], Matthew Hill [4], Richard Dyck [1]

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Different experiences can positively or negatively impact adult hippocampal neurogenesis (AHN). The dentate gyrus of the hippocampus contains the highest concentrations of zinc found in synaptic vesicles, known as vesicular zinc, and we previously showed that vesicular zinc is necessary for enrichment-mediated increases in AHN among male mice. The present study investigates the role of vesicular zinc in modulating AHN in the context of multiple combined experiences in female mice lacking the vesicular zinc transporter, ZnT3, and thus, vesicular zinc. Adult female wildtype (WT) and ZnT3 knockout (KO) mice were administered fluoxetine daily under pair- or individual-housing conditions. After three weeks, hippocampal-dependent behaviours and BDNF levels were examined. Another cohort of mice were administered BrdU, then returned to their cages for an additional three weeks until their brains were collected to assess cell proliferation, survival, and death. Similar to male mice, vesicular zinc is necessary for the positive modulation of AHN in female mice. WT mice, but not ZnT3 KO mice, exhibited fluoxetine-induced increases in the numbers of Ki-67+ and BrdU+/NeuN+ cells and levels of BDNF, as well as improvements in pattern separation. In contrast, isolation-induced reduction in AHN was not modulated by vesicular zinc. Rather, the effects of isolation masked the effects of vesicular zinc and fluoxetine. Together, these results suggest that vesicular zinc mediates positive experience-dependent plasticity and emphasize the complexity of AHN modulation by multiple experiences.

P3-A-11 - Teneurin-3 and Latrophilin-2 are required for somatotopic map formation in the dorsal horn

Kevin Sangster [1], Daniel Del Toro [2], Xinying Zhang [1], Christina Sarantopoulos [1], Ashley Marie Moses [3], Shreya Mahasenan [1], Daniel Tyler Pederick [3], Robert Brian Roome [4], Elena Seiradake [5], Liqun Luo [3], Artur Kania [4]

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Despite first being described nearly a century ago, the mechanisms governing the formation of somatotopic (ST) maps, such as the cortical homunculus of Penfield, remain largely unknown. The mediolateral (ML) axis of the dorsal horn (DH) of the spinal cord is organised as an ST map of the proximodistal (PD) axis of the body surface. We show that this map correlates with complementary ML gradients of Teneurin-3 (Ten3) and Latrophilin-2 (Lphn2) cell surface receptors. A corresponding expression pattern is also evident in the sensory neurons of the dorsal root ganglia (DRG). Since Ten3-Lphn2 mediated repulsion in conjunction with homophilic Ten3-Ten3 mediated attraction control the wiring of hippocampal circuits, we studied the function of these proteins in DRG-DH connectivity. First, we demonstrated that these proteins are capable of guiding DRG axons in vitro. Next, we generated conditional knockouts (cKOs) of Ten3/Lphn2 in the DH and DRG, which revealed a range of ML miswiring phenotypes depending on the ablated gene and location, with the Ten3 DH cKO being the most severe. Furthermore, Ten3 DH cKOs also showed an abnormal distribution of Fos protein, a proxy of neuronal activity, induced by a noxious stimulus. Importantly, such mutants display an impaired ability to accurately attend to a noxious stimulus along the PD axis of the limb. We thus provide the first

evidence that these molecules act in complementary gradients, are required for ST map formation, and disruption of DH ST map development is accompanied by deficits in directed somatosensory behaviours.

P3-A-12 - Within-litter variation is greater than between-litter variation in maternal provisioning, offspring anxiety-like behaviour, and offspring neurophysiology

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Maternal care is crucial for the neurodevelopment of rat offspring. However, the level of care can vary between different litters, even among those with shared genetics. Our lab's previous research identified variations in the care provided to pups within litters. These within-litter differences, in addition to reported between-litter differences, contribute to the heterogeneity of maternal care and consequently to offspring behavior and neurophysiology. We analyzed behavioral variables in Long-Evans rats related to maternal care, offspring behavior, and physiology, including neurotransmitter levels in several brain regions. In ongoing studies, we are analyzing variance in maternal behaviors in the first week of life with exposure to gestational stress, and relative fold expression of microRNAs involved in maternal care and neurodevelopment. These analyses use a standardized coefficient of variation (CV) to capture the effect of within-litter differences on behavioral and physiological outcomes. Variables analyzed in this model showed greater within-litter CVs compared to between-litter CVs for maternal licking behaviors and offspring behavior and neurophysiology. In our stress study, we are assessing within- and between-litter CVs for maternal behaviors across both conditions. Our results show that within-litter variation is greater than between-litter variation for maternal care and offspring behavior and physiology. These findings highlight that variability in maternal care among siblings may be an important source of individual differences in behavior and neurophysiology later in life.

P3-A-13 - Prenatal oxidative DNA damage causes sex-dependent and promoter region-specific postnatal epigenetic dysregulation of Gabra2 in the brains of DNA repair-deficient Brca1 knockout progeny exposed in utero to saline or ethanol

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Oxidative DNA damage (ODD; 8-oxoG) caused by reactive oxygen species (ROS) is implicated in neurodevelopmental disorders (NDDs), but the molecular mechanisms are unclear. We hypothesize that 8-oxoG formation in fetal brains dysregulates promoter methylation (pMe) of neurodevelopmental genes, initiating postnatal NDD-like phenotypes. We have shown that increased fetal ODD either in DNA repair-deficient heterozygous (+/-) breast cancer 1 (Brca1)knockout progeny, and/or following prenatal exposure to the ROS enhancer ethanol (EtOH), was associated with altered promoter methylation and expression of NDD risk genes. This study investigated the relationship among ODD, DNA methylation and NDD gene dysregulation. DNA was isolated from postnatal day (PND) 24 brain cortices of female and male Brca1 +/- and +/+ progeny exposed to saline or 4 g/kg EtOH on gestational day 17, and postnatally

to 1 mg/kg of the methylation inhibitor decitabine (DAC) on PNDs 8, 15 and 22. pMe and expression of the representative NDD risk gene Gabra2 were examined respectively using methylated DNA immunoprecipitation-qPCR and RT-qPCR, revealing sex- and promoter region-specific differences. EtOH decreased Gabra2 expression only in Brca1 +/+ females, which was altered by DAC (p=0.051), and only in Brca1 +/- males. EtOH increased methylation of promoter region (PR) 2 in only Brca1 +/- females, with no changes in PR 1. These preliminary results suggest novel epigenetic effects of ODD in the etiology of NDDs, and reveal potential targets for postnatal therapies to prevent ROS-initiated NDDs.

P3-A-14 - Investigating the developmental impact of hypothalamic tanycytes

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The hypothalamus orchestrates fundamental physiologies and behaviours through a diverse array of neurons and glia which populate an intricate patchwork of nuclei. As in other neural tube-derived brain regions, hypothalamic neurogenesis and gliogenesis occurs through the division of radial glial progenitors at the ventricular zone, followed by radial migration of neuronal and glial precursors into the parenchyma. However, the presence of tanycytes, a population of radial glia-like cells, along parts of the mature hypothalamic third ventricle (3V) raises the intriguing possibility that some, if not all, of these enigmatic ventricular cells may comprise an additional, specialized progenitor pool in the developing hypothalamus. Here, we hypothesized that radial glia and tanycytes work in concert to generate the vast range of hypothalamic neurons and glia. To start, we conducted single-cell RNA sequencing of the developing hypothalamic ventricular zone, with subsequent RNAscope validation, to characterize its cell type composition over time. Alongside classical radial glial cells, tanycytes were detected lining the 3V as early as embryonic day (E) 13.5, a position consistent in both time and place for contributions to developmental neurogenesis and/or gliogenesis. In particular, we identified a putative tanycyte subpopulation residing in the caudal-most regions of the embryonic hypothalamus that uniquely expressed the stem cell marker Tnfrsf19/Troy, and preliminary lineage tracing experiments demonstrated the production of neurons and glia in the mammillary hypothalamus by Troy+ ventricular cells. Taken altogether, these data suggested that tanycytes may indeed serve as an embryonic hypothalamic progenitor, with future experiments aimed at uncovering whether the neural stem capacity of prenatal Troy+ tanycytes is influenced by a maternal high-fat diet.

P3-A-15 - Risk for emotional dysregulation following prenatal alcohol exposure was associated with altered gut microbiota and immune function

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Individuals with Fetal Alcohol Spectrum Disorder (FASD) often have challenges with social interactions and emotional regulation. Increasing evidence highlights the role of gut microbiota-immune system interactions in regulating emotional processes. This study examined how PAE-induced changes in gut microbiota and immune function contribute to risk and/or resilience to emotional dysregulation. Pregnant rat dams were randomly assigned to: PAE – liquid ethanol diet

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ad libitum or Control – pelleted control diet ad libitum. Adult male and female rats (n=48 per/group/sex) were evaluated for social behavior, and anxiety- and depressive-like behavior. Scores from each test were compiled into a single emotionality score, and the 10 animals from each group with the highest (risk) and lowest (resilience) scores were assessed for their gut microbiota composition and immune function. We found a trending difference in bacterial community structure between risk and resilience PAE females. In males, risk animals had lower pro- and anti-inflammatory serum cytokines compared to resilient animals. IL-6 levels in the hypothalamus were higher in risk males compared to resilient males. These findings suggest that changes in gut microbiota and immune function are associated with both risk and resilience to emotional dysregulation. Identifying unique microbial and immune changes in individuals with PAE who are at risk for emotional dysregulation could inform the development of target treatments to mitigate emotional impairments. Support: NIH/NIAAA R01 AA022460 and Azrieli Foundation to CR and TSB.

P3-A-16 - Proteomic insights into synaptic alterations in MDGA2 heterozygous knockout mice

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MDGA2 is a synapse organizing protein shown to have critical roles in synapse development and function. Dysregulation of MDGA2 has been implicated in autism spectrum disorder (ASD) as demonstrated by gene linkage analysis in several human cases of autism and in mice lacking a single allele of the Mdga2 gene, which exhibited social deficits and physiological impairments related to synaptic plasticity. To uncover the molecular underpinnings of these impairments our lab has generated a novel spectral library of synaptic proteins and phosphoproteomics in the CA1 region of the hippocampus in a wildtype (WT) mouse. Using these spectral libraries, we have compared the synaptic proteome and phosphoproteome of the Mdga2+/- ASD model to that of WT through an unbiased full proteomics analysis. This approach was further expanded to include sex-based and hippocampal region-specific comparisons. Preliminary findings identified significant changes in the up- and down regulation, and hypo- and hyperphosphorylation of synaptic proteins in male Mdga2+/- mouse dorsal hippocampus. Gene ontology analyses have highlighted the link to these significant proteins with a myriad of pathways linked to ASD and synaptic plasticity, bringing us new insights into the causal relationship between loss of the MDGA2 allele and onset of ASD-like phenotypes. We hope to use these findings to create a framework to identify new targets for restoring synapses in our ASD model as we expand our analysis into female mice and the ventral hippocampus, answering further questions on sex and region specificity.

P3-A-17 - P2Y2 regulates neurogenesis following spinal cord injury in adult zebrafish

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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

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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 P2Y2 receptor signaling in neurogenesis following SCI in adult zebrafish. Our findings demonstrated that expression of the P2Y2 receptor is upregulated following injury, and inhibition of P2Y2 signaling decreased injury-induced neurogenesis in this species. The long-term impact of P2Y2 inhibition during this crucial neurogenic phase was then analyzed through longitudinal behavioral analysis. Further work will elucidate the impact of this receptor on specific neuronal subtypes and their integration following injury.

P3-A-18 - Arcuate nucleus neurons mediate ventral subventricular zone neural stem cell differentiation through endocannabinoid signaling

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The subventricular zone (SVZ) neurogenic niche holds vast neuroregenerative potential as the largest source of neural stem/progenitor cells (NSPCs) in the adult brain. Despite this, differentiation-facilitating signals within the SVZ remains poorly understood. We previously reported that the endocannabinoid tone set by the enzyme Monoacylglycerol lipase (Mgll) sets the differentiation capacity of SVZ-derived NSPCs in vitro. However, the origin of Mgll signaling in the SVZ niche and how it interacts with SVZ NSPCs in vivo remains unknown. We previously established that axon terminals of satiety activated POMC-expressing neurons from the Mgll-enriched arcuate nucleus (ARC) region form specialized contacts with ventral SVZ NSPCs. As Mgll primarily exists in axon terminals, we hypothesize that Mgll from ARC POMC neurons control endocannabinoid 2-AG signaling to regulate neuronal differentiation of ventral SVZ NSPCs. To test this, we used high-resolution spatial transcriptomics to capture the SVZ from mice where Mgll was removed from ARC POMC neurons (MgllΔPOMC). We report a ventral SVZ-specific increase of neuroblasts in MgllΔPOMC mice. Differential gene expression analysis reveals an enrichment of differentiation genes in MgllΔPOMC mice in the ventral SVZ, including downstream endocannabinoid receptor activation targets. Our results indicate that reducing Mgll in ARC POMC neurons creates a pro-differentiation environment in the ventral subregion SVZ. These findings offer new insights on how niche signaling controls regionally distinct NSPC neuronal differentiation.

P3-A-19 - Examining the impact of early adversity on stress susceptibility in a two-hit model of depression-like behaviour

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Early-life stress (ELS) increases the risk of later-life psychopathology, but not everyone who experiences ELS will develop psychopathology. Recent studies in our lab have investigated a two-hit hypothesis of stress, which posits that a "first hit" of ELS sensitizes an individual to later stressors. Rats were exposed to either neonatal limited bedding/nesting (LBN) stress, or neonatal control (nCON) housing conditions. In late adolescence, offspring from each neonatal condition experienced either subthreshold chronic mild stress (CMS; 2 mild stressors/day for 12 days) or served as an adolescent control (aCON) group. In Experiment 1 (n=12 males/group),

adult offspring from the two-hit (LBN-CMS) group displayed greater immobility in the forced-swim test, as well as decreased social play, and increased aggression compared to the other three groups (nCON-aCON, nCON-CMS, LBN-aCON), which were indistinguishable from each other. In Experiment 2 (n=8 females/group), two-hit offspring displayed decreased sucrose preference relative to the other three groups, displaying the same pattern as Experiment 1. Experiment 3 corroborated these results in both sexes (n=10 males and 10 females/group) via a significant neonatal X adolescent stress interaction, wherein the two-hit group displayed reduced sucrose preference compared to the other three groups. These corroborating findings suggest that early adversity confers a susceptibility to later stress, which manifests as depression-like behaviour.

P3-A-20 - Kirrel3 modulates dendrite morphogenesis in the developing olfactory system

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The formation of dendritic arbors in the nervous system relies on the dynamic outgrowth and branching of dendritic processes, followed by the pruning of exuberant branches. This process is crucial for the refinement of responses in sensory systems, such as the main olfactory system, which processes odorant signals from the environment that are essential for rodent survival. In this system, olfactory sensory neurons project axons to synaptic structures termed glomeruli in the olfactory bulb, where they form synapses with second-order neurons, the mitral cells. The cell adhesion molecule Kirrel3 is expressed on both olfactory sensory neuron axons and in mitral cells of the olfactory bulb, suggesting it may modulate the formation of this circuit through homotypic interactions between their pre- and postsynaptic terminals. Here, we investigate the role of Kirrel3 in modulating the elaboration of mitral cell dendritic arbours in the olfactory bulb. We demonstrate that Kirrel3 is differentially expressed in populations of mitral cells and that its specific ablation in these cells results in improper maturation of their dendritic arborization. Interestingly, Kirrel3 homotypic interactions across the pre- and post-synaptic terminals are dispensable for its effect on mitral cell dendritic arbours, indicating it likely acts through an association with additional, yet unidentified, cell surface receptors. Our findings identify a novel role for Kirrel3 in the regulation of dendritic arborization and characterizes a new molecular mechanism influencing mitral cell dendritic pruning.

P3-A-21 - Epigenetic regulation of neurogenic plasticity in the postembryonic forebrain

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Limiting spontaneous movement in zebrafish larvae by reducing the swimming arena reduces precursor cell proliferation, resulting in decreased forebrain growth. Counterintuitively, these conditions increased the proportion of new neurons in the forebrain, suggesting that a higher proportion of neural stem cells in the telencephalon undergo direct neurogenesis through asymmetrical divisions to generate neurons, at the expense of expanding the proliferative pool through intermediate progenitor cells. We hypothesize that this switch in the modality of neurogenesis is regulated epigenetically, and we are testing this hypothesis using two separate approaches. First, we ask whether inducible over-expression of the de novo DNA methyltransferase dnmt3aa could mimic in untreated larvae the neurogenic plasticity observed

when larval movement is restricted. Preliminary data suggest that precursor cell proliferation is unaffected in the presence of increased dnmt3aa expression, and we are currently assessing neuronal differentiation. Second, we analyzed larvae with a loss of function mutation in the ehmt2 gene encoding the histone H3 K9 methyltransferase, a key regulator of gene silencing, and found that mutants in the restricted movement paradigm demonstrated normal levels of precursor cell proliferation, suggesting a deficit in the neurogenic plasticity response. Together, these approaches are expected to broadly probe the extent to which epigenetic regulation affects the mode of neurogenesis in the forebrain as an underlying mechanism of experience-dependent neurogenic plasticity. Research supported by a grant from NSERC.

P3-A-22 - Growth and characterization of iPSC-derived human cerebellar organoids

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Stem-cell derived human cerebellar organoid models are a promising, recent, and much-needed avenue to model human neurodevelopment, human brain evolution, and developmental growth disorders such as paediatric hindbrain cancer. The unique molecular features of human cerebellar development remain understudied, with almost all molecular studies to date being performed in the mouse. In contrast to stem cell-derived cerebral organoid models, which are well-characterized and have been used to study human brain development, evolution, and disease (3,753 PubMed citations, December 2024), less work has been done in cerebellar organoids (119 PubMed citations). We report the generation of human cerebellar organoids from the human induced pluripotent stem cell (iPSC) line WTC-11, following a recent directed differentiation protocol1. This organoid model has been shown to generate multiple cell lineages resembling the putative cells-of-origin of medulloblastoma2-3, making it a promising laboratory model to study this cancer. Cerebellar organoids at 29 and 32 days express markers of hindbrain identity, including ATOH1 and BARHL1 which mark glutamatergic neuronal lineage and rhombic lip cells; KIRREL2 and SKOR2 which mark the Purkinje cell lineage; and SOX2 and PAX6, which mark neural stem cells and excitatory granule cell progenitors respectively. Our goal is to use this model to understand the impact of genetically perturbing candidate genes controlling human hindbrain development, evolution, and oncogenesis using RNA-based and CRISPR-based technologies. 1. Atamian, A. et al. Cell Stem Cell 31, 39–51.e6 (2024). 2. Hendrikse, L. D. et al. Nature 609, 1021–1028 (2022). 3. Smith, K. S. et al. Nature 609, 1012–1020 (2022).

P3-A-23 - BRCA1 expression is enriched in human neural stem cells and controls proliferation of human neural progenitors and Group 3 and 4 medulloblastoma

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The role of the DNA damage repair pathway in regulating human brain development and neural stem cell fate remains unknown. We propose a role for breast cancer susceptibility type 1 protein (BRCA1) as a driver of proliferation in human neural stem cells and in medulloblastoma (MB), a pediatric hindbrain cancer whose cells resemble undifferentiated neural progenitors. BRCA1 emerged as a top candidate in a computational screen for molecules keeping Group 3 and 4 MB

cells in a state of stalled differentiation. We surveyed over 1.7 million single cells, 142 human brain samples, and four independent datasets, and found that BRCA1 expression is consistently enriched in human neural stem and progenitor cells, relative to differentiating or mature neurons, and in the first trimester of gestation. Downregulation of BRCA1 expression in human induced pluripotent stem cell-derived neural progenitor cells resulted in a reduction of cell viability. In MB, BRCA1 expression is enriched in carriers of i17q aberrations and in Group 4 MB tumours. Increased BRCA1 expression is associated with worse prognosis in Group 3 and 4 MB, and knocking down BRCA1 in two Group 3 MB cell lines resulted in reduced cell proliferation and cell viability. In the developmental and the cancer context, BRCA1 expression is correlated with the cell cycle and DNA damage repair pathways. Taken together with previous mouse studies, our work provides evidence that BRCA1 promotes genome surveillance during human neural stem cell proliferation and brain growth, and that BRCA1 activation drives Group 3 and 4 MB growth.

P3-A-24 - Revisiting the unipolar brush cell during cerebellar embryonic development through in-silico perturbation

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Unipolar brush cells (UBC) are excitatory interneurons in the cerebellum that facilitate sensorimotor processing for eye, head, and body position. Its classic morphology is a single short dendritic brush. UBCs are one of three glutamatergic cell types from the rhombic lip. Early in development, these glutamatergic lineages require the transcription factor Pax6—UBCs specifically need Pax6 for proper cell production. Gene regulatory networks (GRNs) model how cells arrive at their observed state—an apt approach for studying developmental trajectory. GRN perturbations are simulated gene expression changes that serve as an in-silico discovery tool for genes of interest, and GRNs of specific glutamatergic lineages have not yet been modeled. UBCs are a promising lineage of interest because of Eomes/Tbr2 as its cell specific marker, its two molecularly distinct subtypes, and its key developmental timepoints (E10.5 to P14) are represented in publicly available datasets. The present study creates and perturbs GRNs using scRNA-seq and snATAC-seq data. CellOracle and SCENIC+ are perturbation algorithms that simulate gene of interest in-silico knockouts. The in-silico knockouts are compared with in-house Pax6-null scRNA-seq data and a curated set of nine developmentally relevant genes (Eomes, Wls, Atoh1, Pax6, Lmx1a, Calb2, Plcb1, Grm1, Plcb4) to evaluate alignment. The pipeline provides the basis for studying other cerebellar cell types through an inference-based developmental model.

P3-A-25 - Uncovering novel protein-coding isoforms in human iPSC-derived cortical neurons and their relevance to ASD

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Precise gene regulation is critical for human cortical neuron development, yet existing reference databases incompletely capture the diversity of transcripts and proteins expressed in these

cells. To address this gap, we used a multifaceted functional genomics approach to analyze human induced pluripotent stem cells (iPSCs) differentiated into cortical neurons. We performed long-read RNA sequencing to capture full-length transcripts, short-read RNA sequencing to validate splice junctions, and proteomics to confirm translation of novel open reading frames.Our data reveal a rich landscape of 200,000 splice isoforms (~100,000 novel). These mRNA isoforms code for 65,000 protein isoforms (40,000 novel), many of which are directly validated by proteomics, underscoring their biological relevance. Notably, mRNA and protein expression dynamics often differ across genes, in part, explained by extensive mRNA isoform switching across neuronal development, including changes in untranslated regions and splicing events that may influence mRNA stability and translation. Importantly, whole-genome sequencing from autism spectrum disorder (ASD) cohorts suggests that some variants previously deemed noncoding may disrupt newly identified protein-coding exons. Reclassifying these variants as potentially pathogenic could refine ASD risk assessments and implicate novel disease mechanisms. In sum, our work provides a high-resolution view of transcript and protein complexity in human cortical neurons and underscores the importance of isoform-centric analyses for understanding neurodevelopment.

P3-A-26 - Role of DSCAM in the development of the spinal locomotor circuit

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Down's syndrome-associated cell adhesion molecule (DSCAM) is involved in axonal guidance and cellular organization during development. Dscam2J mice, with a ubiquitous loss of function of DSCAM, show an impaired coordination of front and hind legs, characterized by difficulty maintaining trotting: a common and stable gait in mice. Coherent coupling of front and hind legs is controlled by propriospinal excitatory (glutamatergic, Vglut2+) and inhibitory (GABAergic, Vgat+) interneurons linking cervical and lumbar locomotor circuits. We hypothesized that mutation of DSCAM might alter the development of propriospinal networks differently depending on their neurotransmitter. Using Cre-Lox technology, we studied the axonal projections of excitatory (Vglut2+) and inhibitory (Vgat+) cervico-lumbar interneurons from mice carrying a conditional mutation in DSCAM (Vglut2-DS and Vgat-DS mice). Compared with their controls (Vglut2-cre), the spatial distribution profile of excitatory cervical-lumbar interneuron axons is preserved in Vglut2-DS mutant mice, but their spinal cord is more voluminous, and the number of excitatory axons is higher. Compared with Vgat-cre control mice, the axonal distribution pattern of inhibitory cervico-lumbar interneurons is altered in Vgat-DS mutant mice, with accentuated lateralization of axons within the white matter, suggesting impaired axonal guidance. In conclusion, mutation of DSCAM differentially affects the development of excitatory and inhibitory cervico-lumbar interneuronal pathways.

P3-A-27 - Septotemporal differences in hippocampal-prefrontal cortex circuit maturation inform a sensitive period for cognitive flexibility

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The hippocampal(HPC)-medial prefrontal cortex(mPFC) circuit is vital in the expression of higher order cognition. Anatomical studies suggest more ventral HPC regions project to more ventral mPFC regions. Very little is known about the maturation of the HPC-mPFC pathway and whether septotemporal differences exist in development. Here, we used viral tracing and electrophysiology to examine changes in HPC-mPFC projections along the septotemporal axis from postnatal day (P)10 to P60 in mice. We found topographical differences in projection patterns with adult-like ventral CA1(vCA1) terminal density already present at P15 for both prelimbic(PL) and infralimbic(IL) mPFC subregions, and an increase in intermediate CA1(iCA1) terminal density only in the PL at P30. Optically-evoked CA1 responses onto mPFC subdivisions showed that vCA1-PL synapses display a delayed sex-specific increase in presynaptic efficacy starting at P30, while vCA1-IL synapses show stable responses starting at P15. iCA1-PL presynaptic efficacy increases from P21, with iCA1-IL synapses showing a slightly delayed increase at P30. vCA1-mPFC(-IL & -PL) synapses display equivalent AMPA:NMDA ratios from P15, but iCA1-mPFC synapses show increased ratios starting at P30. Finally, chronic inhibition of the vCA1-mPFC pathways during juvenility led to a deficit in extradimensional set shifting exclusively in females, whereas a similar manipulation in adulthood had no effect. These findings identify a sex- and subprojection-specific developmental sensitive period with important implications for cognitive function.

P3-A-28 - From formation to pruning: Investigating transient axonal swellings in the developing cerebellum

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Axonal development is crucial for the proper formation of functional brain circuits. Myelination is an important part of this process ensuring high-fidelity saltatory conduction of action potentials. We have previously demonstrated that axonal swellings appear transiently on cerebellar Purkinje cell during postnatal development in mice and improving action potential propagation fidelity in those axons. Thus, suggesting an enhancement of cerebellar function. Since myelination occurs concurrently with the transient axonal swellings, we wondered whether they were functionally involved with formation or retraction of axonal swellings. Using immunofluorescence, we found that at postnatal day (P)11, when axonal swellings density peaks, over 50% of swellings were myelinated. Interestingly, most swellings were in close contact with immature oligodendrocytes processes. Next, we depleted oligodendrocytes precursor cells with injections of 5-azacytidine (5ug/g) from P6 to P15. Although the number of immature oligodendrocytes was reduced at P11, the total number of axonal swellings was not affected. However, extending the treatment to P15, when the axonal swellings density has decreased, resulted in higher density of axonal swellings and decreased myelin. These results suggest that oligodendrocytes that are myelinating Purkinje cell axons are not involved in the formation of axonal swellings but are required for their pruning. This argues that the presence of axonal swellings may occur during a critical period when axonal function is especially vulnerable during myelination.

B - NEURAL EXCITABILITY, SYNAPSES, AND GLIA: CELLULAR MECHANISMS

P3-B-29 - SNAP-23 mediated vesicular trafficking in oligodendrocytes is necessary to maintain adult myelin integrity

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Adult myelin is dynamic and requires continuous support from oligodendrocytes. Inability to sustain myelin health results in demyelination, evident in multiple sclerosis and neurodegenerative diseases. However, the molecular mechanism by which oligodendrocytes recycle and replenish materials to the myelin sheath remains unclear. Here, we hypothesize that SNARE-dependent vesicular trafficking is critical for myelin turnover. We conditionally removed SNAP-23, one of the target SNAREs, in mature oligodendrocytes in adult mice to study the consequences of blocking materials transport in myelination. Induction of SNAP-23 deletion in adult oligodendrocytes causes demyelination with motor deficits and delayed axonal conduction within 5-10 weeks. Demyelination in these mice is linked with a significant increase in immune cells including infiltrated T cells in the brain. Mechanistically, the removal of SNAP-23 in oligodendrocytes impairs vesicle fusion and trafficking myelin oligodendrocyte glycoproteins and myelin-associated glycoproteins to the myelin. Thus, our results indicate that SNAP-23-dependent transport in oligodendrocytes is necessary for adult myelin maintenance. Failure in vesicular and protein addition to myelin leads to inflammatory demyelination, providing insights into the pathophysiological role of oligodendrocytes in demyelinating diseases.

P3-B-30 - Investigating the role of secreted systemic proteins in synapse formation

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Synapse number and function decline in aging, leading to memory loss and neurodegenerative disease. It is postulated that age-related synapse loss and subsequent cognitive decline could be catalyzed by brain-intrinsic factors or by extrinsic, secreted factors in the blood. Heterochronic parabiosis experiments in mice showed introduction of young serum into aged mice reversed cognitive deficits. Changes in the systemic levels of the extracellular matrix proteins cathepsin B, TIMP1-4, and osteonectin, and hormones osteocalcin, CSF2, and irisin result in pro-cognitive effects like improved memory and plasticity in vivo. These experiments do not address whether changes in synapse formation and function underlie the pro-cognitive effects of these systemic factors. To explore these questions, we cloned the factors, expressed them as recombinant protein, and verified expression by immunoblotting. We treated primary cortical neurons with the secreted factors and quantified synapse number by immunostaining and confocal imaging. We found osteonectin and TIMP2 increased excitatory synapse number, cathepsin B increased inhibitory synapses, and irisin and TIMP1 increased total synapse number, while CSF2 had no effect on synapse density. To test if concomitant changes in synapse properties like network activity and firing frequency occur, we will use calcium imaging. Findings will inform high-throughput investigations into the direct synaptogenic effects of the factors in human neurons and mechanistic studies into the neuronal receptors and signaling cascades by which the factors act.

P3-B-31 - Use-dependent facilitation of postsynaptic electrotonic potentials by uniform vs random ~1 Hz stimulation in electrically coupled bag cell neurons

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Gap junctions are intercellular channels that pass ions from pre- to postsynaptic neurons at electrical synapses. In the marine snail, Aplysia californica, reproductive behaviour is controlled by a group of electrically coupled neuroendocrine cells called bag cell neurons. Subsequent to a brief chemical input, bag cell neurons undergo an ~30-min afterdischarge of synchronous action potential firing. While the overall frequency of the afterdischarge is ~1 Hz, the actual firing pattern is often quite variable. The present study examined the impact of potential differences in spike timing on electrical transmission using dual whole-cell recording from pairs of electrically coupled bag cell neurons in culture. A 1-Hz, 10-sec stimulation with regular vs irregular patterning was delivered presynaptically to evoke postsynaptic electrotonic potentials. In agreement with our previous work (J Neurophysiol 130:69-85; 2023), the electrotonic potential underwent use-dependent facilitation. Moreover, the regular input consistently produced significantly larger responses as the stimulus progressed, whereas those evoked by the irregular input were only occasionally different from baseline. Facilitation arises from a progressive increase in postsynaptic input resistance, due to use-dependent run-down of voltage-gated potassium current. Thus, it is likely that the more even timing provided by the regular input leads to greater incremental run-down and thus facilitation. These outcomes highlight the role of spike timing in ensuring maximal signalling efficiency at electrical synapses.

P3-B-32 - PI3K/AKT and PKA signaling cascades mediate THBS4 and SPARCL1-induced synapse formation in primary cortical neurons

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Age-related synapse loss drives cognitive impairment and neurodegeneration. Recent studies have shown that systemic bloodborne proteins, enriched in young blood, boost cognition in vivo and directly regulate synapse number and function in vitro. The secreted extracellular matrix proteins THBS4 and SPARCL1 directly promote synapse formation in human neurons. However, the signaling cascades by which these proteins induce synapse formation are unknown. Previous work indicates that the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) and protein kinase A (PKA) pathways regulate synapse formation. Thus, we investigated whether the PI3K/AKT and PKA pathways regulate THBS4 and SPARCL1 synaptogenesis, respectively. We cloned and expressed recombinant THBS4 and SPARCL1 in human embryonic kidney (HEK) cells. We treated primary cortical neurons with crude HEK cell lysates and supernatants containing THBS4 and SPARCL1, followed by chemical inhibitors of the PI3K/AKT and PKA pathways. Preliminary results indicate inhibition of the ATP-binding site on AKT blocks THBS4-mediated increases in excitatory and inhibitory synapses. Additionally, inhibition of the ATP-binding site on PI3K selectively prevents THBS4 from promoting inhibitory synapse formation. SPARCL1 selectively increases the number of inhibitory synapses, and this effect is attenuated by PKA inhibition. Next, we will perform calcium imaging to measure the effects of THBS4 and SPARCL1 on network activity. We will also identify neuronal cell surface receptors that mediate the synaptogenic effects of THBS4 and SPARCL1.

P3-B-33 - Diversity decline accompanies hypo- and hyper-excitable state of the brain

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Within cell-type neuronal electrophysiological, morphological, and transcriptomic diversity is fundamental to brain function. We previously showed a decline in intrinsic biophysical diversity in human epileptic brain tissue, leading to reduced information processing and unstable network dynamics. We hypothesize this decline results from shared past activity histories via intrinsic plasticity. We confirmed this in both quiescent and active-synchronous states. The quiescent brain state was examined in whole-cell patch clamp recordings of L2/3 pyramidal neurons from human cortical slice cultures from patients with epilepsy. The active-synchronous brain state was studied in deep subicular neurons and L5 medial prefrontal cortex (mPFC) of the rodent kainic acid (KA) epilepsy model. Both models showed significant declines in biophysical diversity and spiking dynamics. Contrary to prior assumptions that epileptogenic conditions primarily alter mean intrinsic properties, our findings highlight the impact of seizure on diversity and information encoding, implying a more complex disruption of neuronal diversity than previously understood. In addition, applying ZD-7288, a Hyperpolarization-activated Cyclic Nucleotide-gated (HCN) channel blocker, restored biophysical diversity. These findings reveal potential therapeutic targets for restoring neuronal diversity, offering new insights into epilepsy and related neurological disorders.

P3-B-34 - Exercise counters stress effects on CRH-PVN neurons and anxiety without affecting fear recall

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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 (CRHPVN). Exercise (treadmill) for one hour after foot shock (FS) increased CRHPVN activity and circulating corticosterone (CORT). Next, we obtained electrophysiological recordings from CRHPVN 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. However, contextual fear memory recall was not affected by exercise. 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-B-35 - Alternative splicing of GluN1 exon 5 gates NMDA receptor blockade and antidepressant activity of ketamine

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Ketamine has emerged as a rapid-acting and robust antidepressant. However, the mechanism of its antidepressant action remains enigmatic. Ketamine is an open channel blocker of NMDA receptors (NMDARs). However, NMDARs are diverse in structure and function depending on their subunit composition. GRIN1 encoding the obligatory GluN1 subunit undergoes alternative splicing to exclude exon 5 (GluN1a) or include this exon (GluN1b), which encodes the N1 cassette. Here we investigated whether alternative splicing of GluN1 exon 5 has consequences for the NMDAR blockade and antidepressant activity of ketamine. In this study, we used mice generated to lack GluN1 exon 5 (GluN1a mice) or express this exon (GluN1b mice). We found that ketamine prevented NMDAR-dependent long-term potentiation (LTP) in the CA1 region of the hippocampus in GluN1a mice, while ketamine had no effect on LTP in GluN1b mice. In addition, ketamine inhibition of synaptic NMDAR currents in CA1 pyramidal neurons was significantly greater in GluN1a than in GluN1b mice, and the voltage-dependent relief of ketamine blockade was significantly slower in GluN1a than in GluN1b neurons. Furthermore, ketamine treatment, via systemic administration or local infusion into the hippocampus, induced an antidepressant effect in GluN1a mice but had no effect in GluN1b mice. We therefore conclude that ketamine blockade of GluN1a-containing NMDARs mediates its antidepressant activity. This work was supported by scholarships from CIHR and Restracomp to A.T.H and grants from CIHR and the Krembil Foundation to M.W.S.

P3-B-36 - Developmental defects in nanoscale reorganization of AMPARs and quantal transmission in a mouse model of fragile X syndrome

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Excitatory synapses undergo rapid remodeling during early sensory development by changing the abundance, composition, and nano-organization of postsynaptic glutamate receptors to enable fast neurotransmission. Dysregulation of synaptic remodeling can lead to neurodevelopmental disorders such as fragile X syndrome (FXS), caused by a mutation in the Fmr1 gene. It is entirely unknown how FMRP deletion impacts nano-organization of AMPARs and quantal transmission during early synaptic development. AMPARs on the Calyx of Held synapse in the auditory brainstem undergo a developmental subunit switch from slow-gating GluA1-dominant to fast-gating GluA4-dominant. We applied expansion microscopy (ExM) to map nanoscale differences in subsynaptic localization of homomeric GluA1- and GluA4-AMPARs between wild-type (WT) and Fmr1-/- mice at pre- (P8-10) and post-hearing (P16-19) stages. Patch-clamp recordings from the principal MNTB cell were performed to measure miniature excitatory postsynaptic currents (mEPSCs). We found nanoclusters of GluA1- and GluA4-AMPARs in peri-synaptic and synaptic domains were localized in a mutually exclusive pattern in WT synapses, which was altered in Fmr1-/- synapses. In parallel, bimodal distribution of fast and slow mEPSCs in immature Fmr1-/- synapses phenocopied that of mature WT synapses. Basal mEPSC frequency was also altered, indicating a phenotypic difference in presynaptic modeling. These findings suggest a loss of FMRP accelerates developmental organization of both pre- and postsynaptic elements underlying quantal transmission

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P3-B-37 - The rescue of neurodevelopmental disorders (NDDs) with novel potassium channel modulators

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Ion channelopathy is a leading cause for excitation-inhibition (E/I) imbalance and NDDs by disrupting ion homeostasis and neuronal excitability. Loss-of-function (LOF) mutations of the KCNA2 gene, encoding potassium channel Kv1.2, causes epileptic encephalopathy, ataxia, seizures, autism spectrum disorder (ASD) etc. In Fragile X Mental Retardation 1 (Fmr1) gene knockout (KO) mice, downregulation of presynaptic Kv1.2 in GABAergic interneuron terminals leads to excessive inhibitory overtone underlying behavioural deficits associated with Fragile X syndrome (FXS), which can be rectified by upregulating the level and function of Kv1.2. However, few drugs are available to treat its LOF mutations and hypoexpression.We discovered a new class of positive allosteric modulators (PAMs), e.g. C1, C2 and C3, that target Kv1.2 and potentiate its activity at nanomolar range. In the stable Kv1.2-GFP CHO cell-line, electrophysiological recordings revealed that C2 has the highest potency among the analogs. In silico simulation and site-directed mutagenesis revealed a novel binding cavity of C2 and its analogs on the Kv1.2 channel. In the Fmr1KO cerebellum, C2 attenuated hyperexcitability of interneurons and enhanced the spike frequency of Purkinje neurons by reducing its inhibitory overtone. In vivo pharmacokinetic study demonstrates that both C2 and C3 can pass the blood-brain barrier without showing toxicity.This project rationalizes Kv1.2 PAMs as a viable approach to rectify E/I imbalance. It will bring a new class of drugs for NDDs associated with Kv1.2 channelopathy.

P3-B-38 - Dysregulation of astrocyte-mediated purinergic signaling in fragile x syndrome

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Fragile X Syndrome (FXS) is a neurodevelopmental disorder and the leading genetic cause for inherited intellectual disability and autism spectrum disorders (ASD). While the underlying genetic cause is known, the subsequent cellular and molecular mechanisms of the disorder remain unclear. Recent work has identified significant changes to the purinergic signalling system within the FXS brain. Specifically, activation of the purinergic receptor P2X7 may contribute to elevated neural activity and oxidative stress reported in models of FXS. Our findings to date indicate that P2X7 is upregulated within the cortex of the FXS animal model, Fmr1 knockout (KO) mouse, compared to wild-type (WT) mice early in development. Given the integral role of astrocytes in regulating neuronal signaling and oxidative homeostasis, we examined the potential effect of P2X7 dysregulation on FXS astrocytes. Here, we found that aberrant activity (calcium flux) of FXS cortical astrocytes, isolated from Fmr1 KO mice, can be normalized to WT values with P2X7 antagonism (JNJ). In addition, P2X7 antagonism prevented FXS-associated upregulation of active signal transducer and activator of transcription 3 (STAT3), a transcription factor involved in inflammation and cellular stress, as well as NADPH oxidase 2 (NOX2), an enzyme involved in producing superoxide. Taken together, P2X7 dysregulation in FXS astrocytes appears to have detrimental effects on both activation levels as well as stress responses. The findings of this study will give us important information about the molecular mechanisms that contribute to FXS cellular pathology and potentially other disorders, such as ASDs.

P3-B-39 - Implications of astrocyte purinergic dysregulation in a human model of fragile x syndrome

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Fragile X syndrome (FXS) is the leading monogenic cause of intellectual disability and autism spectrum disorder (ASD). FXS arises from reduced expression of Fragile X messenger ribonucleotide protein (FMRP). While much research has focused on neuronal dysfunction in FXS, growing evidence suggests the communication of neurons and astrocytes plays a critical role in its pathology. One major pathway involved in astrocyte and neuronal communication is facilitated by purinergic signaling system. Previous research conducted in our lab has shown that P2Y2 expression is dysregulated in the Fmr1 KO mouse model, which has correlated with increased calcium activity in astrocytes and aberrant neuronal circuitry. The application of a P2Y2 receptor antagonist restores neuronal bursting patterns and aberrant astrocyte calcium activity. However, it remains unclear whether P2Y2 is similarly dysregulated in human FXS astrocytes and furthermore, whether astrocyte purinergic dysregulations contribute to the abnormal glial neuron interactions. This study focuses on determining levels of P2Y2 expression in FXS patient-derived astrocytes, as well as its role in aberrant astrocyte activation and neuronal circuit function. Overall, our findings demonstrate that dysregulation of purinergic signaling in human astrocytes and neurons may contribute to the foundation of molecular mechanisms underlying FXS pathology within a human model.

P3-B-40 - Do Voltage-Gated Calcium Channel-associated bacterial chemotactic domians play a key role in astrocyte signaling mechanisms triggered by glutamate?

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Astrocytes play a pivotal role in synaptic modulation, with voltage-gated calcium channels (VGCCs) contributing to their diverse functions. The alpha2delta-1 ($\alpha2\delta$-1) subunit of VGCCs is emerging for its unique role in astrocytes in synaptogenesis; however, its involvement in astrocytic glutamate signaling remains largely unexplored. $\alpha2\delta$-1 is one of the five known bacterial chemotactic domain-containing proteins (CDCP) within our nervous system. Here, we investigate the role of $\alpha2\delta$-1 in glutamate-induced morphological changes and calcium signaling in U118 MG astrocytoma cells, which serve as an astrocyte model, due to their sequence similarities with glutamate sensors in bacteria. Employing an in vitro approach, we stimulate cells with glutamate and assess morphological alterations alongside intracellular calcium dynamics using live-cell imaging. Pharmacological blockade of $\alpha2\delta$-1 with a selective antagonist reveals the interconnection between glutamate-driven changes in cellular morphology and calcium transients, implicating $\alpha2\delta$-1 as a mediator of astrocytic responses to excitatory neurotransmission. This study is to highlight $\alpha2\delta$-1 as a mediator of glutamate-induced astrocytic plasticity, expanding its known functions beyond a modulator for voltage-dependent calcium signaling. By elucidating a novel role for this VGCC subunit in astrocyte physiology, our findings contribute to the growing understanding of glial involvement in CNS signaling and may offer new perspectives for therapeutic interventions targeting astrocyte-related pathologies.

P3-B-41 - Leak potassium channels underpin input-specific excitability of fast-spiking inhibitory neurons

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Heterogeneity of presynaptic input and postsynaptic intrinsic excitability shape excitation-spike (E-S) coupling. Yet, how they interplay to diversify firing patterns remains poorly understood. In the auditory brainstem, principal neurons in the medial nucleus of the trapezoid body (MNTB) are fast-spiking inhibitory neurons that each receive excitatory input from one calyx of Held nerve terminal. Since the presynaptic release probability (Pr) is inversely correlated with the complexity of calyx morphology, we characterized the intrinsic excitability of postsynaptic neurons and their morphologically identified inputs using whole-cell patch clamp recordings and axon tracing in acute mouse brainstem slices. We discovered that morphologically simple calyces with higher Pr preferentially innervate principal neurons that exhibit lower fidelity of E-S coupling and display phasic firing patterns, while neurons contacted by complex calyces with low Pr exhibit higher fidelity of E-S coupling and are predominantly associated with tonic firing. Phasic and tonic firing neurons have similar action potential shape and composition of voltage-gated potassium currents but display marked differences in their input resistance and potassium leak conductance at rest. These results suggest that leak potassium channels tune the intrinsic excitability of postsynaptic neurons to complement the strength of presynaptic inputs. This synergistic mechanism may expand the coding capacity of a single population of neurons to support multiple streams of auditory processing.

P3-B-42 - General anesthesia and surgery dysregulate inhibitory synaptic signaling in CA1 pyramidal neurons

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Perioperative neurocognitive disorders occur frequently and are associated with poor long-term outcomes and significant healthcare costs, yet, the underlying mechanisms remain poorly understood. α5 subunit-containing GABAA receptors (α5GABAARs) are predominately located in extrasynaptic regions of neurons where they generate a tonic inhibitory current. We previously showed that anesthesia and surgery increased cell-surface expression of α5GABAARs; however, the tonic current was not increased. The goal of this study was to determine if the increased cell-surface expression of α5GABAARs is associated with changes in phasic inhibitory synaptic transmission. Male young adult C57BL/6 mice underwent sevoflurane anesthesia (2.3-5%) and laparotomy with intestinal exteriorization with physiological monitoring. Ex-vivo coronal hippocampal slices were prepared 72 h after surgery and miniature inhibitory post-synaptic currents (mIPSCs) were recorded from CA1 pyramidal neurons using whole-cell patch-clamp technique. Unexpectedly, preliminary data show a decrease in the amplitude, area, and frequency of mIPSCs following surgery (vs. controls) while rise and decay times were unchanged. Ongoing experiments will further unravel these changes and identify underlying molecular mechanisms associated with anesthesia and surgery. We anticipate our results to provide critical mechanistic insights into the role of synaptic inhibition and α5GABAARs in persistent cognitive deficits following surgery and may identify novel therapeutic targets to mitigate these deficits.

P3-B-43 - Investigating non-canonical roles of clustered protocadherins in the regulation of Purkinje cell firing

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The clustered protocadherins (cPcdhs) are a family of cell adhesion molecules with notably large diversity, consisting of 58 isoforms in mice. This diversity can give neurons unique molecular identities – orchestrating neurodevelopmental roles in multiple neuron types. Following circuit development, cPcdh expression remains high through maturity. However, it is unclear what functions cPcdhs have in mature neurons and how they may contribute to circuit function, e.g. by regulating electrical properties or synaptic function. To describe such roles of the cPcdhs, we are investigating their regulation of cerebellar Purkinje cell (PC) firing. In mice with deletion of alpha- and gamma-protocadherins, PCs exhibited depressed firing and excitability. Additionally, these mice were impaired in motor coordination assays (rotarod, gait analysis), but not in non-motor tests. In mutant PCs, we found upregulated potassium currents with properties of the voltage-gated potassium channel Kv3.3, which are highly expressed in PCs and mediate their high frequency firing. I will present ongoing in vitro investigations into the putative regulation of Kv3.3 by cPcdhs. Furthermore, to obtain a broader understanding of cPcdh functions in PCs, we are profiling their sub-cellular localization and assessing the impact of their deletion on PC connectivity, such as the climbing fibre to PC synapse. Altogether, this work identifies and seeks to describe a novel role of the cPcdhs in regulating firing, expanding our knowledge of these molecules beyond their canonical developmental functions.

P3-B-44 - Sevoflurane persistently increases cell-surface α5GABAA receptor expression and drives synaptic clustering via gephyrin

MeiFeng Yu [1], Dian-Shi Wang [1], Beverley Orser [1]

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Cognitive deficits occur frequently after surgery and anesthesia, yet the underlying mechanism remains unclear. α5 subunit-containing γ-aminobutyric acid type A receptors (α5GABAARs) are highly expressed in hippocampal pyramidal neurons where they control cognitive processes. Though α5GABAARs are primarily extrasynaptic, synaptic α5GABAARs can dampen Schaffer-collateral burst firing, regulating mechanisms of memory formation. We previously showed sevoflurane triggers excess cell-surface accumulation of α5GABAARs, via astrocyte-dependent mechanisms, causing persistent memory impairment. Here, we tested whether sevoflurane promotes excess expression of α5GABAARs at inhibitory synapses via its synaptic anchoring protein, gephyrin. Briefly, cultured astrocytes were treated with sevoflurane (2.4%, 1 h) or medical air followed by media washout and a 2 h incubation. Then, conditioned media was transferred to neurons for 24 h. Neurons were immunostained for α5GABAARs, gephyrin, and a dendritic marker for colocalization analyses. Sevoflurane increased synaptic expression of α5GABAAR clusters across secondary dendrites compared to control in two independent experiments. Interestingly, sevoflurane also enhanced gephyrin expression, suggesting increased expression of synaptic α5GABAARs is driven by greater expression of its anchoring protein. Ongoing experiments will assess extrasynaptic α5GABAARs via radixin colocalization. These findings may clarify underlying mechanisms driving cognitive impairment and highlight novel targets for therapeutic development.

P3-B-45 - Aging with fragile x syndrome: Examination of astrocyte senescence and purinergic signaling in wildtype and Fmr1 knockout mice

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Investigation of Fragile X Syndrome (FXS) and other neurodevelopmental disorders is focused on developmental stages whereby symptoms are most noticeable. However, individuals with FXS present with accelerated age-related cognitive defects, consistent with altered excitatory neuron activity in the 12-month-old Fmr1 knockout mouse model of FXS. Neuron activity is modulated by glial cells such as astrocytes through the purinergic signaling system. Purinergic receptors display altered expression in aging and in FXS. Yet, these receptors on astrocytes have not been explored in the context of aging FXS. We hypothesize that aged FXS astrocytes will display a senescent phenotype earlier than wildtype astrocytes, and that this will coincide with variations in purinergic receptor expression. To elucidate the effect of aging on FXS astrocytes, primary cortical astrocyte cultures from wildtype and Fmr1 knockout FVB mice (postnatal day 1-3) will undergo serial passaging to induce a senescent phenotype. Markers of aging will be used to validate this procedure as an aging model. Astrocyte morphology will be visualized by immunofluorescence to determine the state of astrocytes (reactive vs senescent). Lastly, Western blots will be used to compare the relative purinergic receptor expression across passages in wildtype and Fmr1 knockout cultures. It is expected that Fmr1 knockout cultures will display astrocytic senescence at an earlier passage compared to wildtype cultures and that purinergic receptors will display passage- and genotype-dependent alterations in expression.

P3-B-46 - Dynamic maturation of astrocyte calcium signals in the developing telencephalon

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Despite their critical role in neural circuit development and function, the maturation of astrocyte signaling mechanisms remains poorly understood. While astrocytes have many functions in the developing brain such as assisting neuroblast migration, synapse formation, maturation, pruning, and neuronal circuit modulation through calcium-dependent processes, there are contradictory findings on the primary drivers of astrocyte calcium dynamics. Work in cultured astrocytes and acute slice preparations from young animals has suggested that calcium dynamics are driven by responses to synaptic activity and neurotransmitters. However, some evidence in adult slice preparations suggests that calcium dynamics are not associated with synaptic and neurotransmitter activity but rather are primarily driven by neuromodulators. We hypothesize that these discrepancies stem from developmental changes in the function of astrocytes. Astrocytes undergo significant morphological and transcriptomic maturation in the first three weeks of mouse postnatal development, however, there have been no studies examining the functional maturation of astrocytes with respect to their calcium dynamics. In this study, we used a modified stereotaxic neonatal viral delivery of a genetically encoded calcium indicator in mice to discern the properties and contributors of astrocyte calcium activity at six developmental time points. We examined spontaneous astrocyte calcium activity at postnatal day (P)8, P14, P21, P28, P35, and in young adults (>P41). We then examined astrocyte calcium activity in the absence of neuronal activity and in response to neuromodulators at these time points. Our work provides the first developmental atlas of astrocyte functional maturation and of the conditions that drive their calcium dynamics.

P3-B-47 - Defining the impact of interneuron-specific ablation of the receptor Neogenin on synaptic function and behaviour

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The normal function of neuronal networks relies on a functional balance between excitation (E) and inhibition (I) of neuronal activity. Disruption of this balance is proposed to underlie behavioural symptoms associated with various neurodevelopmental and neuropsychiatric disorders, such as autism spectrum disorder and schizophrenia. At the cellular level, the E/I balance is achieved through the generation and maintenance of appropriate ratios of excitatory and inhibitory synapses, as well as through the modulation of postsynaptic strength and presynaptic neurotransmitter release probability. Using in vivo loss-of-function approaches, we have identified the transmembrane receptor Neogenin as a novel regulator of inhibitory synaptic transmission in cortical circuits. We demonstrate that the specific ablation of Neogenin in inhibitory interneurons (Neo1;Dlx5-Cre), but not in excitatory neurons (Neo1;Emx1-Cre), increases inhibitory synaptic strength in the somatosensory cortex. Furthermore, we are currently performing a battery of behavioural assays to examine how the loss of Neogenin in inhibitory interneurons may affect mouse behaviour. We will present the findings of these analyses.

P3-B-48 - Common and distinct roles of gsk-3α and gsk-3β in synaptic plasticity, subcellular localization, and tau regulation

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Glycogen synthase kinase-3 (GSK-3), a serine/threonine kinase, is a key regulator of synaptic signalling and is implicated in brain disorders including Alzheimer's disease. One of the main limitations in understanding the function of GSK-3 is the common and differential roles of GSK-3 paralogs: GSK-3α and β. This study explored the contributions of GSK-3 paralogs in the adult hippocampus using conditional knockout (cKO) mouse models targeting excitatory neurons via postnatal Cre-lox recombination. Electrophysiological recordings in acute hippocampal slices revealed that both paralogs are crucial for DHPG-induced long-term depression (LTD) but not for NMDA-induced LTD, suggesting a mechanism-dependent role in mGluR- and protein synthesis-dependent pathways. Subcellular fractionation of wild-type hippocampus highlighted paralog-specific localization and activity: GSK-3α levels and activity were higher in cytosolic and nuclear-enriched fractions, while GSK-3β levels and activity were greater in nuclear and synaptic fractions. Interestingly, synaptoneurosome analysis revealed that synaptic tau was specifically phosphorylated (at Ser396) by GSK-3β, not GSK-3α. Overall, these results suggest that both GSK-3 paralogs are required for a form of LTD triggered by the activation of mGluRs, whereas their subcellular localization and tau regulatory roles are distinct. These insights enhance our understanding of how GSK-3 paralogs contribute to neuronal plasticity and cellular processes, which may offer potential therapeutic strategies for targeting GSK-3 in brain disorders.

P3-B-49 - Spontaneous and miniature inhibitory postsynaptic currents in medium spiny neurons of dorsomedial striatum from Bmal1 knock-out mice

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Multiple lines of evidence show that circadian rhythm disruption affects alcohol consumption, and can favor susceptibility to alcohol abuse. Evidence has emerged linking clock genes such as Bmal1 with alcohol-drinking behavior in humans and animals. Our behavioral results indicate that conditional deletion of Bmal1 from medium spiny neurons (MSNs) in the dorsomedial striatum (DMS) significantly decreases voluntary alcohol consumption in females versus control animals, without affecting consumption in males. Bmal1 expression in DMS therefore influences alcohol consumption in a sex-specific manner. To study the underlying mechanism of the behavioral alterations, we investigated the electrophysiological characteristics of MSNs in DMS in Bmal1 knocked-out female mice using whole-cell patch-clamp recording and assessed the effects of acute application of alcohol on recordings. Alcohol has been previously shown to alter GABAergic transmission in the dorsal striatum. We recorded spontaneous inhibitory post-synaptic currents (IPSCs), changes in miniature IPSCs, and membrane resistance and capacitance induced by the application of alcohol. Results showed that Bmal1 deletion did not alter spontaneous IPSCs, and that application of alcohol did not alter the amplitude or frequency of sIPSCs and mIPSCs. Membrane resistance and capacitance also remained unchanged. We conclude that Bmal1 deletion in MSNs of the DMS does not readily modulate the effects of acute alcohol exposure. However, the effects of Bmal1 in DMS in relation to chronic alcohol exposure remain to be determined.

P3-B-50 - New insights into Cholinergic transmission in the interpeduncular nucleus

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The interpeduncular nucleus (IPN) is a cholinergically relevant brain region characterized by the expression of a unique set of cholinergic receptor subunits, and high levels of acetylcholinesterase. The IPN receives cholinergic innervation from the habenula (Hb), as well as the nucleus of the diagonal band (DBN) of the medial septum. Previous studies show bath application of acetylcholine or exogenous nicotinic agonists elicits robust responses in IPN neurons. However, a distinct cholinergic response of IPN neurons to optogenetically-elicited endogenous acetylcholine release remains equivocal. Additionally, endogenous cholinergic signalling in the IPN has primarily been examined in the context of inputs from cholinergic habenular afferents, while the impact of the combined GABAergic/cholinergic innervation from the DBN remains largely unexplored. Here, we examine the paradox of cholinergic excitation/inhibition with opto-physiological experiments, and more specifically, we assess the potential modulatory impact of GABAergic/cholinergic DBN afferents on endogenous IPN responses. These manipulations in mouse acute brain slices are an important next step to clarify the impact of endogenous cholinergic signalling in the interpeduncular nucleus and the influence of GABAergic modulation on this circuit.

P3-B-51 - Alternatively spliced N1 cassette in NMDA GluN1 receptor subunit evolved in early vertebrates

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NMDA receptors (NMDARs) are a major subtype of neurotransmitter receptor in the CNS. The functions of NMDARs in mammals are known to be regulated by alternative splicing of exon 4, which encodes the N1 cassette in the GluN1 subunit. We found previously that the nucleotide and primary amino acid sequences are highly conserved in species extending to bony fishes. But,beyond this, how the N1 cassette and its alternative splicing has evolved is unknown. Genomic analysis has identified NMDAR homologs only in bilaterians and cnidarians. Here, we identified a putative exon 4 sequence in the genomes of vertebrates, and investigated a representative species Petromyzon marinus (sea lamprey), one of the earliest-diverging vertebrates. We confirmed alternative splicing of the putative exon 4 in transcriptomes from sea lamprey brain. We identified full-length sequences of GluN1 without (GluN1a) and with (GluN1b) exon 4, and of the lamprey GluN2 homologs. These were cloned into pcDNA3.1 vector and expressed in HEK293 cells. Cells expressing either GluN1a- or GluN1b-containing lamprey NMDARs displayed currents activated by NMDA and glycine. The currents were inhibited by the competitive NMDAR antagonist D-APV. The current-voltage relationship was linear, reversing near 0 mV, without Mg2+. Thus, we demonstrated for the first time, functionality of cloned NMDARs from sea lamprey. Our future work comparing GluN1a- and GluN1b-containing lamprey NMDARs will determine whether the homologous N1 cassette has the functionality of N1 in mammals.Support from CIHR and the Krembil Foundation.

P3-B-52 - The wiring and synapse specificity of cerebellar mossy fibers to inhibitory targets is regulated by atypical cadherins

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A critical step in brain circuit formation is the precise assembly of excitatory and inhibitory connections. The cerebellum receives cortical and vestibular information via mossy fiber (MF) projections from the brainstem to the cerebellum. Presynaptic terminals of MFs connect excitatory cerebellar granule cells (GC) and inhibitory Golgi cells (GoCs). Adhesion molecules involved in the formation of MF-GC synapses have been identified but factors required for MF-GoC synapses are unknown. Lineage tracing and RNA sequencing reveal the selective expression of the adhesion molecule, Cadherin-23 (Cdh23), in cerebellar GoCs and MF afferents. We propose that Cdh23 specifies the synaptic targeting of MFs with GoCs. Here, single-molecule fluorescence in situ hybridization shows Cdh23 transcripts in cells co-expressing markers of GoCs. Moreover, retrograde-AAV labeling reveals the expression of Cdh23 in MFs. Next, we use a novel Cdh23 mutant allele to assess the role of Cdh23 at the MF-GoC synapse. In support of the hypothesis, in vivo/in vitro mis-expression and knockout studies indicate that Cdh23 promotes MF-GoC connectivity. I will present work involving conditional Cdh23 mouse mutants to determine whether cellular and synaptic defects result from pre- or post-synaptic loss of Cdh23. I will present ongoing work on the role of Cdh23 via homophilic or heterophilic interactions with its known binding partner. Together, these studies are the first to investigate synapse formation on cerebellar GoC and the specificity of targeting inhibitory versus excitatory cells.

P3-B-53 - selective inhibition of TrkC-PTPσ interaction impairs synaptic plasticity in the mouse hippocampus

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Tropomyosin receptor kinase C (TrkC) is a postsynaptic cell-adhesion molecule that regulates synaptic function through interaction with the presynaptic protein tyrosine phosphatase sigma (PTPσ). To probe the importance of this complex in synaptic function, we generated a point mutation knock-in that selectively reduces binding between TrkC and PTPσ (TrkC-KI). We previously established that TrkC-KI mice demonstrate behaviours consistent with impaired cognition, altered synapse properties and changes in the phosphorylation status of synaptic proteins linked to neurodevelopmental disorders. However, the effects of selectively preventing TrkC-PTPσ interactions on synaptic plasticity, an activity-dependent alteration of synapse strength implicated in cognitive processes, remains unexplored. This project aims to assess how loss of TrkC-PTPσ binding impacts synaptic plasticity in the CA1 area of dorsal hippocampus. We hypothesize that preventing TrkC-PTPσ binding alters long-term potentiation (LTP) at Schaffer collateral-CA1 synapses. Field excitatory postsynaptic potential recordings in ex vivo hippocampus slices form TrkC-KI mice revealed that both transient (single train) and enduring (multiple train) LTP were impaired. Additionally, heterozygote TrkC-KI slices displayed partial LTP deficits, consistent with an intermediate phenotype. These results indicate that TrkC-PTPσ binding is required for both multiple forms of synaptic potentiation, and suggests that neurodevelopmental disorders resulting from loss of TrkC-PTPσ complexes could be due to impaired synaptic plasticity.

P3-B-54 - Nep1 metallopeptidase appears to suppress learning by limiting synaptic vesicle numbers

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Synaptic vesicles are well-known regulators of synaptic plasticity and overall neuronal function. Stromalin, a core subunit of the cohesin complex, has been shown to function as a learning suppressor in Drosophila melanogaster. It does so by restricting synaptic vesicle pool size in dopaminergic neurons. We hypothesized that this effect of Stromalin was caused by cohesin complex's role in regulating gene expression. Thus, we identified differentially expressed genes resulting from knockdown of Stromalin using RNA-sequencing, followed by an RNAi screen to identify the gene(s) responsible for Stromalin's learning and synaptic vesicle effects. Through these efforts, we identified a downregulation of Nep1, a gene encoding a metallopeptidase, as a promising mediator of Stromalin's effects. We show that knockdown of two cohesin complex proteins, Stromalin and SMC1, led to a downregulation of Nep1 mRNA levels. Replicating this reduction in Nep1 levels in dopaminergic neurons using Nep1 RNAi reproduced the behavioral and neuroanatomical effects we saw with Stromalin knockdown. Conversely, increasing Nep1 expression in Stromalin knockdown flies restored both learning and synaptic vesicle protein levels to normal. Thus, Nep1 acts downstream of the cohesin complex to suppress learning and limit synaptic vesicle numbers. However, how a metallopeptidase like Nep1 regulates synaptic vesicles to affect learning remains unclear.

P3-B-55 - Tracking synaptic density loss using [18F]SynVesT-1 in a mouse model of Multiple Sclerosis

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Background: Synaptic loss is a hallmark of multiple sclerosis (MS). The presynaptic protein synaptic vesicle glycoprotein 2A (SV2A) is a key target for imaging synaptic density. SV2A Positron Emission Tomography (PET) with [18F]SynVesT-1 has been successful in mapping synaptic changes in neurological diseases. Its application in MS, however, remains unexplored. This study assesses [18F]SynVesT-1 in MS using the experimental autoimmune encephalomyelitis (EAE) mouse model.Method: EAE was induced in C57BL/6J mice. PET imaging was performed with a PET-CT scanner, capturing 90-minute dynamic scans. Regional volume of distribution (VT) was calculated using a one-tissue compartment model (1TCM) and Logan plot. In vitro autoradiography (ARG) was conducted on brain sections post-mortem, with Levetiracetam assessing non-specific binding.Result: PET imaging showed reduced tracer uptake in EAE mice compared to controls across selected regions (Fig. 1a). Significant VT reductions were observed in both sexes, with decreases of 23–27% in females and 28–30% in males (Fig. 1b, 1c). ARG confirmed significant binding decreases in females ($p < 0.05$), while males showed a non-significant trend (Fig. 1d). Both PET and ARG indicated consistent results in females with significant decreases, while males showed non-significant trends.Conclusion: This study demonstrates the potential of [18F]SynVesT-1 PET imaging to effectively monitor synaptic density changes in MS. The findings highlight the SV2A tracer's versatility and efficacy in capturing synaptic loss in MS.

P3-B-56 - Cholinergic persistent firing activity in parasubicular neurons in mice

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The parasubiculum (PaS) can potently modulate synaptic responses in the superficial layers of the entorhinal cortex, which contain grid cells and forms the origin of the perforant path. This suggests a potential role for the PaS in modifying neural circuits involved in spatial navigation. The PaS receives inputs from multiple different brain regions, including the anterodorsal thalamus, hippocampus, and cholinergic neurons of the medial septum/vertical limb of the diagonal band of Broca (MS/vDBB). Previous work from our group has demonstrated that muscarinic receptor activation in the PaS triggers strong membrane depolarization through inhibition of the Kir2 and Kv7.2/3 channels. Cholinergic receptor activation has also been linked with the emergence of Ca2+-dependent plateau potentials and persistent firing activity in the adjacent MEC, which has been proposed to serve as the cellular mechanism underlying short-term spatial working memory. However, whether PaS neurons also show cholinergic receptor-mediated persistent firing remains unclear. Here, we show dense immunostaining for vesicular acetylcholine transporter (vAChT) and acetylcholinesterase (AChE) throughout the hippocampal formation including the PaS. Septal injections of AAV2/9-EF1a-DIO-hChR2(H134R)-EYFP) in adult ChAT-IRES-Cre mice revealed strong cholinergic projections in the PaS, indicating that cholinergic inputs to the PaS originate primarily in the MS/vDBB. Using whole-cell patch clamp recordings from PaS neurons, we show that cholinergic receptor activation using carbachol (10 μM) triggers persistent firing in ~85% of parasubicular neurons. Moreover, persistent firing in PaS

neurons was blocked in the presence of the muscarinic receptor antagonist, atropine sulfate (1 µM). Together, these findings provide insight into the cellular mechanisms that promote cholinergic persistent firing in the PaS.

P3-B-57 - Effects of amyloid beta protein on the density and morphology of dendritic spines in the lateral entorhinal cortex

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Amyloid beta protein (Aβ) may play a role in learning-related neuroplasticity. In Alzheimer's Disease Aβ accumulates in the entorhinal cortex and temporal lobe. Typically, high concentrations of Aβ promote the simplification and arboration of dendritic spines[CC1] . We have previously found that 1–3 hour application of soluble Aβ to acute rat brain slices can facilitate evoked excitatory synaptic responses in Layer II of the lateral entorhinal cortex. This effect was blocked by the application of the NMDA glutamate receptor antagonist APV. Here, we assessed if Aβ might also result in rapid morphological changes in dendritic spines in layer II entorhinal stellate neurons. Aβ protein was administered via the cisterna magna in anesthetized rats, and tissue was obtained one hour later for Golgi-staining and analysis of spine morphology and density. Preliminary data from two brains indicate that, in comparison to a control group that did not receive Aβ protein, there were no marked differences in morphological characteristics of spines, but that the mean density of dendritic spines in the lateral entorhinal cortex was increased in rats that received Aβ. Data from 10 brains will be presented. These preliminary findings suggest that Aβ may contribute to lasting increases in synaptic strength in the entorhinal cortex by increasing spine density.

P3-B-58 - Preclinical evaluation of cell-specific GLT-1 overexpression in a mouse model of Alzheimer's disease

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Glutamate, the brain's most abundant excitatory neurotransmitter, is essential for synaptic transmission, plasticity, and memory. Glutamate uptake is mediated primarily by GLT-1 and rapid uptake is required to prevent the toxic accumulation of glutamate in the extracellular space. While most GLT-1 is expressed in astrocytes, GLT-1 can localize to presynaptic terminals at select synapses in the mature brain; however, the precise role of neuronal GLT-1 in the healthy brain and its putative contribution to disease states are largely unknown. Here, we developed a super-resolution confocal imaging and analysis strategy to quantify the relative amount of neuronal and astrocytic GLT-1 in fixed brain tissue. In the healthy brain, we found evidence of regional differences in neuronal GLT-1 abundance, with Schaffer collaterals (SC) expressing significantly more presynaptic GLT-1 compared to perforant path synapses. In the 3xTg mouse model of Alzheimer's disease, glutamate clearance rates were significantly slowed at presynaptic microenvironments, an effect that was restored by ceftriaxone, a beta lactam antibiotic known to increase GLT-1 expression. Currently, we are quantifying the cell-type specific expression of GLT-1 in 3xTg mice, and testing whether neuronal overexpression of GLT-1 can help prevent some of the AD-like pathology in this model. Overall, this research aims to reveal how neurons and astrocytes work together to regulate extracellular glutamate dynamics, and may offer insight into novel AD therapies.

P3-B-59 - Site and cell-specific miRNA and mRNA genes and networks across the CNS

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Biological rhythms drive diverse functions on both physiological and molecular levels. However, rhythms in cells and structures not typically associated with rhythmic biological functions (e.g. sleep/wake, metabolism) remain undefined. This is especially the case across central nervous system (CNS) regions and cell types. In most studies of biological rhythms, circadian (24-hour) rhythms are featured. However, many genes have expression periods that are greater or less than 24-hours and fluctuate depending on region and cell-type. Identifying patterns of gene rhythmicity across the CNS is an important step in elucidating the structural and cellular rhythms of neural activity. Using RNA sequencing data from tissue collected every three hours over the course of 36 hours from male mice, we have identified cycling mRNAs, miRNAs, gene networks and novel mRNA-miRNA co-expression pairs in the cortex, hypothalamus, and corpus striatum using high-dimensional datasets. We have created a searchable catalogue (https://www.ghasemloulab.ca/chronoCNS) to help refine the analysis of cellular/molecular rhythmicity across the CNS. To validate our findings at the protein level as well as determine cell-type specificity, we used immunofluorescence staining to measure protein levels of key targets and their co-localization with microglia, astrocytes, oligodendrocytes and neurons in the cortex and hypothalamus. One of our key findings is that there are strong cycling signatures in resting oligodendrocytes. Our study sheds light on the contribution of circadian, ultradian, and infradian rhythms and mRNA-miRNA interactions to CNS function.

P3-B-60 - Zona incerta regulation of superior colliculus cells by GABA and dopamine

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The dynamic between hunger and fear is vital for prey survival and requires rapid neuronal adaptation. The zona incerta (ZI) is a GABAergic brain region that suppresses fear and promotes feeding. A subset of ZI-GABA cells expressing tyrosine hydroxylase (TH) also produce dopamine (DA) and ZI-GABA/DA cells project densely to midbrain motor regions like the motor-related superior colliculus (SCm). The SCm expresses excitatory and inhibitory DA receptors, but whether ZI-GABA/DA cells regulate SCm activity by both GABA or DA is yet unknown. We transduced ZI Th-cre cells with a Cre-dependent virus encoding channelrhodopsin (ChR2) and photostimulation (470 nm, 5 ms) of ChR2-expressing Th-cre fibers in the SCm elicited a light-evoked, monosynaptic GABAergic current at 33.6% of SCm cells surveyed. To assess DA release, we transduced ZI Th-cre cells with either the red-shifted opsin ChrimsonR or chemogenetic activator hM3Dq, as well as the DA sensor dLight1.1 in the SCm. Photostimulation (617 nm light, 20-Hz pulses, 0.5 Hz train) or chemogenetic (3 µM Compound 21) activation of ChrimsonR- or hM3Dq-expressing fibers in the SCm for 5 min increased dLight1.1 fluorescence, which persisted following light or drug offset then returned to baseline levels. Furthermore, by expressing the calcium indicator GCamp6s in the SCm, we found that chemogenetic activation stimulated or inhibited SCm cells and may be consistent with the activation of D1 or D2 DA receptors, respectively. In sum, ZI-GABA/DA cells released GABA and DA and may thus permit acute and prolonged regulation of SCm function.

P3-B-61 - Lithium isotope effects in murine neuronal-like cells and human iPSC-derived neurons

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Lithium has been used as a treatment for bipolar disorder since the mid 20th century and is ubiquitously found in the environment and the diets of all organisms. Comprised of two stable isotopes, 92.41% 7Li and 7.59% 6Li, there is some research that indicates these two isotopes of lithium may have differential effects on rat behaviour and neurophysiology. An early work found differences in general activity, brain-wide distribution, and blood cell fractionation between 6Li and 7Li-treated rats. More recently, a large differential effect of lithium isotopes on excitatory post synaptic potentials in rat hippocampi was reported by our collaborator at University of Waterloo. The observed differences between the lithium isotopes are hypothesized to be due to quantum tunneling through closed cation channels, kinetic isotope effects, or nuclear spin effects. Since there are many hypotheses by which lithium isotopes may modulate behaviour and neurophysiology, we have examined multiple lithium isotope mechanisms across three cell lines: HT22s, SH-SY5Ys, and human iPSC-derived neurons. To ascertain whether lithium isotopes have differential biological effects in these models, we examined the inhibition of GSK-3-β and other kinases, determined whether lithium isotopes are differentially regulated with respect to neuronal membrane passage, and have also investigated lithium isotope effects on human iPSC-derived neuronal growth, differentiation, and electrophysiology.

P3-B-62 - Insulin modulates glutamate transmission in the rat dorsomedial hypothalamus

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Insulin, an important regulator of peripheral blood glucose levels, can also act in the brain to influence food intake and metabolism. The dorsomedial hypothalamus (DMH) contains neurons that regulate appetite and express insulin receptors, but nothing is known about the effects of insulin on synaptic function in this region. We used whole-cell patch clamp electrophysiology to examine the effect of insulin on evoked and spontaneous glutamatergic currents and action potentials in DMH neurons of young male and female Sprague-Dawley rats. Insulin decreased evoked glutamate currents in both sexes (independent of spatial location within the DMH), but blockade of insulin receptors only abolished this effect in female rats. Both insulin and insulin-like growth factor receptors were required for the insulin-induced decrease in glutamate signaling in males. Tonic insulin appears to modulate glutamate transmission, as spontaneous current frequency and evoked current amplitude increased in the presence of both insulin receptor antagonists. Variance analysis suggests that insulin presynaptically affects quantal release. Insulin also decreased neuronal excitability by reducing action potential frequency and magnitude. In fasted animals, insulin still suppressed glutamate transmission and action potential frequency, suggesting that it may regulate physiological processes beyond appetite. Overall, this research contributes to our understanding of how insulin acts in the hypothalamus to alter synaptic function, with potential implications for energy intake or expenditure.

P3-B-63 - Enhanced calcium-permeable ampar-dependent long-term potentiation in the hippocampus of C9orf72 knockout mice

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Hexanucleotide intronic repeat expansions in chromosome 9 open reading frame 72 (C9orf72) represent a major genetic driver of amyotrophic lateral sclerosis with frontotemporal dementia (ALS-FTD). These expansions induce reduced C9orf72 protein expression, that has been associated with neuronal hyperexcitability and dysregulation of synaptic activity. In line with this, we have found that C9orf72 knockout (C9orf72-/-) mice exhibit an increase in postsynaptic GluA1 but not GluA2 levels within the hippocampus relative to WT mice (PMID: 31651360). We tested whether increases in GluA1 lead to more calcium-permeable AMPA receptor (CP-AMPAR)-dependent synaptic plasticity using field electrophysiology recordings in the Schaffer collateral-commissural pathway from 9-to-11-month-old WT and C9orf72-/- mice. We found that C9orf72 deletion significantly enhanced the expression of long-term potentiation (LTP) induced by spaced, but not compressed, theta-burst stimulation, implying the involvement of CP-AMPARs. We next applied the CP-AMPAR selective antagonist, IEM-1460 (20 µM), in interleaved experiments and found that the increased portion of LTP in C9orf72-/- was restored to WT levels. C9orf72-/- mice did not exhibit altered axonal activation, basal synaptic transmission, nor paired-pulse facilitation relative to WT mice. In summary, these findings indicate C9orf72 deletion upregulates a CP-AMPAR-dependent LTP mechanism.

P3-B-64 - Investigating anesthetic-induced neurotoxicity: Molecular mechanisms and neuronal activity alterations

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Anesthetics are required for surgical procedures and induce unconsciousness by suppressing excitatory activity or augmenting inhibitory neuronal communication. However, anesthetic exposure has recently been shown to induce neurotoxicity in various animal models, potentially leading to cognitive decline in the elderly human population. The search for non-toxic agents or strategies to mitigate neurotoxic effects is ongoing. Evidence on how anesthetics affect neuronal and network activity in the hippocampus (memory center) and other brain regions remains limited. A preliminary Nanostring analysis of hippocampal tissue from animals exposed to sevoflurane revealed differential expression of 39 genes associated with synaptic plasticity and memory. Three genes—Tbr1, Aldh1a1, and Plp1—were selected for further investigation due to their roles in synapse formation and learning. qPCR results showed gene regulation changes at the neonatal level, which were not observed in adults, while proteomic validation is ongoing. To investigate neuronal activity changes from sevoflurane exposure, multiple electrode arrays (MEAs) were used to record real-time alterations in hippocampal neuronal cultures. This demonstrated that sevoflurane perturbs neuronal communication in vitro, effectively modeling anesthetic induction and recovery. This approach enabled real-time monitoring of brain connectivity, offering unique insights into how anesthetics disrupt brain communication. Taken together, the findings from this study will pave the way for developing safer anesthetic agents by mitigating neurotoxic effects.

P3-B-65 - Interaction of plasticity paradigm and social isolation in mouse prefrontal cortex

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Theta burst paradigms are increasingly applied to prefrontal cortex (PFC) in mood disorder treatments. These protocols originated in hippocampal preclinical research, where it reliably induces robust long-term potentiation (LTP). However, theta burst stimulation results in lower amplitude outcomes in the PFC and may benefit from paradigm refinement. Here, we developed a wide-field calcium imaging approach to observe and refine plasticity experiments in prefrontal cortical brain slices from male and female Thy1-GCaMP6f mice. This approach offers a novel window into the spatial spread of potentiation. In these experiments, we conduct real-time monitoring of calcium dynamics before, during, and after plasticity induction protocols, including low-frequency test pulses and theta burst stimulation aimed at eliciting LTP. By capturing both the magnitude and a spatial map of potentiation, our approach elucidates PFC plasticity dynamics. We use this method to contrast a typical theta burst paradigm used in patients with a reduced stimuli spaced paradigm. Interleaving gaps between theta burst episodes has been shown to boost LTP in other brain regions. We pursue these experiments in group-housed mice and prolonged social isolation stress littermate controls. Both clinical and spaced paradigms elicit LTP of modest amplitude in PFC brain slices, but only the spaced theta burst protocol yields consistent area-based LTP in slices from socially isolated mice. This research indicates that a spaced approach with relatively few stimulation episodes may be effective in the prefrontal cortex.

P3-B-66 - Optophysiological imaging of theta burst stimulation-induced calcium signals in mouse prefrontal cortex

Hsin-Yun Hsieh [1], Angela Zolis [1], Sridevi Venkatesan [1], Rachael Ingram [2], John Georgiou [2], Tarek Rajji [3], Graham Collingridge [4], Evelyn Lambe [1]

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Treatment of mood disorders with transcranial magnetic stimulation often employs theta burst stimulation (TBS) of the prefrontal cortex (PFC) with a substantial number of episodes delivered per session. Despite this approach originating from rodent hippocampal plasticity experiments ex vivo, the typical paradigm used in humans has not been examined preclinically in the rodent PFC. Intriguingly, the growing body of plasticity literature from rodent PFC suggests this region tends to resist changes in synaptic strength. Here, we use wide-field imaging in PFC brain slices from transgenic Thy1-GCaMP6f male and female adult mice to investigate optophysiological measures of calcium signalling during TBS applied using electrical stimulation. This approach permits detection of changes to the peak and the spatial spread of the dendritic calcium response within and across episodes of TBS. We show that TBS paradigms yield calcium responses that increase in both amplitude and area across a well-defined number of episodes, followed by a marked decrease in both parameters with additional episodes. We are now pharmacologically interrogating the molecular mechanisms underlying this characteristic response pattern. Ongoing research is probing the sensitivity of TBS-induced calcium responses to PFC perturbation via social isolation, a condition suggested to modify excitatory-inhibitory balance.

P3-B-67 - Dextroamphetamine alters synaptic connectivity in rat hippocampal cultures

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Between 2005-2015 there was a four-fold increase in stimulant prescriptions in Canada, despite the rate of attention deficit hyperactivity disorder (ADHD) diagnoses minimally increasing. Amphetamine (AMP) is one of the most common prescribed stimulants for ADHD, and primarily functions to increase the levels of dopamine, norepinephrine, alongside serotonin that are available in the synaptic cleft to reduce deficits in attentiveness associated with ADHD. While AMP can be effective in treating symptoms of ADHD, the long-term effects of AMP on cognitive processes remain poorly understood. Chronic AMP exposure has been linked to neuronal death in the hippocampus, which is integral for learning and memory processes. It is therefore imperative to investigate how repeated amphetamine use may alter synaptic connectivity within the hippocampus. Here we exposed hippocampal cultures derived from P0 Sprague Dawley rat pups to either 5/10/20μM dextro-amphetamine (d-AMP) or methanol (control) on DIV 9, 11, and 13. A cell-viability assay was performed on DIV 15 which revealed no significant differences in cell viability between conditions. This was followed by immunocytochemistry for synaptic markers synaptophysin and postsynaptic-density-protein-95. Interestingly, we observed a decrease in PSD-95 expression paired with an increase in synaptophysin expression for cultures exposed to 20μM d-AMP. These findings suggest that repeated d-AMP exposure may reduce the embedding of excitatory receptors in the hippocampus.

P3-B-68 - Computational network analysis reveals distinct antiseizure drug effects in an in vitro zero-mg^{2+} seizure model

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Epilepsy affects over 65 million people worldwide, and while antiseizure medications (ASMs) can suppress seizure activity, their effects on long-term network stability and functional connectivity remain poorly understood. Seizures disrupt neuronal interactions, leading to hyper-synchronization, altered network topology, and changes in functional clustering. However, how ASMs with different mechanisms of action restore disrupted network organization remains unclear. To investigate this, we applied computational network analysis to microelectrode array (MEA) recordings from primary rat hippocampal cultures using a zero-Mg^{2+} model to induce seizure-like activity (SLA). Using network topology metrics, we quantified SLA-induced changes in hub node activity, modularity, and functional clustering. SLA significantly increased hub connectivity, amplifying network-wide synchrony and disrupting functional organization. Valproic acid (VPA), a widely used ASM, reduced network participation, leading to a less functionally distinct system. In contrast, cannabidiol (CBD), a newly approved adjuvant ASM, preserved clustering coefficients, maintaining local efficiency and functional modularity. These findings provide a novel, computationally driven perspective on SLA-induced network reorganization and how ASMs with distinct mechanisms influence functional connectivity. By establishing a direct link between seizure dynamics and network topology, this study advances our understanding of seizure pharmacology in vitro.

P3-B-69 - Characterization of electrophysiological properties of retinoic acid /GLP-1 differentiated SH-SY5Y cells

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Dysfunctions in glutamatergic signalling underlie several deleterious neuronal pathologies. Current stem cell models capture many aspects of glutamatergic signalling but are expensive and labour intensive. SH-SY5Y neuroblastoma cells are widely used as an accessible "neuronal" model. Sequential treatment with retinoic acid (RA) and glucagon-like peptide-1 (GLP1) promotes differentiation of these cells to a phenotype with morphological and electrophysiological features of mature dopaminergic and glutamatergic neurons, but the identities of the major voltage-gated cation channels present in the differentiated cells, and the kinetic characteristics underlying their observed activity, are currently unknown. This preliminary study begins to address this gap by using whole-cell voltage-clamp electrophysiology to isolate and characterize the kinetics of the ionotropic currents in service of their development as a model for studying glutamatergic signalling. SH-SY5Y cells were differentiated with RA and GLP1, and voltage clamp protocols were applied to characterize the kinetics of activation, inactivation, deactivation, and recovery of cell currents. Different pharmacological agents were used to isolate channel conductances. Application of external tetrodotoxin eliminated most inward currents, indicating that voltage-gated sodium channels are responsible for the majority of excitation. Further work will continue to characterize the remaining electrophysiological components.

P3-B-70 - Exploration of cellular phenotypes and lipid dysbiosis in ABCD1 deficiency, using human stem cell derived microglia, to understand the mechanisms of X-ALD

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Leukodystrophies involve progressive CNS white matter degeneration. Many, including X-linked adrenoleukodystrophy (X-ALD), result from peroxisomal dysfunction and remain debilitating and incurable. X-ALD is the most prevalent monogenic demyelinating disease, affecting ~1 in 15,000 births, primarily boys with ABCD1 mutations. This gene encodes a peroxisomal transporter of very long-chain fatty acids (VLCFAs), whose accumulation leads to toxicity in all tissues, including the brain. Severe cases trigger neuroinflammation, destroying myelin-forming oligodendrocytes and culminating in widespread neurodegeneration.Microglial dysregulation plays a key role in neuroinflammation, as these CNS-resident immune cells become reactive and lose normal function. It remains unknown how VLCFA accumulation drives pathophysiology or which cells initiate and propagate neurological damage.We hypothesize that ABCD1-deficient microglia exert cytotoxic effects on oligodendrocytes and neurons due to impaired peroxisome function, leading to cytokine release, inflammatory lipid production, and oxidative stress. We further propose that these microglia exhibit enlarged lipid droplets, altered peroxisome proliferation, defective mitochondrial respiration, and impaired phagocytosis, disrupting lipid metabolism.Using isogenic ABCD1 knockout/wild-type iPSCs, we developed novel models to study inflammatory demyelination. We are currently investigating how this mutation affects lipid-related organelles, including mitochondria, lipid droplets, and peroxisomes. Phenotypic discoveries, combined with lipidomic analyses, will help assess VLCFA accumulation and

metabolic disruptions. This platform will support drug screening, therapeutic interventions, and better disease severity prediction in patients.

P3-B-71 - Using LIPv and LIPd to divulge the microcircuitry of persistent activity in visuospatial working memory

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Working memory is the cognitive ability to store temporarily limited information for goal-directed behaviour and it is essential for our daily functioning. Its neural substrate is the subject of intense investigation. One mechanism proposed for visual working memory is persistent neuronal activity, which has been observed in a cortical network that includes the posterior parietal cortex, particularly the lateral intraparietal (LIP) area. This area is subdivided into a dorsal (LIPd) and a ventral (LIPv) portion based on connection with the processing streams for object and spatial vision respectively, as well as quantitative differences in neurotransmitter receptor density and myelin content. Here we characterize the spiking statistics of LIPv and LIPd neurons recorded while rhesus monkeys perform a memory-guided saccade task, in which a saccade must be made to the remembered location of a visual stimulus. We found that the persistent activity of LIP neurons located more dorsally possess stronger tuning, larger signal-to-noise ratio, and lower signal variability. Neurons located more ventrally showed greater bursting activity. These findings provide new constraints for modelling the mechanisms underlying persistent activity. In addition, they suggest that the cortical processing streams for object and spatial vision, including the oculomotor system, rely on neural signals of different qualities.

P3-B-72 - Lactate modulates brainstate differently when administered centrally vs. systemically

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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), the activation of which reduces neural activity. Using simultaneous Local Field Potential (LFP) and enzymatic biosensor recordings in Urethane anesthetized rats, we demonstrated that brain lactate levels increase during the Rapid Eye Movement (REM) -like state, and decline during the slow wave sleep-like state, similarly to what has been found previously in natural sleep. When supplementing brain lactate levels however, we found differential effects when lactate was administered Intravenously (IV) or Intracerebroventricularly (ICV). Specifically, IV L-Lactate increased brain lactate levels, and increased the amount of time spent in the slow wave state, but ICV L-Lactate administration decreased the amount of slow wave state. Interestingly, D-Lactate, a non-metabolizing isomer of lactate but an agonist of the HCAR1, enhances the slow wave state in both the IV and ICV administrations. This leads us to conclude that the slow wave enhancing effect of IV lactate is mediated by its signalling mechanisms, not its oxidative effect, but that lactate in its metabolic capacity may be doing different things in the brain and the body. With its dual roles in helping sustain neural activity, but also being a signal to tone down activity, lactate could be one of the key homeostatic dials in neurophysiology. Our research illuminates its surprisingly different actions on the two sides of the blood brain barrier.

P3-B-73 - Cav1.2 in tripartite astrocytes: Relevance to acute and disease-driven hippocampal neuroinflammation

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Neuroinflammation is increasingly recognized as a contributing factor in Alzheimer's disease (AD). The hippocampus is vulnerable to inflammation, and synapse loss here is the number one predictor of cognitive impairment in AD. Over half of these synapses are tripartite and include an astrocyte with pre/post-synaptic neural counterparts, all of which rely on intricately orchestrated Ca2+ signals to coordinate synaptic plasticity; excess Ca2+ can weaken synapses and lead to cell death. Reactive astrocytes expressing high levels of Cav1.2, a voltage-gated Ca2+ channel, have been observed in vitro following acute inflammation, and proximal to amyloid-β (Aβ) plaques in an Aβ-specific model of AD. We therefore sought to determine how hippocampal astrocytic Cav1.2 expression is altered by ex vivo acute neuroinflammation versus disease-driven inflammation in 3xTg mice, a transgenic model of AD with both tau and Aβ pathology. Acute inflammation impaired long-term potentiation at Schaffer collateral synapses in a time- and Cav1.2-dependent manner, and increased Cav1.2 expression in astrocytes relative to dendrites. The same preferential increase in astrocytic Cav1.2 was seen in 9-12 month-old 3xTg mice. Further preliminary data suggests that this expression profile may be evident as early as 6 months of age, correlating with the documented onset of synaptic plasticity deficits in this model. The switch in Cav1.2 expression from predominantly neuronal to astrocytic may represent a molecular signature indicating a shift to a neuroinflammatory environment, which may remain permanent in AD.

P3-B-74 - Developing a human iPSC-based 3D co-culture system to investigate microglia-mediated synaptic elimination in health and disease

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During normal brain development, microglia can control the number of synapses by phagocytic elimination of supernumerary synapses. Excessive synaptic elimination, or synapse loss, is a feature of many neurodegenerative diseases, including Alzheimer's disease and amyotrophic lateral sclerosis. As the brain resident macrophages, microglia can respond to pathological stimuli which can drive aberrant microglia-mediated synaptic elimination. Previous studies in mice partially revealed the cellular and molecular mechanism of microglia-mediated synaptic elimination. Human microglia share basic similarities to their rodent counterparts, but it has not been fully investigated how human microglia contribute to synaptic elimination under healthy and pathological conditions. Therefore, I built a human induced pluripotent stem cell (iPSC)-based co-culture system, which contains both neural cells and microglia in a 3D environment. In my system, human iPSC-derived microglia-like cells (MLCs) showed ramified morphology and became ameboid in response to LPS stimulation. Current data showed that both naïve and LPS-treated MLCs can decrease the synaptic density on neurons by phagocytic engulfment of synaptic components. I am currently investigating how pro-inflammatory MLCs drive synapse loss. Mouse studies showed that the complement protein C1Q can tag synapses to be eliminated by microglia ("eat-me" signal). Meanwhile, microglia can secrete C1Q in response to their extracellular environment. I created C1Q-deficient cell lines by knocking-out the C1QA subunit

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using CRISPR-CAS9. LPS treatment can upregulate C1Q production significantly in wildtype MLCs. My further studies will focus on whether C1Q deficiency in MLCs can affect the microglia-mediated synaptic elimination in naïve and LPS-treated 3D co-cultures.

P3-B-75 - Ketamine enhancement of surface expression of TrkB modulates α5GABAA receptor activity

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Disruption of γ-aminobutyric acid type A (GABAA) receptor function contributes to perioperative neurocognitive disorders (PNDs). Specifically, commonly used general anesthetic drugs trigger a persistent hyperactivity of α5GABAA receptors. We recently showed that ketamine prevents excess α5GABAA receptor-mediated tonic inhibition in vitro and memory deficits in vivo, through brain-derived neurotrophic factor (BDNF)/tropomyosin receptor kinase B (TrkB) signaling pathways. However, it remains unknown whether ketamine increases expression levels of BDNF and TrkB after anesthesia. The goal of this study was to investigate the effects of ketamine and etomidate on BDNF and TrkB levels. Astrocyte-neuron co-cultures were treated with etomidate (1 μM, 1 h) alone or with ketamine (10 μM). Two hours later, conditioned media and cell lysates were analyzed for BDNF using ELISA and Western blotting. Cell-surface and total TrkB levels were assessed via biotinylation and Western blotting. The results showed BDNF levels in cell lysates and media were no different between treatments. However, ketamine significantly increased cell surface accumulation of TrkB without altering total TrkB levels. Thus, ketamine enhances retention of TrkB on the cell surface, rather than increasing overall production. Increased surface TrkB may underlie ketamine's BDNF-TrkB-dependent modulation of α5GABAA receptor activity. Enhancing TrkB signaling could offer a novel strategy to alleviate PNDs.

C - DISORDERS OF THE NERVOUS SYSTEM

P3-C-76 - The impact of alpha-synuclein pathology on sustained and selective attention

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Synucleinopathies are a group of neurodegenerative disorders characterized by the abnormal misfolding and aggregation of the protein alpha-synuclein (α-syn) throughout the nervous system. Evidence suggests that α-syn aggregation disrupts critical neural networks, which may underlie many motor and non-motor deficits. Recently, there has been a shift towards understanding cognitive impairments, as they have the potential to serve as early biomarkers for these disorders. A key cognitive domain of interest is attention, as patients may present with impaired sustained and selective attention, yet the molecular basis underlying these deficits is poorly understood. This study aims to explore the impacts of α-syn pathology on attentional processing. To investigate this, human-derived α-syn pre-formed fibrils will be injected unilaterally into the striatum of a transgenic M83 mouse line, creating a model of synucleinopathies. Then, using cutting-edge rodent touchscreen cognition devices, the mice will perform a rodent paradigm of the Continuous Performance Task, a test widely used to assess

sustained and selective attention in human patients. The results highlighted that as α-syn spreads, there is a time-dependent decline in attentional processing. Synucleinopathies are currently classified as an uncurable group of disorders, and the development of cognitive impairments is severely debilitating. A stronger understanding of how α-syn pathology can impair attention can guide the development of novel disease-modifying treatments and improve the quality of life of patients.

P3-C-77 - Diffuse and cell-specific transcriptomic changes after mild traumatic brain injury

Aditya Swaro [1], Brianna Bristow [1], Mehwish Anwer [1], Angela A Zhang [1], Larissa Kraus [1], Sarah Erwin [1], Tara Stach [1], Kaitlin Sullivan [1], Riya Gandhi [1], Jianjia Fan [1], Wai Hang Cheng [1], Cheryl Wellington [1], Mark Cembrowski [1]

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Understanding how traumatic brain injury (TBI) alters the brain is critical, yet challenging due to its complexity and variability across individuals. To address this, we used the murine CHIMERA model to induce mild diffuse TBI in a controlled and reproducible manner during the subacute phase. We then applied Visium spatial transcriptomics to identify the multiscale spatial molecular changes in the diffusely injured mouse brain. From an initial dataset of over 1 billion reads, we identified cell types with dysregulated genes previously linked to TBI, along with novel differentially expressed genes and impacted regions. Notably, we observed unexpected astrocyte vulnerability in the molecular layer of the dentate gyrus, neocortical dysregulation, and pronounced gene expression changes in thalamic neurons. This transcriptomic dysregulation was further validated at the single cell-level through immunohistochemistry and multiplexed fluorescent in situ hybridization experiments. To allow the use of this dataset by the wider TBI community, we developed TBIseq (http://tbiseq.com/), a web portal that hosts our transcriptomic data and allows researchers to explore TBI's complex effects across different brain regions, cell types, and molecular pathways.

P3-C-78 - TSC2-mTORC1 axis regulates morphogenesis and neurological function of Gli1+ adult-born dentate granule cells

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Aberrant adult hippocampal neurogenesis is implicated in neurological and mood disorders associated with dysregulation of the mechanistic target of rapamycin (mTOR). Understanding how the mTOR pathway shapes the functional development of different subpopulations of adult-born hippocampal neural stem cells will enable insight into potential therapeutic pathways for these disorders. Here we study how loss of TSC2, a regulator of mTOR pathway and a causal gene for tuberous sclerosis complex (TSC), affects dentate gyrus granule cell morphogenesis and hippocampal-dependent function. We found that Tsc2KO mice with TSC2 specifically ablated from Gli1+ adult-born neural stem cells showed neuronal hypertrophy, reduced NEUN expression, increased dendritic arborization, premature cellular senescence, and hypervascularization of the dentate gyrus. Neurologically, Tsc2KO mice showed altered exploratory behavior, impaired spatial learning, abnormal contextual recall, and hypersensitivity to kainic acid–induced seizures. Importantly, genetic reduction of Raptor, essential for mTORC1 signaling, rebalanced mTORC1 signaling and mitigated molecular, cellular, and neurological

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deficits in Tsc2KO mice. This study uncovered functions of TSC2 in Gli1+ adult-born neural stem cells and highlights RAPTOR as a potential therapeutic target for reversing disease features associated with TSC2 mutations.

P3-C-79 - Early cortical hyperexcitability and altered sensorimotor processing in a mouse model of Huntington disease

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Huntington Disease (HD) is a genetic neurodegenerative disorder, characterized by early neuronal circuit changes, followed by overt motor impairments. To investigate these early circuit dysfunction, we measured cortical calcium activity along with striatal glutamate release in head-fixed pre-manifest zQ175 mouse model of HD and their wild-type (WT) littermates performing a lever pulling task. Despite reduced uninstructed movements, zQ175 mice exhibited higher cortical area activation, and altered pattern of cortical activity during lever pulling compared to wildtype controls. Pupilometry revealed a paradoxically enhanced dilation in zQ175 mice, a potential indicator of increased attention. In contrast, striatal glutamate signals remained comparable. Slice electrophysiology demonstrated an elevated excitatory-to-inhibitory ratio in layer 2/3 pyramidal neurons of zQ175 mice, potentially driving the widespread overactivation. Consistent with heightened cortical excitability, sensory mapping revealed enlarged regions of activity beyond visual cortices upon pure visual stimulation. These findings highlight that an early-stage cortical hyperactivity can alter sensorimotor processing in zQ175 mice prior to pronounced motor impairments.

P3-C-80 - Lineage tracking and a novel dual-reporter AAV reveal in vivo astrocyte-to-neuron reprogramming coincident with functional recovery following ischemic stroke

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Neuronal loss following ischemic stroke results in lifelong disability. Direct conversion of astrocytes to neurons (AtN) is a potential strategy to replace lost neurons and promote recovery. To date, targeting astrocytes for conversion using adeno-associated virus (AAV) strategies in vivo has led to off-target transduction of pre-existing neurons, challenging interpretations of neuronal reprogramming. Here, we use two independent strategies to confirm AtN conversion in vivo and test the hypothesis that AtN conversion is coincident with functional recovery. First, we used lineage tracking with Aldh1l1-CreERT2;tdTomato reporter mice to prelabel astrocytes prior to motor cortical stroke. At one-week post-stroke, mice received AAV delivery of neurogenic transcription factors (TFs) (Ascl1 or NeuroD1) expressed from the GFAP promoter to target astrocytes. We observed tdTom+ (prelabeled astrocytes) expressing the neuronal marker NeuN at 3-weeks post-AAV with both TFs and motor recovery was observed in mice with AtN conversion. Second, we used a dual-reporter AAV to differentially label pre-existing vs reprogrammed neurons in vivo. The AAV expresses Ascl1 under the GFAP promoter and floxed mCherry from the hSyn promoter, followed by EmGFP, thereby distinguishing pre-existing neurons (mCherry) vs AtN converted neurons (EmGFP) in mice, with and without stroke. Significantly more AtN conversion (EmGFP+) was seen with Ascl1 compared to controls. Our findings definitively reveal AtN conversion in vivo, coincident with functional recovery following stroke.

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P3-C-81 - Disrupted temporospatial noradrenaline dynamics in motor cortex underlie motor learning deficits in an ASD mouse model

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Children with autism spectrum disorders (ASDs) frequently experience delays in motor development. In the 16p11.2 deletion mouse model, which mimics a common copy number variation associated with ASDs, we previously demonstrated delayed motor learning alongside abnormally elevated neuronal activity in the primary motor cortex (M1). Remarkably, activating locus coeruleus noradrenergic (LC-NA) neurons rescued both circuit deficits and delayed motor learning. In this study, we used in vivo two-photon microscopy to monitor LC-NA calcium axonal activity during motor learning and identified the temporal of LC-NA activity, in which non-behavioral related 'rapid' axonal activity (sub-second duration events) profoundly affect the behavior-induced 'persistent' axonal activity (second duration events). In addition, we performed two-photon imaging of the NA sensor in M1, and we further uncovered that behavior-induced NA release is spatially heterogeneous at the scale of local microcircuitry. However, these temporospatial specificities were disrupted in 16p11.2 deletion mice. Intriguingly, pharmacological and closed-loop optogenetic interventions designed to mimic the temporal and spatial NA disruptions observed in 16p11.2 deletion mice were sufficient to induce motor learning delays in WT mice. These findings shed light on previously unrecognized patterns of NA release dynamics within M1 at temporal and spatial scales, underscoring their pivotal role in motor skill learning and their disruption in ASD-related conditions.

P3-C-82 - Decoding the bipolar disorder neuronal secretome: identifying a biomarker for lithium responsiveness and assessing network activity

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Bipolar disorder (BD) is a chronic mental illness that typically begins in adolescence and leads to recurring episodes of mania and depression. BD is often misdiagnosed and mistreated. Lithium (Li) is the most effective BD treatment for preventing both manic and depressive episodes; however, its mode of action is not well defined. Furthermore, only 30% of patients are lithium responders, and no method currently predicts a patient's lithium response. Thus, we aim to identify a biomarker within neuronal extracellular vesicles (EVs) to monitor diagnosis and lithium response and determine whether the BD neuronal secretome affects lithium responsiveness and network activity. Previously, we developed a hiPSC cortical neuron model for male BD patients who are lithium responsive (LR), lithium non-responsive, and healthy age-matched controls (ctl). We have successfully isolated EVs from these neurons and confirmed the presence of proteins and miRNAs within them. We will compare the proteomic and miRNA sequencing results among the 3 groups -/+ lithium treatment to identify a biomarker for Li responsiveness and/or disease state. Lastly, previous work from our lab reproduced a lithium-reversible hyperexcitability phenotype in our LR BD neurons. Using GCaMP6f, a genetically encoded calcium indicator, we aim to assess whether the BD neuronal secretome affects ctl

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neuronal network activity and influences lithium responsiveness. Overall, this study aims to identify a biomarker for lithium responsiveness and gain a deeper understanding of BD pathology from the lens of the neuronal secretome.

P3-C-83 - Understanding brain mechanisms underlying descending pain modulation in pediatric participants with headache: Use of functional MRI

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Introduction: Chronic pain from pediatric headache is a significant symptom with unclear central nervous system involvement. One mechanism of evaluating pain modulation within the CNS is through offset analgesia (OA) – a mechanism where a change in pain perception is disproportionate to a change in nociceptive stimulation. This study aims to evaluate central pain modulation using OA in an fMRI scanner in pediatric patients with chronic headache. We hypothesize that greater brain activity will be observed during the OA paradigm in persons with chronic pain relative to healthy controls. Methods: A cohort of twenty-four persons with headache, and eight healthy controls underwent an MRI session at Boston Children's Hospital. Three different pain paradigms were assigned to each participant: offset analgesia, control, and constant temperature paradigms. Each paradigm was separated by a rest condition.Results: Findings from the fMRI task showed that when comparing the OA task relative to rest, the headache cohort showed greater activation in the precentral gyrus. Increased activation of the lateral occipital cortex was observed in the headache group compared to controls. Comparing our control task relative to rest demonstrated greater activation in the headache cohort in the occipital pole. Conclusion: Preliminary findings suggest that pediatric headache participants exhibit a unique activation pattern across pain paradigms. Notably, alterations during the OA task suggest changes in pain-modulation circuitry which may underscore how pain is processed in this chronic cohort.

P3-C-84 - A novel Usher Syndrome type 1F model to uncover the pathomechanism of retinal dystrophy

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Usher syndrome type 1F (USH1F) is a rare, autosomal recessive genetic disease caused by mutations in PCDH15 (Protocadherin-15). In addition to sensorineural hearing loss and vestibular disturbance, USH1F causes blindness due to a progressive rod-cone photoreceptor dystrophy (retinitis pigmentosa). In healthy photoreceptors, Protocadherin-15 localizes to actin processes that surround the photosensitive outer segment, however, its role in this subcellular compartment is poorly understood. One reason for our limited understanding is the lack of an adequate animal model: Pcdh15 mutant mice fail to recapitulate the retinal dystrophy phenotype. Here we describe a novel zebrafish disease model of USH1F which lacks the zebrafish orthologs pcdh15a and pcdh15b. Double mutants demonstrated a diminished response to the visual background adaptation and optokinetic reflex tests, consistent with visual deficits. Although photoreceptor number was normal in larval double mutants, the outer nuclear layer was severely disorganised. Outer segments of both rod and cone photoreceptors were dysmorphic with conspicuous holes, and there was detachment from the inner segment. The

actin processes surrounding outer segments were disorganized and detached in double mutants. Our results demonstrate that zebrafish PCDH15 disease models more closely recapitulate human disease and suggest a structural role for Protocadherin-15 at the inner segment / outer segment boundary. Supported by FBC and Ush1F Collaborative.

P3-C-85 - Widespread transcriptomic reorganization following CHIMERA traumatic brain injury

Mehwish Anwer [1], Aditya Swaro [1], Brianna Bristow [1], Angela A Zhang [1], Larissa Kraus [1], Sarah Erwin [1], Tara Stach [1], Kaitlin Sullivan [1], Riya Gandhi [1], Jianjia Fan [1], Wai Hang Cheng [1], Cheryl Wellington [1], Mark Cembrowski [1]

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Traumatic Brain Injury (TBI) is highly heterogeneous and results in long-term neuropsychological deficits. Understanding the cellular architecture and TBI-induced molecular changes across different brain regions has critical implications for early and region-specific biomarker identification. 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 investigated transcriptomic changes in murine brain at 7 days after mild CHIMERA injury (2.1J). Injured mice showed delayed righting reflex recovery and poor performance on neurological tests. Blood biomarker analysis showed increased plasma glial fibrillary acidic protein (GFAP) and neurofilament light levels (NfL) in the TBI compared to sham mice. Using Visium spatial transcriptomics, we identified widespread (6614 DEGs: 82% downregulated, 18% upregulated) as well as regional gene dysregulation in cortex, hippocampus and thalamus, which were validated using multiplexed fluorescence in situ hybridization. Cell type specific gene dysregulation was found in clusters including optic tract where the astrocytic marker Gfap, microglial marker Aif1 and several disease-associated microglia (DAMs) genes like Apoe, Ctsd, Trem2 were upregulated. GFAP immunohistochemical labelling confirmed astrogliosis in optic tract and hippocampus. Our data-rich spatial transcriptomics approach identified molecular and cellular substrates crucial to TBI pathology and its comorbidities including Alzheimer's disease.

P3-C-86 - Stress-induced alterations in astrocyte properties and trophic signaling contribute to blood-brain barrier adaptations in depression

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Major depressive disorder (MDD) is a psychiatric illness for which chronic stress exposure is a main environmental risk factor. Loss of blood-brain barrier (BBB) integrity is observed in MDD patients and chronic social defeated stress (CSDS), a mouse model of depression. Astrocytic morphological changes, namely reactivity and reduced end-feet coverage, occur in MDD but their contributions to pathogenesis and stress responses remain unknown. Male mice were subjected to 10-day CSDS producing two subpopulations: stress-susceptible (SS) animals characterized by depression-like behaviors and resilient (RES) behaving like unstressed controls. CSDS induces BBB hyperpermeability in a region and sex-specific manner, leading to infiltration of inflammatory mediators and depressive-like behaviors in SS but not RES animals. Following CSDS, we observed reduced gene expression of connexin gap-junctions and vascular growth

factor, linking neuronal and vascular activity in the nucleus accumbens of SS male mice. Increased expression of a glial-derived trophic factor was observed in the prefrontal cortex of stress-resilient (RES) animals, suggesting compensatory mechanisms in this brain region. Cultured mice primary astrocytes were treated with blood serum collected from CSDS cohort. RES serum-treated astrocytes showed increased gene expression of Gdnf, a key astrocytic growth factor. In contrast, SS serum-induced upregulation of Aldh1l1, indicating astrocyte reactivity. Additional morphological and calcium signaling analysis are ongoing. Altogether, our results suggest that astrocyte signaling actively contributes to stress responses and MDD-associated alterations, representing a promising target for innovative therapies for mood disorders.

P3-C-87 - Use of machine learning for identification of Parkinson's disease and mild cognitive impairment through neuroimaging and biofluid biomarkers: A study from the PPMI cohort

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Since current diagnostic tests for PD and MCI focus on individual biomarkers, misdiagnoses can be frequent. This research evaluates the use of combined neuroimaging and biofluid data as biomarkers for PD and MCI progression. Using the support vector machine (SVM) and random forest (RF) machine learning techniques, models were created based on neuroimaging and biofluid biomarkers for a subset of PD and healthy subjects (HC) from the Parkinson's Progression Markers Initiative dataset. Striatal binding ratios extracted from DaT-SPECT imaging were used as neuroimaging biomarkers. Proteomic concentrations, including beta-amyloid-42 (Aβ42) and phosphorylated-tau-181 (p-tau), derived from cerebrospinal fluid (CSF) represented the biofluid biomarkers. We performed statistical analysis of biomarker and demographic information to determine significant differences. We found that there were statistically significant differences between subjects with PD and HC in SBRs regardless of cognitive status. When differentiating subjects with PD from HC, both SVM and RF techniques perform with high accuracy using DaT-SPECT alone. These techniques did not perform as well when proteomic biomarkers from CSF were used alone. Models combining DaT-SPECT with Aβ42 and/or p-tau tended to have higher performance. No biomarkers performed well when detecting MCI. Diagnostic performance may be improved through combining neuroimaging with biofluid markers to distinguish subjects with PD from HC. This study's next steps involve investigating other techniques and biomarkers that can apply to both PD and MCI.

P3-C-88 - The neurophysiological underpinnings of brain resilience: a deep phenotyping fMRI study of AD pathology progression

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Disease modifying therapies for Alzheimer's disease (AD), targeting hallmark pathologies, amyloid beta and tau, have hitherto produced modest cognitive benefits. The complex link between AD pathologies and cognition is reflected in cognitive reserve (CR), i.e. individuals

maintaining cognitive abilities despite AD pathology. While proxies of CR such as education, exercise and socialization are known, cellular mechanisms of resilience to cognitive decline remain elusive. At the microscopic scale, functional properties of neurons are continuously changing even in environmentally stable conditions, manifesting in neuronal excitability changes. The level of neuronal response variability to stimuli has been suggested as a predictor of the brain's adaptive capacity, but neuroimaging markers of such variability are yet to be defined. We collected large amounts of intrasubject BOLD fMRI responses and investigated whether trial-by-trial spatiotemporal variability in activation patterns to repeated somatosensory stimuli correlates with cognitive performance in aged vs young and transgenic vs non-transgenic rats in the Fischer 344 model of AD. Barnes Maze testing showed that 30% of aged TgF344-AD rats showed no cognitive flexibility impairment, compared to non-transgenic littermates. From fMRI, we computed parametric activation maps and effect-size series such that the variability in voxel-wise effect sizes for all pairs of stimulation trials was quantified. Grouping by cognitive performance revealed that average activation variability increased with age and cognitive impairment.

P3-C-89 - Uncovering behavioral differences in GRIN1-related disorder mice using machine learning tools

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Characterizing behavioral differences in rodent models of neurodevelopmental disorders is crucial for testing treatments. Recent machine learning advances provide a more efficient and accurate alternative to traditional methods. This study applied computational ethology tools to assess behavior in mice with a patient-specific GRIN1-variant. The Y647S mutation in the GluN1 subunit of the NMDA receptor disrupts its function, leading to seizures, intellectual disability, and hyperactivity in patients. Grin1-Y647S+/- transgenic mice show similar symptoms, previously characterized using traditional methods. This study replicated previous findings with machine learning tools and uncovered finer behavioral differences. For this, male and female Grin1-Y647S+/- mice and littermate wild-type controls (N = 16/genotype) were each filmed twice in an open arena. Keypoint coordinates were extracted with DeepLabCut, and locomotion data analyzed with Simple Behavioural Analysis, DLCAnalyzer, and keypoint-Motion Sequencing. Grin1-Y647S+/- mice showed prominent differences in center avoidance, habituation, and distance travelled. Behavioral syllable analysis revealed a profile for the Grin1-Y647S+/- mice that is dominated by greater long-range, fast movements and more frequent transitions. Interestingly, correlations among age, seizures, and locomotion were discovered. These results highlight the potential of computational ethology to improve behavioral quantification and advance the study and treatment of neurodevelopmental disorders.

P3-C-90 - Selective potentiation of NaV1.1 channels by XPC-A in Dravet mice suppresses spontaneous seizures, prevents SUDEP, and increases long term potentiation

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Dravet Syndrome is marked by diminished expression of NaV1.1 in inhibitory neurons, resulting in heightened neuronal excitability. Impairment of inhibitory activity leads to epilepsy and delays in cognitive and motor function. We are developing potent, isoform-selective small molecules to directly potentiate the remaining NaV1.1 channels in Dravet Syndrome. A selected, Xenon-developed, NaV1.1 compound, XPC-A, exhibited robust and selective enhancement of NaV1.1 channels. Automated patch clamp studies established that XPC-A disrupts the inactivated state of NaV1.1 channels. XPC-A increased the firing activity of fast-spiking cortical PV+ interneurons and reinstated the equilibrium between spontaneous excitatory and inhibitory synaptic input to pyramidal neurons in brain slices obtained from Scn1a+/- mice. In vivo experiments show that XPC-A effectively mitigates 6Hz electrically induced seizures in Scn1a+/- mice and improves their motor performance deficits. Chronic oral dosing of animals led to significant suppression of spontaneous seizures, protection from SUDEP, and increases in hippocampal long-term potentiation. Potent and selective enhancers of NaV1.1 can augment the excitability of fast-spiking inhibitory neurons and rebalance excitation in brain slices from Scn1a+/- mice. From these studies, XPC-A emerges as a pioneering mechanism for enhancing voltage-gated sodium channels, offering promise as a therapeutic approach that could modify the course of Dravet Syndrome.

P3-C-91 - Exploring the role of non-ionotropic NMDAR-Panx1 signaling in stroke-induced dementia

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Stroke is one of the leading causes of death and disability worldwide. Those affected by stroke have a dramatic increased risk of developing dementia, likely due to a reduction in neuronal plasticity. The N-methyl-D-aspartate receptor (NMDAR) plays a central role in numerous physiological signalling, being critical for synaptic plasticity and cell survival. However, NMDAR signalling becomes pathological when its overactivity causes neuronal death, like in ischemic stroke. Though targeting NMDARs directly have been met with limited success, recent efforts focusing on targeting auxiliary proteins that are downstream from NMDAR appear promising. Our laboratory identified a non-ionotropic (ni) signaling mechanism of NMDARs and the ion/metabolite channel Panx1, which is important for neurodegeneration. Since Panx1 has a known role in neuronal plasticity, we hypothesized that niNMDAR-Panx1 signalling contributes to the alterations of plasticity following stroke that leads to dementia.We have implemented the proximity-based (i)BioID2 strategy which allows for potential interactors (interactome) to be tagged with biotin and identified by mass spectrometry. Here, we ligated BioID2 to Panx1 and NR1. Expression was confirmed in N2a cells and trafficking to the plasma membrane remained intact. Functionally, the BioID2-tagged proteins display an increase in labelling interacting candidates upon the addition of 50µM biotin. In this system we expect to elucidate the niNMDAR-Panx1 interactome and test the efficacy of targeting these candidates in reducing stroke-induced cognitive decline.

P3-C-92 - Orthosteric and allosteric negative CB1-R modulation in hyperdopaminergic states

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Negative modulation of the cannabinoid receptor 1 (CB1-R) is being explored as a therapeutic approach for various conditions. We previously demonstrated that the CB1-R NAM (or PAM antagonist) ABM300 alleviates psychosis-like phenotypes in genetic mouse models of hyperdopaminergia1. However, its limited brain penetration (Kp, brain = 0.77) and inability to block cannabimimetic behaviours raise questions about the mechanisms underlying its antipsychotic effects. Here, we examine ABM300's effects on psychosis-like behaviours, CB1-R involvement, and whether its effects are mediated peripherally or centrally. We compared ABM300 (10 mg/kg) with AM6545 (CB1-R peripheral orthosteric antagonist, 10 mg/kg) and SR-141716 (CB1-R orthosteric inverse agonist, 3 mg/kg) in two hyperdopaminergic models: Dopamine Transporter Knockout (DAT-KO) mice and amphetamine-pretreated (AMPH, 3 mg/kg) mice. We also assessed the compounds' effects on locomotion, CB1-R-mediated tetrad behaviours and ABM300's impact on serum corticosterone in the DAT-KO mice. At the doses tested, ABM300 reduced hyperlocomotion in both DAT-KO and AMPH models, while AM6545 and SR-141716 were effective only in DAT-KO mice. Neither ABM300 nor AM6545 blocked CB1-R agonist-induced tetrad behaviours. ABM300 had no significant effect on serum corticosterone in DAT-KO or wildtype mice. These findings suggest that peripherally restricted CB1-R modulators and orthosteric blockers influence hyperdopaminergic behaviours, though the contribution of central and peripheral mechanisms remains unclear. [1] Mielnik, C.A., et al. (2021) Neuropsychopharmacol. 46, 413–422.

P3-C-93 - Prenatal alcohol exposure in rats: A longitudinal analysis of cognitive performance and neuroinflammatory processes in aging males and females

Sunny Qureshi [1], Carolina Luft [1], Madeleine 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 cause long-lasting deficits in the developing brain, including cognitive impairments and neuroinflammation. However, little is known about how these deficits change as an individual ages and which mechanisms might be playing a key role. We examined the relationship between inflammation and learning and memory performance in males and females with PAE as they age. Pregnant rats were randomly assigned to: ad libitum PAE (liquid ethanol diet throughout gestation) or control (pelleted diet). Offspring were tested in the Novel Object Recognition task at 6 and 12 months of age. Brain sections were immunostained with Iba1 to label microglia, and confocal microscopy was used to evaluate microglia morphology in hippocampal regions (dentate gyrus, CA1, CA3), and the perirhinal cortex. Blood samples were collected for analysis of cytokine levels. Behavioral findings indicated that PAE females exhibited recognition memory deficits as early as 6 months, while PAE males showed similar deficits by 12 months. Cytokine data showed sex-specific alterations in immune functioning with PAE females displaying lower levels of IL-10, IL-13, and IFN-γ. Preliminary microglia analysis showed that PAE females had a higher number of microglia cells in the dentate gyrus, indicating potential sex-dependent neuroinflammatory mechanisms. Morphological assessments of microglia using segmentation are currently ongoing. Collectively, our findings thus far suggest that PAE leads to sex- and age-dependent alterations in both cognitive function and inflammatory markers.

P3-C-94 - The role of neuronal stochasticity in cognitive impairment due to Alzheimer's disease pathology

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Healthy brain undergoes continuous remodeling of neuronal functional properties even in environmentally and behaviorally stable conditions. This basal plasticity leads to ongoing, activity-independent synaptic changes that contribute to the stochastic responsiveness of neurons to stimulation. We set out to investigate the significance of trial-dependent variation in the responding neurons in the level of cognitive impairment in AD progression. We inserted the ultra-high density Neuropixels 1.0 electrode (10-mm long, single-shank, 384 active channels, 70 x 24 μm cross-section) so as to span both primary somatosensory cortex and hippocampal CA1 and CA3 regions in Fischer 344 rats. The neuronal stochasticity was quantified via the neuronal engagement index (NEI), calculated as the average ratio between the subset of neurons activated on a given trial and the total population of neurons activated across all trials. Our preliminary results showed that cognitively impaired rats exhibited a 96 % higher NEI in putative inhibitory cortical neurons and a 43 % lower NEI in putative excitatory hippocampal neurons compared to their cognitively maintained littermates, independent of the transgene. Additionally, the cognitively impaired rats showed a 68 % reduction in total number of monosynaptic connections and a 26 % decreased variability in the hippocampal oscillatory power compared to their cognitively maintained littermates. Our data suggests that neuronal stochasticity may play a significant role in cognitive impairment and thus may be a novel target for presymptomatic AD interventions, and stratification of mild cognitive impairment subjects by their susceptibility to progression to dementia.

P3-C-95 - The role of MANF/CDNF in dopamine neuroprotection in pre-clinical animal models

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[1] McMaster University

Cerebral Dopamine Neurotrophic Factor (CDNF) and Mesencephalic Astrocyte-derived Neurotrophic Factor (MANF) are crucial regulators of neuronal survival and cellular balance and aid in promoting neuroprotection by mitigating cellular stress. Pre-clinical studies highlight their therapeutic potential in neurodegeneration, though the literature on the mechanisms remains unclear. My project involves a comprehensive review of data from different animal models, evaluating the neuroprotective roles of MANF/CDNF.A systematic literature search using Web of Science, Embase, and OVID Medline followed PRISMA guidelines. Studies utilized pre-clinical animals, neuronal cell cultures with genetic modifications, and recombinant MANF/CDNF administration. Studies assessing their expression, localization, and function in neurodegeneration were included, while non-neuronal models, unrelated factors, and clinical studies were excluded. Comparative analyses between treated and untreated models were reviewed.While my analysis continues, current findings indicate that MANF/CDNF modulation affects neuronal stability and cellular stress responses. Upregulation enhances neuroprotection by improving neuronal resilience and mitigating cellular stress, while deficiency contributes to neurodegeneration. Research suggests their involvement in pathways regulating protein homeostasis and neuronal survival.My project provides insights into MANF and CDNF as key neuroprotective factors, contributing to understanding neurodegeneration and potential

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interventions. To further explore their roles, an experimental study will be carried out to assess how the manipulation of MANF/CDNF in animal models affects neuronal survival, motor function, and stress responses.

P3-C-96 - Investigating changes to the endocannabinoid system following adolescent repetitive mild traumatic brain injury in male and female rodents

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Repeated mild traumatic brain injuries (RmTBIs) are very prevalent among adolescent populations. RmTBIs lead to neuroinflammation and varying motor and cognitive deficits. Previous literature has demonstrated that the endocannabinoid system (eCB) may be a modulator of neuroinflammation following injury, however further research is needed to fully elicit these effects. The aim of this study is to establish any changes to eCB ligand levels (AEA & 2-AG) in both male and female Sprague Dawley rats with regional and temporal specificity. Adolescent rats were administered 5 mTBI at 72-hour intervals. Hippocampus, amygdala, frontal and motor cortex regions were taken to assess eCB levels (AG & AEA) via mass spectrometry immediately or one week following the last hit. Motor strength was assessed pre and post injury as well. Following all behavioral analyses, brains were perfusion-fixed and processed for immunohistochemistry to quantify microglia (density & morphology). RmTBI male animals significantly decreased time to hang compared to their baseline (p=0.0152), yet females demonstrated no differences post injury. 2-AG was significantly decreased in male TBI animals a week following hits in the prefrontal cortex (p=0.0104) however no significant differences were seen in 2-AG in the hippocampal and amygdala regions in females. AEA levels were elevated in the hippocampus of TBI male animals at the immediate time point, females demonstrated no differences in AEA. Iba1 staining for microglia density is ongoing. Further studies will investigate the role the eCB plays following injury.

P3-C-97 - Modulating the levels of alpha-synuclein and Parkinson's disease pathogenesis using a novel dual-hit hypothesis model

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One of the key pathological hallmarks of the neurodegenerative disorder Parkinson's disease (PD) is the misfolding/aggregation of alpha-synuclein (αSyn), however, where, and how alpha-synuclein pathology begins is unknown. The prevailing theory of pathogenesis is Braak's Dual-hit hypothesis, which states that alpha-synuclein pathology initiates within the gut and olfactory mucosa. This then spreads towards the midbrain via the vagus nerve and olfactory bulb, respectively, where the bulk of degeneration occurs. What cells initially contribute to this misfolding and how they pathologically progress from one to the other remains unclear. To explore the cellular underpinnings of Braak's hypothesis, we are using AAV and genetic models to monitor and modulate αSyn expression in regions of interest within the gut and olfactory systems to quantify their roles in PD pathogenesis. We previously characterized the endogenous levels of αSyn expression with αSyn reporter line, which showed mixed levels of expression within the gut. With this data in hand, we now seek to determine if targeted expression of αSyn in these regions will lead to development of PD symptomology and cell-to-cell spreading in mice. For this,

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we are using a Cre-dependent αSyn overexpression approach using both AAV and murine models. We have determined effective approaches for AAV administration for the enteric nervous system and gut and olfactory mucosa. Now, we aim to produce a reflective model of Braak's hypothesis that can be used to address questions related to the peripheral systems role in PD development.

P3-C-98 - Behavioural effects of chronic prefrontal cortex inhibition in mice: sex differences and stress susceptibility

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Chronic stress is major risk factor for many psychiatric disorders including major depressive disorder (MDD). Brain imaging studies consistently report hypoactivity and loss of volume of the prefrontal cortex (PFC) in MDD. Chronic stress is used in rodents to model behavioural and cellular features relevant to MDD, such as increased anxiety, depressive-like behaviours, and synaptic loss. Our lab showed reductions of PFC volume in chronic stress mice that correlated with behavioural deficits and synaptic loss. However, the question remains whether these changes are causally linked with PFC hypoactivity. Here, using cell-specific chemogenetic manipulation and longitudinal behavioural testing, we characterized the progressive behavioural effects of chronic PFC hypoactivity and its potential interaction with chronic stress exposure. After validating our PFC hypoactivity model with live calcium imaging, we found that chronic PFC hypoactivity induced anxiety-like, but not anhedonia-like behaviours. Intriguingly, PFC hypoactivity in males and females induced anxiety-like deficits with differing timelines. In males, anxiety-like behaviour appears after 4 weeks of PFC inhibition and was not exacerbated by chronic stress. Females with PFC hypoactivity alone did not display anxiety-like deficits but showed a trending exacerbation when subjected to chronic stress for 5 weeks. Since the PFC plays a key role in stress-related illnesses, this work sheds light on the contribution of PFC dysfunction in the sexually dimorphic emergence of behavioural deficits and susceptibility to stress.

P3-C-100 - Chloride dysregulation and impaired GABAergic signaling in 15q13.3 microdeletion syndrome

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The 15q13.3 microdeletion syndrome is a genetic disorder caused by deletion of several genes on chromosome 15 and is associated with multiple conditions including autism spectrum disorders. The underlying mechanisms are unresolved, but alterations in cortical circuits due to reduced inhibitory function have been implicated in social and cognitive impairments in several neurodevelopmental disorders. Inhibitory synaptic transmission in the brain is largely mediated by GABA acting on Cl- _permeable GABAA receptors. This requires low levels of intracellular Cl- that are mainly achieved by the K+-Cl- cotransporter, KCC2. Decreases in KCC2 expression and/or function reduce synaptic inhibition and contribute to the pathophysiology of neurological disorders including social deficits and repetitive behaviors in other autism mouse models. We used a combination of electrophysiology, biochemistry and behavioral assays to investigate the potential contributions of Cl- regulation and altered inhibitory synaptic transmission to social

behavioral deficits in the 15q13.3 microdeletion. We found that the 15q13.3 mice display impaired social recognition and interaction. These deficits are accompanied by a depolarization shift in the reversal potential for GABA (EGABA), an indirect measurement of KCC2 function. Our results show that depolarization of EGABA contributes to increased neuronal excitability and spiking activity in 15q13.3 brain. Understanding KCC2 function and chloride regulation in 15q13.3 and its behavioral consequences could represent novel insights into the pathogenesis of 15q13.3 microdeletion syndrome.

P3-C-101 - Comparison of neuronal vulnerability to huntingtin conditional knockout in the hippocampus versus striatum

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Huntington 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 wildtype 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 the adult brain.Here, we conditionally knocked out (cKO) wtHTT in 2–4-month-old Httfl/fl mice by AAV-Cre injection targeting either the dorsal striatum or dorsal hippocampus. In HD, striatal neurons are particularly vulnerable to degeneration, and most HD research has focused on this cell type, while the HD hippocampus remains understudied. Our project investigated the consequences of wtHTT depletion on histomorphology, inflammation, electrophysiology, and behaviour in these two brain areas. We found that 1-2 months wtHTT cKO in striatal neurons altered intrinsic cell excitability and produced an inflammatory response, while gross morphology and nuclear density remained unaffected. Unexpectantly, wtHTT cKO in the hippocampus had profound effects on morphology and behaviour in addition to similar effects as what was seen in striatal cKO animals. These observations contribute valuable insights to our evolving understanding of wtHTT's intricate role in the mature brain. Importantly, our results highlight the hippocampus as a potentially vulnerable region to the effects of non-selective HTT lowering.

P3-C-102 - TrkA reduction and proNGF transport deficits in vitro but not in vivo for a TrkA-R685A knock-in mouse model disrupting TrkA-PTP1B interaction

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Basal forebrain cholinergic neurons (BFCNs) are responsible for memory and are especially susceptible to neurodegeneration in Alzheimer's disease (AD). BFCN survival depends on the neurotrophic signaling of target-derived precursor nerve growth factor (proNGF) through its receptor, tropomyosin-related kinase A (TrkA). TrkA transports proNGF from axon terminals to the soma and, in peripheral neurons, can return to the axon terminal via transcytosis involving TrkA dephosphorylation by protein tyrosine phosphatase 1B (PTP1B). It is unclear if transcytosis occurs in the CNS. The TrkAR685A mouse model prevents TrkA from binding to PTP1B and being dephosphorylated. If transcytosis occurs in BFCNs, this mutation will reduce the transport of TrkA and will cause proNGF accumulation in BFCN target areas. Cultured TrkAR685A/R685A

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BFCNs grown in microfluidic chambers had less TrkA at the axon terminals and less proNGF transport to the proximal axons than wildtype BFCNs. However, in young adult mice, there were no significant differences in TrkA or proNGF protein levels between TrkAR685A/R685A and WT mice by western blotting. Preliminary immunofluorescence data suggest mutant mice have decreased TrkA in BFCN target areas. However, in the novel object test, mutant mice had no recognition memory deficits. The lack of a robust TrkA decrease or cognitive effects in vivo indicates that transcytosis of TrkA may not be the predominant axonal transport pathway for TrkA in CNS neurons. However, because AD is age-dependent, deficits seen in vitro may only become evident in vivo in older mice.

P3-C-103 - Subjective autonomic symptoms and daytime sleepiness in people with drug-resistant focal epilepsy

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People with drug-resistant epilepsy (PwDRE) are most at risk for sudden unexpected death in epilepsy (SUDEP). While mechanisms for SUDEP are largely unknown, autonomic dysfunction and sleep apnea are likely involved. Here, we evaluated subjective autonomic symptoms in PwDRE. People with focal DRE (N=34) undergoing stereoelectroencephalography at an Epilepsy Monitoring Unit completed the Composite Autonomic Symptom Score (COMPASS-31) and Epworth Sleepiness Scale (ESS). The mean total COMPASS-31 score was 27.4 (SD=13.8) and the mean ESS score was 7.1 (higher normal daytime sleepiness; SD=3.4). The scales were significantly correlated (r=.41; p=.02). Females (N=13) scored higher than males on the COMPASS-31 (t=3.41, p<.01) but not on the ESS (t=1.55, p=.13). There were no significant correlations (p>.05) between current age (mean=32.7 years, SD=10.6) and total scores (r(COMPASS-31)= -0.04; r(ESS)= -0.12). Scores on either scale did not differ between seizure onset zones in the temporal lobe(s) (N=20) and multi-focal, extra-temporal or unknown epileptogenic zones (COMPASS-31: t=0.18, p=.86; ESS: t=1.29, p=.21). Participants prescribed 2-3 sodium channel blocking anti-seizure drugs (cardiotoxic; N=17) scored worse than participants prescribed 0-1 sodium channel blockers (N=17) on the COMPASS-31 (t= -2.15, p=.04) and ESS (t= -2.80, p<0.01). Overall, only sex and number of sodium channel blockers prescribed were associated with assessment scores.

P3-C-104 - Proteomic profiling of the human cerebellum in major depression

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Objective: The cerebellum is now recognized as a functionally heterogeneous brain region involved not only in traditional motor coordination but also in executive and emotional functions which are crucial facets disrupted in major depression. Increasing evidence suggests altered functional connectivity between the cerebellum and key brain areas implicated in depression. However, little is known about potential depression-associated molecular alterations occurring in the cerebellum. To gain molecular insights, we used a proteomic approach to profile a cognitive functioning lobule in individuals who had depression and died by suicide. Methods:

Postmortem human cerebellar tissues were obtained from the Douglas-Bell Canada Brain Bank and included 15 neurologically healthy individuals (H) (9 male, 6 female) and 17 individuals who had a diagnosis of depression and died by suicide (DS) (9 male, 8 female). Proteins were extracted from frozen crus I samples and tandem mass tag-based mass spectrometry was performed. Results: Preliminary results suggest protein divergence between males and females in depression occurring in the cerebellum. Most of the differentially abundant proteins were between H females and DS females. Gene ontology and gene enrichment analyses will aid to identify the biological processes and molecular functions that are potentially affected in the cerebellum of DS females. Conclusions: This study is generating original information on potential cerebellar proteomic signatures associated with major depression, and how these may differ between males and females.

P3-C-105 - Spatially resolved transcriptomics identifies intercellular signaling post-ischemic stroke that impacts neural stem cells

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Globally, stroke is the second most prevalent cause of death and disability. Ischemic stroke mobilizes adult neural stem cells (NSCs) out of the quiescent state. The multifaceted responses of endogenous NSCs to ischemic stroke involve proliferation, migration, differentiation, and synaptogenesis. Hence one strategy for recovery after ischemic stroke is to take advantage of the intrinsic mechanisms of endogenous NSC mobilization. However, the survival rate of recruited endogenous NSCs is low. Moreover, the intercellular signals to activate NSCs after ischemic stroke are poorly understood. Here, we hypothesized that after stroke, cells located in the cerebral infarct send signals to the NSC niche to initiate the regenerative response. To test this hypothesis, we used CellChat to computationally infer the signaling between the ischemic infarct region and ventricular-subventricular zone (V-SVZ) NSC niche from the spatial gene expression profile of ligand-receptor pairs. We identified ligand-receptor pairs and signaling pathways involved in the signaling transduction events at 2, 10, and 21-day after stroke. We evaluated the effects of selected ligands on neurospheres cultured from mouse V-SVZ region. We discovered Galectin-9 as a negative regulator of NSC proliferation by intracerebroventricular injection. We further identified the putative receptors for Galectin-9 in the brain and the signaling pathway that mediated the inhibitory effects of Galectin-9 on NSC proliferation.

P3-C-106 - Postnatal stress exacerbates, while enrichment mitigates, locus coeruleus pretangle tau-induced gene alterations in the hippocampus

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Environmental factors play a crucial role in Alzheimer's disease (AD) onset and progression. The locus coeruleus (LC) is an early site of tau pathology, where hyperphosphorylated pretangle tau accumulates and spreads to other memory and modulatory structures decades before clinical symptoms emerge. Using an LC pretangle tau model with pseudophosphorylated human tau transduction, we demonstrated that LC axonal degeneration in the hippocampus impairs spatial learning deficits. Here, we investigated the effects of neonatal stress/enrichment and adult stress/enrichment on hippocampal gene expression using single-nucleus RNA sequencing.

Neonatal stress resulted in the highest number of differentially expressed genes (DEGs) in excitatory neurons, including dentate gyrus, CA1, and CA3, affecting mitochondrial function, ribosomal protein synthesis, and synaptic processes. Glial cells also showed significant DEGs related to mitochondrial function, synaptic transmission and organization. Gene ontology analysis revealed overlapping biological pathways between pretangle tau alone and pretangle tau combined with neonatal stress, emphasizing the exacerbating impact of neonatal stress. In contrast, neonatal enrichment induced minimal gene changes, suggesting a protective effect, while adulthood enrichment was associated with neuroprotection and synaptic plasticity. These findings reveal the strong impact of early-life experiences on tau pathology, with stress worsening and enrichment mitigating pathological changes, underscoring their distinct roles in AD neurodegeneration.

P3-C-107 - An integrated iPSC-derived cortical, cerebellar and choroid plexus organoid model of SCA1

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Spinocerebellar ataxias (SCAs) are a group of ~50 genetic disorders characterized by progressive neurodegeneration, primarily in the cerebellum and often the cerebral cortex. Patients experience balance and coordination deficits, speech impairments, and muscle stiffness. Current treatments are only symptomatic. We aim to develop a multi-regional human stem cell-derived model of SCA1 including cerebral, cerebellar, and choroid plexus (ChP) organoids, to identify disease mechanisms and associated biomarkers in cerebrospinal fluid (CSF). We use induced pluripotent stem cells (iPSCs) to model SCA1 and investigate patient-specific disease mechanisms. Using iPSCs derived from SCA1 patients, which harbour excess poly-glutamine repeats on ATAXIN-1, we generated cerebellar and ChP organoids to study the impacts on neurodevelopment. Our analyses revealed Purkinje cell dysfunction in cerebellar organoids, characterized by altered development, morphology, and metabolism. In contrast, ChP organoids developed normally and secreted CSF-like fluid, though preliminary mass-spectrometry and biochemical analyses have identified altered metabolic and signatures in SCA1 CSF. Our stem cell-derived models provide insights into SCA1 pathology and offer a translational alternative to invasive CSF extraction. This approach may help to identify reliable biomarkers and potential therapeutic targets for ataxia patients.

P3-C-108 - What change require: Assessing mRNA regulation in direct neuronal reprogramming

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By 2050, approximately 1.5 billion individuals worldwide will be aged 65 and older, representing 25% of Canada's population. Advanced age is the main risk factor for neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, and dementia, which are caused by the irreversibly unnatural death of neurons. As neurons in the adult brain cannot be naturally replenished, how to replace lost neurons becomes the key question for developing potential therapies. A promising approach is direct neuronal reprogramming (DNR), which allows the conversion of non-neuronal brain cells, such as astrocytes, into new functional neurons.

However, the gene regulatory mechanisms underlying DNR are still not well understood, hindering the efforts to improve reprogramming efficiency and specificity. To overcome the technical hurdles for gene expression assessment during DNR, we developed Tag-N-Convert, a new technique that allows cell enrichment for multi-omics analyses. Using fluorescent noncanonical amino acid tagging (FUNCAT) and immunostaining, we found that protein synthesis transitions dynamics during the conversion of human astrocytes into neurons. These changes were accompanied by alterations in processing bodies (PBs), subcellular organelles involved in mRNA stability and translation regulation. Our findings suggest that protein synthesis is dynamically regulated to support the transition from astrocytic to neuronal states, which is potentially facilitated by selective mRNA sequestration through PBs. Our study provides new insights into the molecular control of DNR.

P3-C-110 - Ketamine hampers corollary discharge signals in lateral prefrontal cortex neurons: Implications for models of Schizophrenia

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Disrupted corollary discharge (CD)—the neural signal that helps distinguish self-generated from external sensory input—is a key deficit in schizophrenia. Subanesthetic doses of ketamine are commonly used to model aspects of schizophrenia; however, its effects on corollary discharge signals remain unexplored. In this study, we investigated whether ketamine affects CD signals associated with saccadic eye movements in the primate lateral prefrontal cortex (LPFC). We recorded activity from 1,292 neurons in LPFC areas 8a and 9/46 in two male macaques (Macaca mulatta) performing a virtual working memory task, both before and after ketamine administration (0.25–0.4 mg/kg). Ketamine administration induced working memory deficits and increased overall neuronal firing rates. However, for neurons modulated by saccade direction, ketamine selectively suppressed the responses, indicating a reduction in the saccade-related CD signal within LPFC circuitry. Our results support the use of ketamine as a model for schizophrenia and further suggest that deficits in CD signals play a fundamental role in the development of the disease's core symptoms.

P3-C-111 - Neural markers of social interaction deficits in neurodevelopmental disorders: A normative modelling approach

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Neurodevelopmental disorders (NDDs), such as autism, affect over 3% of children globally and are characterized by deficits in social interaction, communication, and cognition. Recent advances in neuroscience, coupled with shifts toward the neurodiversity paradigm, highlight the need to study NDDs as natural variations in brain function rather than purely as deficits. To address this, we apply normative modelling, a data-driven approach that examines biological variation across both clinical and healthy populations, to identify brain-based markers associated with social interaction impairments. Using cognitive and naturalistic task data from the Healthy Brain Network, we analyse task-based EEG data to measure neural dynamics during

sensory encoding. Specifically, we focus on temporal dynamics such as the autocorrelation window (ACW) and power-law exponent (PLE) which capture how neural activity unfolds over time and have been previously linked to sensory processing and cognitive function. By comparing these markers across individuals with NDDs and the broader population in tasks, we aim to identify specific neural patterns related to social deficits. Preliminary results suggest that participants with NDDs, particularly autism, show shorter ACWs and lower PLEs compared to the population generally. Other EEG measures of task-related neural activity will be discussed, with a focus on their utility as potential biomarkers for identifying and understanding deficits in social interactions. This research contributes to the growing field of neurodiversity-affirming neuroscience by mapping neurocognitive variation in NDDs beyond traditional neurotypical comparisons. Identifying neural markers associated with social impairments could lay the groundwork for more personalised and biologically informed interventions, ultimately improving outcomes for individuals with NDDs.

P3-C-112 - Maternal exposure to acetaminophen affects neuronal morphology within the cerebellum and associated behaviours

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The lipid prostaglandin E2 (PGE2) is synthesized by cyclooxygenase 2 (COX-2) and is important for healthy brain development. Its abnormal levels are linked to Autism Spectrum Disorder (ASD). Prenatal exposure to acetaminophen (APAP), a COX-2 inhibitor, was reported to increase the risk of ASD incidence. We previously showed that COX-2-knockin mice (COX-2KI) exhibit ASD-like behaviour. In this study, we use COX-2KI mice and mice prenatally exposed to APAP to examine neuronal morphology in the cerebellum and related motor deficits. We measured cell density, primary branch density and length within the molecular, Purkinje, and granular layers of the cerebellum at postnatal day 30 using Golgi-Cox staining. We used the grid walking test to examine motor skills. We observed a male-specific increased cell density in COX-2KI mice in the Purkinje layer and APAP-exposed mice in the Purkinje and granular layers. COX-2KI male mice had increased branch density and length in the molecular layer. APAP-exposed males had decreased branch density in all three layers but increased molecular layer branch length. APAP-exposed female mice had decreased branch density in the Purkinje and granular layers. APAP-exposed mice slipped more often during the grid-walking test indicating motor deficits similar to those found in COX-2KI mice. This study shows that reduced prenatal PGE2 levels contribute to sex-dependent neuronal abnormalities and motor deficits in COX-2KI and APAP-exposed mice. We propose that maternal APAP exposure negatively impacts brain development and contributes to ASD in offspring.

P3-C-113 - Acetaminophen-exposure affects dendritic morphology in the developing medial prefrontal cortex

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Prostaglandin E2 (PGE2) is a major lipid signalling molecule in the developing brain. It plays an important role in neuronal proliferation, migration, differentiation and synaptic plasticity. Various environmental risk factors known to increase (infections) or decrease PGE2 levels (acetaminophen (APAP)) have been linked to Autism Spectrum Disorder (ASD). Previous research

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in our lab in mouse offspring prenatally exposed to increased PGE2 levels and knockin mice lacking the PGE2 producing enzyme COX-2 (COX-2KI; low levels) showed altered dendrite and dendritic spine morphology in the hippocampus and cerebellum along with ASD-like behaviour. The objective of this research is to examine the sex-dependent effects of prenatal APAP exposure on dendritic morphology in developing medial prefrontal cortex (mPFC). Pregnant mice were exposed to APAP on gestational day 11 (GD11) until birth. At postnatal day 30 brain tissue were stained using the Golgi-Cox technique to examine dendritic morphology including cell density, soma size and primary branch length and density. Our findings show that APAP-exposed females had significantly increased cell density coupled with decreased soma size compared to age matched control females. Moreover, both APAP-exposed males and females had significantly reduced primary branch lengths. There were significant sex differences observe in branch density, but there was no effect of treatment. Overall, there is a female-specific effect of APAP-exposure on cell density and soma size and an effect of treatment on primary branch length in the developing mPFC.

P3-C-114 - Investigating the nuclear alpha-synuclein interactome using TurboID

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Parkinson's disease (PD) is a prevalent neurodegenerative disorder characterized by motor impairments as well as non-motor symptoms. There is considerable neuropathological and genetic evidence that links the protein alpha-synuclein (aSyn) with PD. aSyn is predominantly localized to the presynaptic terminal in neurons, but a small percentage also exists in the nucleus. Work from our lab and others suggests that the nuclear accumulation of aSyn is neurotoxic and may contribute to PD pathogenesis. Despite these observations, both the native role of nuclear aSyn and potential PD-specific roles remain unclear. We thus set out to uncover the interactome of nuclear aSyn using TurboID proximity labelling with mass spectrometry in mouse primary cortical neurons. Protein interactors of wildtype, nuclear localized, and nuclear excluded aSyn were evaluated. Analysis of the nuclear aSyn interactome revealed an enrichment for RNA-binding proteins (RBPs), particularly those involved in mRNA splicing. Validation experiments in HEK293T cells confirm the interactions between aSyn and several splicing factors using co-immunoprecipitation and bimolecular fluorescence complementation assays. Building on this data, we are using RNA sequencing to explore the functional outcomes of these aSyn-RBP interactions on splicing integrity in neurons and whether changes are paralleled in PD. Collectively, we present a resource for exploring cell compartment-specific interactomes of aSyn and identify novel aSyn protein interactors involved in mRNA splicing.

P3-C-115 - The effect of amygdala kindling on underlying pain sensitivity and pain-related emotional behaviours

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Pain conditions are reportedly highly comorbid with epilepsy, including chronic headache disorders, migraines, neuropathic pain, and fibromyalgia. Despite this, few studies have directly examined differences in pain sensitivity in epilepsy. To address this gap, Long-Evans rats underwent short-term (30 stim) and extended (99 stim) amygdala kindling—an animal model of

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epilepsy in which repeated daily electrical stimulations within a specific brain region lead to long-lasting increases in fear- and anxiety-related behaviour. Given the overlap in neurocircuitry involved in nociceptive pain and fear processing, we hypothesized that recurrent kindled seizures might sensitize brain regions that regulate pain responses, potentially impairing the processing of sensory and affective aspects of pain.We conducted sensory and reflexive nociceptive measurements at multiple time points (pre-kindling, 24 hours, 48 hours, and one week post-kindling), including von Frey hair stimulation and the Hargreaves test for noxious stimulation. Initial results indicate that nociceptive thresholds were significantly elevated during the 24-hour period following the last kindling session in both short- and long-term kindling groups. This hyperalgesic effect persisted one week post-kindling in rats that underwent extended kindling. However, all rats exhibited aversion to the formalin-paired chamber. Investigations into the nociceptive and affective components of kindling-enhanced pain are ongoing.

P3-C-116 - Prenatal valproic acid exposure: Gestational timing and sex-specific effects in an autism spectrum disorder model

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Autism spectrum disorder (ASD) is a developmental condition affecting social interactions, communication, and stereotype/repetitive behaviours. Its increasing prevalence has raised questions about environmental factors, including prenatal medications exposure such as valproic acid (VPA), commonly used to treat epilepsy and migraines. Prenatal exposure to VPA can increase the risk of ASD, with a pronounced male bias, and a similar pattern is observed in a rodent model of maternal VPA exposure. However, the reasons for this sex difference remain unclear.This study explores how the timing of VPA exposure during pregnancy may differentially impact males and females' risk of developing ASD-like traits. In mice, fetal males experience a surge in testicular hormones, such as androgens, during late pregnancy, which may interact with environmental exposures like VPA to heighten their vulnerability. To test this, pregnant mice were exposed to either a vehicle or VPA in early (E12.5) or late (E17.5) embryonic day, analogous to the first and second trimester in humans. We then examined the effects of these maternal exposures on offspring development.Preliminary findings reveal that the timing of VPA exposure is crucial. E17.5 exposure dramatically reduced offspring survival rates, with fewer than 20% of pups surviving compared to over 60% for E12.5 and 100% of controls. We are now analyzing the surviving offspring for differences in social behavior, learning, and anxiety, as well as examining neuroimmune hormonal and epigenetic brain changes to understand the biological mechanisms involved and the sex-specific effects in behaviour and brain development.This research sheds light on how prenatal exposure timing and biological sex contribute to ASD risk, potentially informing early interventions and prevention strategies.

P3-C-117 - Palmitoylation plays a role in multi-system Proteinopathy

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Multisystem proteinopathy (MSP) is a neurodegenerative disease marked by degeneration in any combination of the brain (FTD), bone (Paget's Disease of Bone), and muscle (inclusion body

myopathy). MSP is caused by point mutations in the gene encoding for the Valosin-Containing Protein (VCP). More than 50 known mutations of VCP lead to MSP nd other neurodegenerative diseases. VCP is required to clear p62 and TDP-43 aggregates, and its disease variants are linked to TDP-43 and ubiquitin aggregates. Importantly, VCP's disease-causing variants may be linked to VCP mislocalization, which co-factors and protein modifications may regulate. Indeed, we found a new role for the lipidation of the Small VCP-interacting protein (SVIP) in regulating VCP turnover. We confirmed SVIP is palmitoylated and myristoylated, and both modifications differentially regulate SVIP localization. Myristoylation is needed for SVIP localization to membranes, while palmitoylation partially regulates this. Myristoylated SVIP induces VCP localization to membranes, while amino acid substitution of key glycines and cysteines abolishes this localization pattern. Intriguingly, when R155H-VCP is co-expressed with SVIP, there is an aberrant interaction between the two proteins, resulting in cell death. This is rescued when myristoylation of SVIP is blocked. Here, we investigate the cell death mechanism by examining ER stress and apoptosis markers. We further investigate the role of palmitoylation in regulating this interaction. Using acyl-biotin exchange assays (ABE) and click chemistry reactions, we have also identified key palmitoylating enzymes of proteins in MSP.

P3-C-118 - Spatial and single cell transcriptomics reveal developmental neural circuit deficits in human forebrain assembloids modeling the 15q13.3 microdeletion

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Cortical circuit dysfunction is strongly linked to neurodevelopmental disorders (NDDs). How disruptions in early cortical development lead to defects in human neural circuit formation are not well understood. To elucidate cell type-specific and organizational deficits related to human neural circuit abnormalities, we generated forebrain organoids and assembloids from individuals with the 15q13.3 microdeletion syndrome, a NDD associated with epilepsy, schizophrenia, and autism. Employing single-cell transcriptomics in organoids, we identified dysregulation of synaptic signaling and distinct gene networks in excitatory and sub-populations of inhibitory neurons. We validated this imbalance in organoids with virally labelled excitatory and inhibitory neurons, and functional analysis using calcium imaging showed hyperactive neural networks. Next, using forebrain assembloids, we found reduced medial ganglionic eminence (MGE)-interneuron migration by lightsheet imaging and increased synaptic connectivity by monosynaptic rabies virus tracing. Finally, we leveraged unbiased spatial transcriptomics and revealed regional transcriptional and cell-cell signaling disturbances to various neural lineages, sensitivity to endoplasmic reticulum stress, and elevation of DNA damage in inhibitory neurons. Collectively, we establish a framework in human brain organoids and assembloids to identify that early microenvironment and cell type-specific defects are linked to neural circuit dysfunction, which may contribute to neurodevelopmental deficits in 15q13.3 microdeletion disorder.

P3-C-119 - Investigating altered somatosensory function in a peripheral nervous system organoid model of autism spectrum disorder

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Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by changes in social communication and patterns of restricted, repetitive behaviours. Sensory alterations are reported in up to 95% of individuals with ASD, however the biological pathways driving these changes are not well understood. Intriguingly, genetic perturbation of several strong ASD-associated genes (e.g., FMR1) in animal models also manifests in somatosensory (touch and pain) phenotypes, and emerging evidence underscores a more direct contribution of the peripheral nervous system (PNS) in ASD pathogenesis than previously understood. To examine the cellular and molecular mechanisms underlying altered human PNS somatosensory development in a monogenic model of ASD, we generated hPSC-derived isogenic FMR1-knockout and patient-derived PNS somatoSENSORy organoids (SENSORs) reminiscent of dorsal root ganglion (DRG) development. To characterize SENSOR cell type and structural features, we performed immunohistochemistry and single-cell RNA sequencing, and ongoing work is underway to compare their functional properties using established organoid pipelines in our lab (e.g., patch-clamp electrophysiology, calcium imaging). Further, we have performed preliminary studies xenografting SENSORs into rodent DRG that may offer an unprecedented opportunity to examine more complex humanized somatosensory innervation, function, and circuitry in vivo. Findings from this project can provide novel insight into understudied PNS somatosensory pathways in ASD and other conditions involving sensory dysfunction.

P3-C-120 - Age-dependent susceptibility to seizure-like activity and antiseizure medications in developing neuronal networks

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Epilepsy is a debilitating neurological disorder that affects over 130,000 Canadians and 50 million people worldwide. Seizures disrupt neuronal networks, increasing the risk of long-term cognitive impairment. Neonates and infants exposed to seizure-like activity (SLA) or antiseizure medications (ASMs) face risks of altered synaptic development, disrupted receptor expression, and excitatory-inhibitory imbalance, potentially leading to lifelong neurological deficits. This vulnerability may stem from the unique biochemistry of the developing brain, including GABAergic giant depolarizing currents, which shape network maturation differently than in adults. However, the precise mechanisms underlying these age-dependent differences in seizure susceptibility, network remodeling, and drug efficacy remain unclear.To investigate this, primary hippocampal cultures at different developmental stages (young: DIV 8; mature: DIV 15) were used to assess SLA-induced changes in network excitability, synaptic receptor regulation (GLUR1, NMDAR, PSD-95, and GABAAR), and cell viability. To analyze functional responses, both neonatal and adult rat brain slices were recorded using 3D microelectrode arrays (MEAs) and exposed to zero-magnesium aCSF to induce SLA. Network activity, burst properties, and recovery dynamics were analyzed, followed by ASM treatment with cannabidiol and valproate to determine how SLA modulation differs across developmental stages.By comparing SLA and ASM effects in young vs. mature neuronal networks, this study provides insight into how the brain's developmental stage influences seizure susceptibility and drug response. Understanding these differences may inform the development of safer, age-specific treatment strategies to mitigate the long-term impact of seizures and ASMs in neonates and adults.

P3-C-121 - Key Therapeutic Target for pathological mechanisms leading to blindness: B1R wins over B2R

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Finding new therapies for wet age-related macular degeneration (AMD) is crucial, as choroidal neovascularization (CNV) causes severe vision loss. The proinflammatory kallikrein-kinin system (KKS), particularly the kinin B1 receptor (B1R), contributes to CNV damage, while B2R plays a role in angiogenesis. This study evaluates the effects of B1R, B2R, or dual antagonism on pathological changes in laser-induced CNV.CNV was induced via 5 laser burns in the right eye of 24 C57Bl6 mice. Visual function was assessed pre-CNV and on days 2 and 7 using electroretinography (ERG), Optokinetic Response and Visual Cliff. Mice received 7-day topical treatment with vehicle, R-954 (B1R antagonist), HOE-140 (B2R antagonist), and both. Retinas and choroids were analyzed via immunohistochemical staining (Iba-1, isolectin B4, B1R) and confocal microscopy. Laser-induced CNV triggered microglial invasion, B1R expression, and ERG deficits in the vehicle group. R-954 abolished B1R/Iba-1 staining, reduced CNV volume (20 ± 2 vs. 152 ± 5 × 104 µm3, R-954 vs. Vehicle), and rescued a-wave (-47 ± 20 vs. -34 ± 14 µV) and b-wave (101 ± 27 vs. 64 ± 17 µV) amplitudes on day 7. HOE-140 and combination therapy showed no significant effect on inflammation, CNV volume, or ERG impairment. R-954 partially restored visual acuity and depth perception, unlike HOE-140.Our findings highlight KKS's role in CNV progression, suggesting new AMD therapies. B1R antagonism (R-954) is effective, while B2R inhibition (HOE-140) shows limited impact, and combination therapy offers no added benefit. Further research is needed.

P3-C-122 - Examining the role of IL-1R antagonism in treating postpartum depression using a rodent model

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Background: Depression risk is highest during the postpartum [postpartum depression (PPD)]. Selective serotonin reuptake inhibitors (SSRIs) are often prescribed for PPD, however, only 3.2% of females with PPD achieve remission with SSRIs. In our preclinical model of PPD, we administer high corticosterone (CORT) during the postpartum. We found increased levels of the proinflammatory cytokine IL-1β in the hippocampus was commensurate with reduced SSRI efficacy, indicating this may be an important target to boost SSRI efficacy. Our central hypothesis is that antidepressant efficacy in the postpartum is mediated by inflammatory signalling. Methods: High CORT was administered during the postpartum period to dams starting on postpartum day 2 along with fluoxetine (FLX) and/or anakinra (KIN), an IL-1R antagonist. FLX efficacy was measured using the forced swim test (FST), and maternal care observations. All dams were euthanized 23 days later to examine inflammation and neuroplasticity in the hippocampus. Results: Dams treated with KIN (with or without FLX) failed to rescue passive coping behaviours in the FST. However, FLX and KIN together were able to rescue reductions in neuroplasticity as noted in hippocampal perineuronal net (PNN) expression and doublecortin (DCX+ cells) expression. Current analyses are in progress to quantify PNNs in the frontal cortex, alongside postsynaptic density-95 (PSD-95) in both hippocampal and frontal cortex tissue. Lastly, we will quantify the percentage of phagocytic microglia (Iba1+/CD68+) in the

hippocampus and frontal cortex. Conclusions: These findings indicate that IL-1β may serve as a potential target for increasing antidepressant efficacy in people with PPD

P3-C-123 - A deficit in GABAergic inhibition in the ACC as a substrate of chronic pain-induced depression

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Chronic pain often occurs alongside mood disorders, sharing common neural pathways. A key player in this comorbidity is the anterior cingulate cortex (ACC), a region responsible for processing the emotional, sensory, and cognitive aspects of pain and mood regulation. Notably, ACC dysfunction in comorbid pain and depression is characterized by hyperexcitability. GABAergic neurons seem central to these changes. We have shown that optogenetic activation of ACC GABAergic neurons reduces depressive-like behaviors in rodents with chronic pain-induced depression (CPID), suggesting that impaired GABAergic signaling may underlie certain mood disorders. Hence, we hypothesize that chronic pain disrupts GABAergic neuron function in the ACC, reducing their ability to inhibit neural circuits. This leads to hyperexcitability, which may drive anxiodepressive-like behaviors. To explore this, we performed in vivo fiber photometry in the ACC from mice where a genetically encoded GABA sensor was expressed to measure GABA release during anxiodepressive-like behavior. Control mice showed a decrease GABA release in the ACC during feeding motivation (e.g., NSF) and innate behaviors (e.g., grooming), suggesting a role for GABAergic inhibition in initiating these behaviors. In CPID mice, we found a greater decrease in GABA release during these behaviors. This loss of inhibition in the ACC during behavior initiation in CPID mice likely contributes to the expression of depression-like phenotypes in chronic pain conditions, highlighting GABAergic neuron dysfunction as the key player in this pathology.

P3-C-124 - Pre-operative neuroimaging biomarkers for DBS response: Advancing patient selection with network-based approaches

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Deep Brain Stimulation (DBS) offers significant benefits, yet outcomes remain variable. For example, 1/3 of Parkinson's Disease (PD) patients show no significant improvement. Beyond targeting accuracy, patient factors must be considered, highlighting the need for objective preoperative biomarkers to improve patient selection.We systematically reviewed DBS studies on preoperative neuroimaging features linked to postoperative outcomes. 53 studies (1,758 patients), mostly on PD and major depressive disorder (MDD), were analyzed. Brain regions were extracted, weighted by appearance, mapped onto standardized atlases, and disease- and target-specific brain maps were generated.In PD, regions linked to favorable motor outcomes included the subthalamic nucleus, thalamus, globus pallidus, and cerebellum, while suboptimal motor

outcomes were associated with the precentral gyrus, putamen, and lateral ventricle. Non-motor outcomes were linked to the prefrontal cortex, limbic structures, and occipital lobe.In MDD, mood improvement was associated with the subcallosal cingulate cortex, anterior cingulate cortex (ACC), insular cortex, thalamus, and superior temporal gyrus, while poor mood improvement correlated with atrophy in the prefrontal cortex and ACC.Our findings show that motor outcomes in PD align with the motor circuit but also involve cognitive and emotional regions. MDD outcomes are linked to the limbic circuit, insular cortex, and thalamus. Data on other DBS indications remain limited. These brain maps provide a framework for future research and improved DBS patient selection.

P3-C-125 - Role of the MEN1 gene in cellular and molecular mechanisms underlying Alzheimer's disease in human autopsied brain tissue

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Alzheimer's disease (AD) is the leading cause of dementia, affecting over 55 million people worldwide. Despite decades of research, AD etiology remains unclear, with therapies targeting amyloid β and tau pathology showing limited success. This underscores the need to explore alternative mechanisms. Synaptic dysfunction is one of the earliest pathological features of AD, particularly in cholinergic circuits in the hippocampus and cortex. As such, the cholinergic hypothesis attributes AD-related cognitive decline to impaired cholinergic synaptic integrity, yet the molecular regulators of this vulnerability remain unclear. Menin, a protein that modulates synaptic plasticity has been implicated in cholinergic signaling crucial for learning and memory. To investigate the role of menin in AD-related synaptic pathology, we analyzed human postmortem hippocampal and cortical brain tissue with immunohistochemistry and quantified synaptic (synaptophysin, PSD-95) and AD-related (tau, menin) biomarkers. We found significantly reduced PSD-95 expression in AD samples, indicating synaptic loss. Menin was also diminished, correlating with synaptic alterations and increased tau accumulation. Strong tau-menin colocalization in both AD and control samples suggests a potential interaction in disease progression. These findings highlight menin as a potential regulator of cholinergic synapse vulnerability. By measuring protein alterations in human AD brain tissue, this study provides key insights into AD pathogenesis and suggests new avenues for therapies targeting synaptic resilience in AD.

P3-C-126 - A multimodal molecular characterization of the human postmortem uncinate fasciculus

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The uncinate fasciculus (UF) is a long-range white matter (WM) tract connecting the anterior temporal lobe and orbitofrontal cortex. Though implicated in psychiatric disorders, including depression, it remains understudied as it exists only in humans and non-human primates. Despite prior findings of long-lasting myelin-related alterations in cortical WM of individuals with childhood abuse (CA), whether such changes occur in long-range fiber bundles is unknown. We

aimed to characterize the UF's cellular and molecular properties—including cell type composition, lipids, and ultrastructure—and compare these measures in depressed individuals with or without severe CA vs. controls.Fresh frozen left hemisphere UF samples were obtained from the Douglas-Bell Canada Brain Bank. snRNA-seq: Extracted nuclei were captured using 10X Genomics technology and sequenced on the NovaSeq6000. Lipid analysis: Lipids were extracted (Folch method), phospholipids separated by thin-layer chromatography, and fatty acids/cholesterol quantified via gas chromatography. Ultrastructure: 300 um thick UF sections were imaged via spectral-focusing coherent anti-Stokes Raman scattering microscopy, and axon/myelin metrics were extracted using a custom AxonDeepSeg model.No significant gene expression, fatty acid, or cholesterol alterations were observed with CA or depression. However, age-related gene expression patterns and declines in omega-6 fatty acids across the lifespan were detected in multiple phospholipid fractions. Interestingly, snRNA-seq revealed neuronal clusters, which were validated as non-artifactual via NeuN immunohistochemistry and in situ hybridization.This first ever molecular characterization of the human UF presents gene expression, lipid, and ultrastructure data. The absence of group differences underscores the importance of regional specificity in psychiatric research.

P3-C-127 - Circadian rhythmicity of chronic pain in a mouse model of multiple sclerosis

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Multiple sclerosis (MS) is a central nervous system demyelinating disease, with over 50% of people with MS (pwMS) citing pain as their primary symptom. A diurnal rhythm of pain intensity is often reported by pwMS. When normal circadian rhythms are disrupted, such as among firefighters, nurses and people living at high altitudes, the risk of MS increases significantly. We therefore sought to investigate the role of circadian rhythms in MS-related chronic pain using the experimental autoimmune encephalomyelitis (EAE) model. In EAE and sham mice, mechanical sensitivity was assessed at multiple days post-immunization every 6 hours using the von Frey assay. Our results show a circadian rhythm in mechanical sensitivity of EAE mice, with highest intensity at ZT8. Accordingly, inflammatory cytokine expression, spinal cord infiltration by myeloid cells and myeloid activation in the dorsal horn all showed circadian rhythms at peak disease, with their troughs at ZT8. Given the reciprocating circadian oscillations in mechanical hypersensitivity and neuroinflammation, their interaction as a pain mediator in MS was further explored. EAE mice were given indomethacin or vehicle at ZT11 before being assessed at ZT14. After indomethacin injection, EAE mice exhibited increased mechanical hypersensitivity at ZT14, similar to ZT8. Our results showed unexplored interactions between pain and inflammation that are under circadian control. This avenue of research could lead to novel pain mechanisms that can be targeted to improve the lives of many impacted by MS.

P3-C-128 - Effects of endocannabinoid modulation in an acute mouse model of Parkinson's disease

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Parkinson's Disease (PD) is a neurological disorder marked by dopamine cell loss and motor symptoms such as resting tremor, bradykinesia, and muscle rigidity. L-DOPA, the gold standard pharmacological treatment for PD, can lead to a condition named L-DOPA Induced Dyskinesia

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(LID) with prolonged use and high doses, causing abnormal involuntary movements. The endocannabinoid system (ECS) modulates dopamine in the striatum by releasing endocannabinoids like 2-arachidonoyl-glycerol (2-AG) and activating pre-synaptic CB1 receptors, altering neurotransmitter release. Dysfunction of the ECS in PD is increasingly recognized, with alterations in CB1 receptor expression and endocannabinoid levels observed in patients. This study aims to prolong L-DOPA efficacy and ameliorate dyskinesia by targeting the ECS in a mouse model. To do this, we used the Dopamine Deficient Dopamine Transporter Knockout (DDD) model, inducing full dopamine depletion with tyrosine hydroxylase (TH) inhibitor alpha-methyl-p-tyrosine (aMPT). Mice were treated with ECS modulators and L-DOPA. L-DOPA can bypass TH inhibition and restore locomotion in our mice. Acutely, MAGL inhibitors (ABX-1431/MJN110) enhanced L-DOPA effects, while DAGL inhibitor DO34 reduced them, suggesting that blocking 2-AG metabolism enhances L-DOPA response. Chronic L-DOPA use led to reduced locomotor activity and increased dyskinesia, delayed by ABX-1431. In conclusion, 2-AG modulation via MAGL enhances L-DOPA's effects and may delay dyskinesia, supporting ECS therapies as adjuncts to L-DOPA in PD treatment.

P3-C-129 - Assessing reinforcement of sweet additives in an oral morphine self-administration task in male and female rats

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Introduction: To improve translatability of morphine self-administration (SA), we use a two-lever oral morphine SA task with a reinforcer solution containing grapefruit juice (GFJ), thought to enhance morphine's bioavailability, and varying sucrose concentrations (SUC) to mask morphine's bitter taste. However, the influence of morphine's reinforcing properties in our task is unclear due to the sweet additives and limited knowledge of GFJ's impact on morphine's metabolism in rats. Methods: Exp 1: Male and female rats were trained to lever press for a morphine solution using a SUC fading procedure (20%, 10%, 5%); half with GFJ, half without. Seven 10-session phases followed, each modifying a component of the solution to assess additive-dependent differences. Exp 2: To discern morphine's pharmacological role in SA, a two-day test cycle followed. On Day 1, all rats were pretreated with saline prior to their SA session and on Day 2 were pretreated with 1mg/kg naloxone. Results: Exp 1: Training history altered behavior across phases with lever pressing increasing when GFJ was presented alone, indicating additive-dependent differences driving behaviour. Exp 2: Sex and additive differences were present throughout acquisition. Naloxone decreased consumption in both sexes; however, males had a greater magnitude of difference in active lever pressing. Conclusions: Sweet additives in the solution are more likely to be reinforcing lever pressing than the pharmacological effects of morphine, thus, further understanding is required before characterizing 'morphine-seeking' in this task.

D - SENSORY AND MOTOR SYSTEMS

P3-D-130 - Modular integration of multimodal cues in the cortical reach network

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Real-world behavior requires the integration of multiple cues for coordinated action, for example object location cues to aim a reach combined with object-orientation cues to form a correct grasp. To understand how the brain might integrate these different sensory and motor components, we employed a cue-separation event-related fMRI task in which twelve participants were visually cued to the object shape (square) and Location (L: left or right to center), and verbally instructed how to manually Orient the grip (O: horizontal or vertical), with each cue followed by a delay in randomized temporal order (OL vs. LO). We employed standard univariate analysis and graph theory analysis (GTA) of 200 cortical nodes to understand how the cortex integrates these action cues over time. The univariate analysis revealed order-dependent, sensory-specific activation: during the first delay, early Visual Cortex was activated for the visual instruction and Superior Temporal Gyrus for the auditory instruction, followed by the opposite patterns during Delay 2. Order-independent widespread sensorimotor activation occurred during the action period. GTA revealed two significant network modules: one spanning occipital-parietal cortex (with important hubs in V1, SPOC and pIPS), and one spanning auditory/somatomotor cortex (with important hubs in M1, PMd and ACC). These results show how different instructions are integrated into the reach plan, i.e., bottom-up visual cues through 'dorsal stream' parietal reach areas and top-down auditory task instructions through the frontal cortical reach network.

P3-D-131 - Spatial transcriptomics reveal distinct cell populations in the human balance organ

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The utricle is a sensory organ in the inner ear crucial for detecting balance. Its sensory epithelium consists of specialized sensory hair cells that sense head tilting and linear acceleration on the horizontal plane, along with nonsensory supporting cells that play a key role in maintaining the integrity of the epithelium. Hair cell degeneration is a primary cause of progressive and irreversible balance disorders. Currently, no biological therapies exist to treat such conditions, largely due to a limited understanding of both the molecular characteristics of the utricle and the human-specific mechanisms that govern hair cell regeneration. Recent advancements in spatial transcriptomics provide insight into gene expression patterns within the spatial context of the tissue. We utilized our snRNA-seq data to inform the design of a customized gene panel for an imaging-based spatial transcriptomics platform and processed utricles from gestational weeks 15 and 18. The expression of representative marker genes was mapped to their corresponding cell types and used to characterize their spatial organization within the utricle. Comparative analysis revealed key changes in gene expression occurring between the two ages. Our study presents the first spatial transcriptomic map of the human fetal utricle and provides novel insights into its cellular organization. These findings advance our knowledge of the human utricle and will be critical for defining the spatiotemporal gene expression dynamics that regulate hair cell development and regeneration.

P3-D-132 - Dopamine and GABA from the zona incerta innervates the medial superior colliculus to suppress escape

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The zona incerta (ZI) in the hypothalamus responds to stress, such as from a predator or hunger, by suppressing fear and anxiety. The ZI is primarily GABAergic, and one cluster of ZI-GABA cells are marked by tyrosine hydroxylase (TH) and produce dopamine (DA). These ZI-GABA/DA cells are medially distributed and densely innervate midbrain motor regions, especially the medial motor-related superior colliculus (SCm) that promotes escape from perceived visual threats. We found that ZI-GABA/DA cells can inhibit SCm cells, but it is not known if ZI-mediated inhibition suppresses fear-induced escape at the SCm or how it manages conflict between hunger and safety. We transduced Th-cre ZI cells with a Cre-dependent virus encoding an excitatory DREADD hM3(Dq) to determine if direct activation of ZI fibers at the midline of the SCm suppressed escape from a predator-like looming dot presented overhead. Intra-SCm infusion of C21 delayed escape and suppressed time spent in the home shelter in fasted but not sated female or male mice. Interestingly, GABA-A receptor antagonism robustly suppressed escape only in female mice, while DA receptor antagonism suppressed escape only in male mice; this implicated a mechanism underlying sex differences in ZI-mediated escape. Ongoing studies will elucidate the effects of fasting on the activity of ZI and SCm cells, as well as transmitter release from ZI-GABA/DA fibers in the SCm. Together, our work suggested a sexually dimorphic neural pathway whereby the energy state can regulate innate fear responses.

P3-D-133 - Sensorimotor integration in soft bodies

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Dexterity and agility are behaviors studied in distinct contexts, but which arise from common mechanisms of rapid and precise body and limb control. Dexterous behaviors include gesturing, manipulating objects, or typing and playing musical instruments, and agile behaviors include rapid accelerations and flips. Feedback control of dexterous and agile behaviours requires proprioceptive awareness of the body's configuration in space and time. Classical proprioception of limb positioning through joint angles and muscle stretch is well described. In contrast, 'awareness' of soft tissue configurations, like skin deformations underlying facial expressions or those modulating grip contact forces in grasping, remains enigmatic. Even computationally classifying soft tissue poses is challenging. Bats combine dexterity and agility in a single controlled system, a comparative model of joint positioning and skin deformations involved in precision motor control. Bats' five elongated fingers allow them to manipulate air currents during acrobatic behaviours just like humans deftly manipulate objects, and common neural architectures underlying both. We present a pipeline for reconstructing complex body shape dynamics, applied here to bat morphology throughout the stroke cycle. Our results highlight substantial shape changes that can be attributed to actuation and aeroelastic interactions. This method can be generally applied to identify deformable tissue configurations, a prerequisite for understanding their closed-loop sensorimotor feedback control.

P3-D-134 - Characteristics of neuronal activity in motor and prefrontal areas during a dual task

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Achieving specific behavioral goals often requires maintaining internal action plans against external perturbations. To examine how the brain retains and recalls such plans while responding to local stimuli, we trained monkeys on a memory-guided sequential motor task with interruptions. Experimental sessions were divided into sections based on the temporal order of the motor sequence in the main task. Each section required animals to execute two visually guided movements, chosen from four possible options (e.g., pronation or supination of either forearm). The sequence was performed twice while memorizing it, followed by two memory-guided repetitions. A visual cue then interrupted the main task, directing the monkeys to perform one of the four movements independently of the task (interrupting movement). Afterward, they resumed the main task and recalled the previously performed sequence. After the monkeys achieved recall accuracy of ≥85%, we recorded neuronal activity from multiple motor-related areas and the lateral prefrontal cortex (lPFC) during the performance of the dual task. Apart from neurons encoding the immediate next movement, we found many neurons reflecting discordance or concordance between the first movement in the main task and an interrupting movement, suggesting temporary suspension of the motor plan. Some lPFC neurons predicted recall success, highlighting a supervisory function of this area. Interestingly, neurons in the dorsal premotor area exhibiting tonic selectively for the second movement temporarily lost this selectivity during the interruption.

P3-D-135 - A neuronal pathway for the supraspinal relay of warm and cool sensation

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Thermosensation is a fundamental sense allowing discrimination of external temperature changes and driving homeostatic responses. Activation of peripheral thermoreceptors relay thermosensory information to spinal cord circuits, and from there to supraspinal centres including the cortex. While extensive studies identified nociceptive-specific thermosensitive anterolateral tract (ALT) neurons in the superficial spinal dorsal horn, the relay of innocuous thermal signals remains poorly understood. We found that in mice, spinal lamina I ALT neurons expressing the transcription factor Phox2a have morphological and histological properties consistent with previously identified thermosensitive ALT neurons. Our single synapse retrograde rabies virus tracing of Phox2a ALT neurons and ex vivo patch-clamp recordings confirmed direct inputs from peripheral thermoreceptors. In addition, in vivo calcium imaging revealed that the majority of lamina I Phox2a ALT neurons can be subdivided into two populations, selectively tuned to a wide range of innocuous temperature changes, either in warming or cooling ranges. Their genetic ablation, chemogenetic or optogenetic manipulation impaired temperature discrimination. Together, our findings reveal that two largely non-overlapping subsets of Phox2a lamina I neurons comprise an important ascending pathway transmitting warm and cool sensation.

P3-D-136 - Spinal reflex contributions to multi-finger force control: Insights from transcutaneous nerve stimulation and high-density electromyography

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Understanding how spinal reflex pathways interact with biomechanical constraints to govern finger force control is critical for neuroprosthetic design and rehabilitation. This study combined transcutaneous stimulation of the ulnar/median nerves with high-density electromyography (HD-EMG) and multi-finger force measurements in 12 participants to investigate how spinal reflex engagement (via H-reflex activation), electrode placement, and neuroanatomical variability shape finger interdependence. We evoked H-reflexes in intrinsic and extrinsic finger flexor muscles and quantified spinal excitability using H-reflex/M-wave (HM) ratios while recording force outputs across individual fingers and pairs. The index and middle fingers generated more force than the ring and pinky fingers, and spinal reflex strength showed a significant relationship with force production. Middle finger activation strongly influences neighboring fingers, highlighting biomechanical coupling and neural crosstalk. Electrode configurations shaped the force distribution, with some enabling selective activation and others triggering multi-finger responses. These results demonstrate that multi-finger force coupling arises from spinal excitability, electrode-dependent nerve recruitment, and anatomical differences. By isolating reflex-mediated force dynamics, this study advances hierarchical motor control models and underscores the need for personalized stimulation protocols to optimize neuroprosthetic interfaces and post-stroke rehabilitation.

P3-D-137 - Auditory processing abnormalities in Cntnap2-knockout mice

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Autism spectrum disorder (ASD) is characterized by social difficulties that are often accompanied by altered sensory processing. In the auditory system, disruptions to sensory processing may contribute to impairments in auditory perception and hyperacusis, which are both symptoms seen within ASD (Roth et al., 2012). Previous studies using genetic models of ASD have utilized electrophysiological recordings from auditory brainstem responses (ABR) to investigate this potential pathway dysfunction. In the Cntnap2-knockout (KO) rat model, a delayed maturation phenotype has been observed as evidenced by reduced ABR wave amplitudes and prolonged latencies within juvenile animals (Scott et al., 2020). However, it remains unclear whether this developmental phenotype is conserved across species. To test this, we examined the characteristics of click-evoked ABR waveforms in the Cntnap2 KO mouse model. We recorded ABRs from juvenile (4-6 weeks) and adult (8-10 weeks) KO mice and compared recordings to wildtype littermates. We found that juvenile KO mice (n = 7) exhibited differences in ABR peak amplitudes and generally longer latencies, compared to wildtype littermate controls. However, these abnormalities appear to be normalized in adult mice (n = 8), which is consistent with ABR findings in Cntnap2 KO rats. These results suggest early alterations in auditory pathways as a potentially conserved mechanism across species, which may influence downstream sensory processing and contribute to the sensory deficits observed in ASD.

P3-D-138 - Dominant ICMS-evoked EMG pattern sharing across muscles uncovered by recursive clustering, including a possible echolocation module in sensorimotor cortex of egyptian fruit bats (rousettus aegyptiacus)

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Milad Hafezi [1], Saba Hosseini [2], James Liggins [1], Andrew Halley [3], Carlos Pineda [3], Tobias Schmid [4], Fernando Gomez [3], Robin Boparai [3], Leah Krubitzer [3], Michael Yartsev [4], Dylan Cooke [1]

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Natural behaviors require coordinated muscle activation across the body, a process in which the neocortex plays a key role. Intracortical microstimulation (ICMS) of the sensorimotor cortex (e.g., M1, parietal areas) evokes multi-joint movements resembling essential behaviors like feeding and reaching. However, how the neocortex organizes diverse muscles for motor responses remains unclear.We examined the topography and temporal dynamics of ICMS-evoked electromyographic (EMG) activity in four Egyptian fruit bats (Rousettus aegyptiacus), a species with specialized motor behaviors, including flight and lingual echolocation. EMG recordings from 16 muscles spanning the face, forelimbs, torso, and hindlimbs revealed broadly distributed muscle representations across the motor and somatosensory cortex. Feature analysis (onset, offset, duration) showed systematic variations in activation timing across cortical sites.We observed different temporal EMG patterns coded across the cortex for every muscle. To determine whether these muscles share similar EMG patterns, we applied machine learning techniques to cluster them. This revealed similar waveforms within and across muscles, suggesting the neocortex coordinates functionally linked muscle groups as cohesive units. Combining this method with video analysis of ICMS-evoked movements, we identified a cluster in sensorimotor cortex that may underlie production and precise aiming of echolocating tongue clicks. Moving forward, we aim to map these multi-muscle activation patterns and their behavioral relevance.

P3-D-139 - Comparative mapping of glucagon-like peptide-1 receptor (Glp1r) mRNA in the mouse and human nodose ganglia

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In recent years, the molecular profile of vagal sensory neurons has been characterized in mice through high-throughput quantitative PCR, single-cell RNA sequencing, and RNAscope in situ hybridization. However, it remains unclear whether these molecular findings are applicable to humans. In this study, we isolated nodose ganglia from human donors at the University of Utrecht (The Netherlands). We evaluated the effects of postmortem interval and fixation duration to optimize RNAscope in situ hybridization. We then examined whether vagal neurons expressing the glucagon-like peptide-1 receptor (Glp1r), previously identified in mice, are also present in humans. Our results reveal distinct differences in the distribution of Glp1r mRNA expression between mice and humans. In mice, Glp1r mRNA was highly expressed in a restricted subset of neurons, whereas in humans, low levels of Glp1r were found in most neurons, as well as in non-neuronal cells throughout the nodose ganglion. These findings suggest that molecular data obtained from mice cannot always be directly applied to understanding the human nervous system.

P3-D-140 - Analgesic properties of MDMA in acute and inflammatory pain in mice

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Many patients with chronic pain report anhedonia or the loss of pleasurable sensation from gentle touch, and this remains a largely unaddressed issue in the treatment of chronic pain. The drug 3,4-methylenedioxymethamphetamine (MDMA), colloquially known as ecstasy or molly, is a psychedelic drug with empathy-promoting properties, and has been shown in humans to enhance the pleasantness of gentle, affective touch. This effect has also been correlated with changes in activation in brain areas that process sensory information. This highlights an ability for MDMA to modulate somatosensory processing, with a potential to restore the appetitive value of gentle touch in chronic pain. The analgesic effects of MDMA were tested in mouse models of acute and inflammatory pain induced by intraplantar injections of capsaicin or Complete Freund's Adjuvant (CFA) respectively. Von Frey filaments were used to measure changes in tactile sensitivity after systemic administration of MDMA. Mice exposed to MDMA exhibited increased paw withdrawal thresholds compared to their saline-treated counterparts, with the effect being stronger in males than in females. These findings reveal the potential use of MDMA for acute analgesia, and set the stage for further work in the drug's modulation of pain and touch processing.

P3-D-141 - Influence of handedness on changes in hand muscle representation size and excitability within ipsilateral motor cortex during unimanual contraction

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Introduction: Activation of the ipsilateral motor cortex (M1) with unimanual movement has been well observed. However, the effects of a unimanual contraction on motor representation size and excitability within ipsilateral M1 remain inconclusive. Additionally, no study has investigated the influence of handedness on such modulatory effects within ipsilateral M1. Objective: Our goal was to investigate the effects of a unimanual hand task on the excitability and spatial area of a hand muscle representation within ipsilateral M1, and specifically to examine if these modulatory effects differed between right- and left-handers. Methods: 40 right- and 40 left-handers received transcranial magnetic stimulation (TMS) to each hemisphere separately, under two task conditions: (1) Rest and (2) Active: isometric contraction of the hand ipsilateral to TMS stimulation. Changes in motor threshold and cortical area of the contralateral resting first dorsal interosseous (FDI) muscle between task conditions were examined. Results: The active condition decreased motor threshold and increased cortical area of the FDI muscle within ipsilateral M1. However, these changes did not differ across handedness groups nor between hemispheres. Conclusion: We demonstrate that an ipsilateral hand contraction indeed increases the excitability and spatial area of motor representations within ipsilateral M1, and that these increases are symmetric across hemispheres and between right- and left-handers. Our findings may have fundamental implications for future TMS studies and stroke rehabilitation protocols.

P3-D-142 - VTA dopaminergic pathways to the forelimb motor cortex: Anatomical, molecular, and sex-specific insights

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Learning motor skills and recovering from injuries relies on neuroplasticity, with dopamine playing a key role in this process. The motor cortex receives dopaminergic projections from the

ventral tegmental area (VTA), and the disruption of dopamine release in the motor cortex disrupts motor learning. The VTA serves as a primary dopamine source and contains neurons with combinatorial neurotransmission, influencing their functional output. This study quantifies the proportion and neurotransmitter content of VTA dopaminergic projections to the rostral (RFA) and caudal (CFA) forelimb areas of the motor cortex to elucidate their role in forelimb motor learning.The Long-Evans rats received Fluorogold injection in the RFA or CFA, unilaterally. Immunofluorescent labeling for Tyrosine Hydroxylase (TH) was conducted to quantify projection ratios. Additionally, fluorescent in-situ hybridization (RNAscope) was performed to identify TH, VGAT, and VGLUT2, revealing neurotransmitter content.Approximately 20% of VTA neurons projecting to the forelimb motor cortex are dopaminergic, with 5% of these projecting contralaterally. Most projections to the RFA originated from the mid-VTA (-5.2 to -5.5 mm), whereas CFA projections were located more posteriorly; making these regions ideal targets for functional studies. Notably, sex differences were observed: males exhibited a higher ratio of dopaminergic projections to the RFA than females. Conversely, females received significantly more non-dopaminergic VTA projections to both RFA and CFA, the identity of which remains under investigation.

P3-D-143 - Individual attentional abilities modulate speech motor control systems within children

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Due to the daily importance of speech, understanding the mechanisms involved in speech production is crucial. Feedback and feedforward motor control systems work together to correct for speech production errors and initiate speech, respectively. Attention has been shown to impact the weighting of these systems under experimental manipulations of attention, yet it is unclear if individual differences in attentional abilities may modulate their use. This study measured attentional abilities to probe whether differences in attention might be related to the control of ongoing speech in children ages 4-12. Children were instructed to produce vocalizations while their voice was suddenly shifted ±100 cents, 3 times per utterance. Children's vocal pitch was recorded to assess the magnitude of compensatory responses to the manipulations. Individual differences in attention were measured through performance on the sustained attention to response task (SART). Children who performed worse on the SART produced significantly smaller compensatory responses to the pitch manipulations even when controlling for age. This suggests that attention modulates the weighting of feedback and feedforward control whereby reduced attentional abilities result in a decreased weighting of the feedback control system. Further, this finding is independent of age-related differences in attention and speech production suggesting a unique contribution of attention on speech motor control. Future research is required to help clarify the developmental implications of attentional differences on speech production.

P3-D-144 - Posterior Intraparietal Sulcus activity during a head unrestrained, memory guided reach task

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The purpose of the current study is to investigate how local field potential (LFP) activity along the mid-posterior intraparietal sulcus (IPs) is modulated by visual landmarks before and during reaches to remembered visual targets. We recorded both action potentials and LFPs using 32-channel neural probes in one female Rhesus monkey. A landmark (four identical dots positioned at the vertices of a virtual square) was displayed at one of fifteen locations within reach on a touch screen. A visual target then appears, either within or outside of the landmark square, followed by a visual mask. After the mask disappeared, the landmark reappeared either at the same location (stable landmark condition) or shifted by 8 degrees in one of eight directions (landmark shift condition). Gaze and head position were allowed to move freely, and the animal was rewarded for reaching within 4.7 cm of the target. In the 'no-landmark' control trials, the procedure was the same, but the landmark is not presented. In parallel array recordings from posterior ventrolateral prefrontal cortex, LFP activity displayed a decrease in delta band power during the memory-delay phase and an increase in both delta and theta power during the planning and execution of the reaching movement (Lin et al., CAN-ACN 2025). Preliminary analysis of the current IPS LFP dataset suggests a decrease in beta band power that is time-locked to the reaching movement. Additionally, there appears to be an increase in delta and theta band power before reward in the landmark task conditions compared to no-landmark controls.

P3-D-145 - Motor learning deficits in a mouse model of Fragile X Syndrome

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Fragile X Syndrome (FXS), the most prevalent inherited intellectual disability and monogenic cause of autism spectrum disorder (ASD), causes an array of deficits including impaired motor learning and coordination. Despite its impact on quality of life, how different parameters of movement learning evolve during skill acquisition, and the underlying neural circuit mechanisms, remain incompletely understood. To investigate this, we trained C57BL/6 wild-type (WT) mice and a mouse model of FXS (Fmr1 KO) of both sexes to perform a skilled forelimb reaching task. Fmr1 KO mice exhibited a learning deficit, which manifested as lower overall success rates and differences in detailed reach-types. To elucidate these reach-type differences more precisely, we automated the task with a motorized door cue synchronized with high-speed video recordings to track paw movements using DeepLabCut. Remarkably, Fmr1 KO mice overall achieved success rates similar to WT mice in the forelimb reaching task when the door cue was present, however, reach trajectories between WT and Fmr1 KO mice were different. This suggests that the learning deficit can be alleviated with the right cues to aid learning, and that Fmr1 KO mice may have developed alternate strategies to achieve success. These findings raise important questions about the cellular and circuit-level mechanisms underlying the observed learning improvements and differences. Exploring these mechanisms may provide the foundational knowledge needed to develop targeted treatments for FXS and similar conditions.

P3-D-146 - Profiling the transcriptomic and epigenomic features of human auditory neurons and glial cells to advance reprogramming strategies

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Introduction. Primary auditory neurons (PANs) conduct sound information from the inner ear to the brain. Damage to PANs leads to irreversible degeneration and permanent hearing loss that

currently available treatments cannot reverse. One regenerative strategy involves reprogramming glial cells, which surround PANs and survive neurodegeneration, into functional neurons. However, little is known about PANs and glial cells in the human inner ear, limiting potential reprogramming approaches. Therefore, to characterize the molecular underpinnings of human PANs and glial cells, we performed single-nucleus transcriptomic and epigenomic sequencing. Methods. Human fetal inner ear ganglia were collected at gestational age week 18 (n=4), processed following 10X Genomics' single nucleus sequencing, and bioinformatically analyzed. Results. We found that PANs and glial cells comprise novel molecular subtypes defined by unique gene expression and epigenomic features. Though PANs express mature gene markers, glial cells express genes associated with early-stage development. Discussion. Our study comprehensively profiled the transcriptomic and epigenomic signatures of PANs and glial cells in the human inner ear. We resolve the key gene regulatory networks that must be reprogrammed to convert glial cells into PANs. Thus, our study will advance regenerative strategies capable of reversing neurodegeneration and restoring hearing.

P3-D-147 - Investigating the role of attention in speech motor control

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Speech is a critical aspect of human communication, with speech production involving many complex cognitive and motor control systems. Importantly, auditory feedback is used in speech production to allow for the monitoring and regulation of errors. Previous research suggests that attention is important to our ability to use auditory feedback to correct errors. In light of this, studies have suggested that by dividing a participant's attention, they produce smaller compensatory responses on a frequency-altered feedback (FAF) paradigm and, therefore, are less able to correct for errors in their voice (Tumber et al., 2014; Scheerer et al., 2016). This study aims to explore the role of natural attentional abilities on auditory feedback using an FAF paradigm. A sample of participants with Attention Deficit Hyperactivity Disorder (ADHD) and a sample without ADHD, will complete both an attentional task and a FAF task. The sustained attention to response task (SART) will be administered to measure attention. In the FAF task, participants will complete 100 vocalizations, with 34 vocalizations being shifted up +100 cents and 34 vocalizations being shifted down -100 cents. Within each of these vocalizations, the voice is perturbed 3 times for 200 ms. The researchers hypothesize that participants with lower attentional abilities will compensate less compared to those with greater attentional abilities. This project expands the current understanding of how attention influences speech-motor control and the use of auditory feedback.

E - HOMEOSTATIC AND NEUROENDOCRINE SYSTEMS

P3-E-148 - The medial prefrontal cortex and the rapid effects of estradiol on cognition in male mice

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Sex hormones are involved in regulating cognition via rapid or genomic effects, where the present study investigates their rapid effects. The medial prefrontal cortex (mPFC) mediates higher order cognitive processes and in females the estrogen 17b-estradiol (E2) in the mPFC seems to rapidly modulate short-term social but not non-social cognition. A study in female mice found that when E2 was infused into the mPFC, two social cognitive processes were facilitated (social recognition (SR) and social learning (SL)), however two non-social cognitive processes were not (object recognition (OR) and object placement (OP)). Males have high levels of circulating testosterone that is often converted to E2 by the aromatase enzyme in the brain and thus elicits effects through estrogen receptors. The present study investigates the rapid effects of E2 in the mPFC of male mice on short-term cognition where it was hypothesized that E2 would facilitate social but not non-social cognition. Following castration and cannulation surgeries, mice receive an E2 infusion into the mPFC, targeting the prelimbic and infralimbic subregions, and perform in one of SR, SL, OR or OP paradigms. Interestingly, preliminary results are showing facilitative effects of E2 infusion in SR, as well as the OR and OP paradigms which in females were not facilitated by E2. These results indicate a potential sex difference in the mPFC's regulation of cognition by E2. Future analyses will be assessing whether the facilitation of cognition by E2 in the mPFC depend upon the specific mPFC subregion targeted by the infusion.

P3-E-149 - Homeostatic plasticity during habituation to predictable stress

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Encountering a stressor immediately activates the hypothalamic-pituitary-adrenal (HPA) axis, but constantly mounting full-blown stress response under chronic stress can be detrimental. Indeed, this stereotypic stress response also undergoes experience-dependent adaptation (habituation) to a learned, familiar stressor (habituation) and not due to a generalized hypofunction of the systems involved in stress response. Using patch-clamp electrophysiology, we recently showed that the habituation of the HPA axis in mice is accompanied by hypo-excitability (a decrease in the intrinsic excitability) of output neurons of the HPA axis [corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus of the hypothalamus (PVN)]. While this plasticity can partially explain the habituation of the HPA axis, it also points to the existence of additional homeostatic plasticity to maintain the long-term activity levels of CRHPVN neurons (and hence the HPA axis function) within a normal range and avoid generalized hypofunction of the HPA axis.Here, we replicated the decrease in the intrinsic excitability of CRHPVN neurons (neuronal correlation of habituation); we also show compensating mechanisms involving an increase in the frequency of sEPSCs with no change in amplitude and a decrease in the amplitude of sIPSCs with no change in frequency of inhibitory input on CRHPVN neurons. These synaptic changes partially compensate for the hypo-excitability of the neurons as a stabilizing mechanism to maintain the rest of the HPA axis activity in its normal range during habituation.

P3-E-150 - Endothelin-3 enhances membrane expression of NaX channels and response to hypernatremia in rat magnocellular vasopressin cells of the supraoptic nucleus

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Sodium homeostasis is an important process for proper cellular function. At the central level, sodium detection occurs in the circumventricular organs —structures lacking a blood-brain barrier— as well as in the magnocellular neurosecretory cells (MNCs) of the supraoptic (SON) and paraventricular nuclei (PVN). It has been found that various peptides and channels, including endothelins and NaX channels, play a role in this dynamic process. There are three types of endothelins (ET-1, ET-2, and ET-3), each expressed in different organs, such as the vascular endothelium, brain, and kidneys. They are important for several physiological functions like neural crest development, cell proliferation, sodium excretion, salt homeostasis and regulation of vascular tone. These functions are mediated through ETA and ETB receptors, which are part of the G-Protein Coupled Receptor family. Previous studies have demonstrated that the activation of ETBR by ET-3 in SFO cells induces sodium entry, presumably through NaX channels. NaX is a voltage-independent sodium channel that is insensitive to TTX blockade. It has been shown that NaX channels are expressed in both VP and OT magnocellular cells of the SON, where they play a crucial role as detectors of extracellular sodium concentration. However, modulation of the NaX channel via ETBR has not yet been reported in this system. Our preliminary data indicates that activation of ETBR by ET-3 enhances the sodium detection response by increasing the expression of membrane NaX channels.

P3-E-151 - Light-evoked release of melanin-concentrating hormone in the lateral septum

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Melanin-concentrating hormone (MCH) is an orexigenic neuropeptide produced in the lateral hypothalamus, but the brain regions underlying orexigenic MCH actions are poorly defined. MCH can be released into the ventricles to target widespread regions, but MCH neurons also densely innervate the lateral septum (LS), an anorectic region expressing the MCH receptor MCHR1. MCH directly inhibits LS neurons, and intra-LS MCH infusion increases feeding. However, it is not known whether MCH is released in the LS, and as MCH neurons are glutamatergic, potential opposing effects of MCH and glutamate at their targets must be considered. We transduced Mch-cre neurons with a Cre-dependent virus encoding channelrhodopsin (ChR2) and photostimulated ChR2-expressing fibers in the LS to elicit glutamate and/or MCH release. Dorsally in the LS, blue light pulses (5 ms) evoked monosynaptic glutamatergic events, but these cells seldom responded to puff-applied MCH. Ventrolaterally, MCH-sensitive LS cells hyperpolarized by a MCH puff were more common and may also be innervated by monosynaptic glutamate release from Mch-cre terminals. Photostimulating Mch-cre fibers at MCH-sensitive LS cells with a 1 s, 10-Hz light pulse train (0.3 Hz) for 5 min elicited a MCHR1-dependent membrane hyperpolarization that reversed but persisted past the photostimulation period. Together, these findings indicated that glutamate and MCH release from Mch-cre terminals primarily innervate distinct LS subpopulations and may provide excitatory and inhibitory regulation to encode rapid and prolonged LS-mediated behaviors.

P3-E-152 - Brain-derived neurotrophic factor-induced proteostasis deficit

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Objectives: Neuropeptides (NPs) are small neuromodulatory proteins that are produced on demand in the endoplasmic reticulum (ER) and secreted either constitutively or in an activity-dependent manner to regulate brain function. Post-mortem transcriptomic analysis on young vs. old human brains showed that expression of NPs, including brain-derived neurotrophic factor (BDNF), is disproportionately decreased during aging and in neuropsychiatric disorders. However, the molecular and cellular mechanisms underlying reduced NPs remain elusive. We hypothesized that this vulnerability may originate in the ER, where excess demand on NP synthesis causes ER stress, as in brain disorders. This study aims to determine the contribution of ER stress as a mechanistic origin of cell type-selective vulnerability that may underpin emotional and cognitive deficits universally seen across neuropsychiatric conditions. Methods: Using adeno-associated virus encoding BDNF precursor (preproBDNF), we modelled this excess demand on BDNF by inducing various forms of BDNF affecting ER processing in selective neuron subtypes and evaluated ER stress levels via immunofluorescence (IF) with ER stress markers (p-eIF2α) and behavioral outcomes. Results: Forced expression of preproBDNF induced ER stress (IF) and working memory deficits (Y-maze). Conclusions: BDNF precursor overexpression can exceed ER processing capacity, leading to ER stress-mediated proteostasis deficit and protein aggregation. Ongoing proteomic and transcriptomic studies are underway to further elucidate the underlying mechanisms.

P3-E-153 - Melanin-concentrating hormone inhibited mania-like hyperactivity at the VTA

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There is considerable patient overlap in anorexia nervosa and bipolar disorders. Anorexia and mania in bipolar disorder are related to hyperdopaminergic states via the mesolimbic dopamine (DA) system. The ventral tegmental area (VTA) is a major DA source, but it is a heterogeneous region that also comprises GABA and glutamate cells. Upstream VTA-GABA can inhibit VTA-DA cells, thus loss of VTA-GABA cells leads to a hyperdopaminergic state and elicits mania-like endophenotypes like hyperactivity seen in anorexia. Melanin concentrating hormone (MCH) is linked with anorexia and is a vital regulator of energy homeostasis. MCH inhibits VTA-DA cells and suppresses DA release, thus loss of MCH enhances DA tone and drives hyperactivity. In addition, MCH also inhibits VTA-GABA cells, which may disinhibit VTA-DA cells and recover DA tone. However, it is not known if inhibitory MCH signaling can block DA-mediated hyperactivity at the VTA. We delivered a Cre-dependent virus encoding CRISPR Cas9-Slc32a1 at Vgat-cre VTA cells, which deleted Vgat at 75% of VTA-GABA cells transduced. Compared to Cas9-treated WT mice, Vgat deletion induced a 3-fold greater mania-like hyperactivity that was enhanced by blocking DA reuptake. Intra-VTA MCH infusion via a bilateral guide cannula implanted at the VTA suppressed WT locomotor activity and suppressed hyperactivity seen with VTA-Vgat deletion. Our findings suggested that MCH regulates DA release by direct and indirect GABAergic mechanisms in the VTA and implicated its therapeutic potential for psychiatric disorders related to hyperdopaminergia.

P3-E-154 - Understanding hypothalamic circuits regulating the stress response

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Stress necessitates an immediate boost in physiological functions from their homeostatic operation to an elevated emergency state. Here, we report that corticotropin releasing hormone (CRH) neurons in the paraventricular nucleus of the hypothalamus (PVN), the effector neurons of the hormonal stress response, transition between distinct low- and high-activity states through recurrent inhibition.Specifically, in vivo optrode recordings show that under non-stress conditions, a subset of CRH neurons fire with brief rhythmic bursts (RB), constraining the firing rate due to long inter-burst intervals. Various stressors – including sciatic nerve stimulation, inflammatory signaling by prostaglandin E2, and air puff – rapidly switch RB to continuous single spiking (SS), permitting a large increase in firing rate. Simultaneous recordings revealed that CRH neurons transition firing states together, pointing to a common underlying network. Notably, in awake head-fixed mice, locomotor activity correlated with shifts to the high activity state.Network modeling showed that recurrent inhibition can control this activity-state switch. Moreover, in vivo pharmacological and chemogenetic manipulations of GABAergic inputs to the PVN produced model-predicted switches in activity states. In search for the anatomical substrate for the recurrent circuits, axonal projection targets from CRH neurons were systematically characterized using anterograde tracing of synaptophysin-mRFP expressed in CRH neurons. Together, we present a network mechanism for state-dependent activity dynamics in CRH neurons.

P3-E-155 - Elucidating state-dependent activity dynamics of hypothalamic stress output neurons using spiking network models and dynamic clamp

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Corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus of the hypothalamus (PVN) are critical regulators of the neuroendocrine stress response. Using in vivo single-unit recordings, we recently reported that CRHPVN neurons transition between low- and high-activity states, each with distinct firing patterns. Spiking network models predicted that recurrent inhibitory circuits between CRHPVN and GABAergic neurons govern this state transition. Here, we investigate how these state-dependent activity dynamics influence single-neuron computation in CRHPVN neurons, by using tight combinations of experimental and computational approaches. First, we built a data-driven, spiking network model of the CRHPVN-GABA microcircuit. Under the low activity state, fluctuating synaptic inputs from the recurrent inhibitory network dampen the gain of individual CRHPVN neurons and constrain their overall firing rate. By contrast, release from recurrent inhibition dramatically changed firing pattern and increased the gain, revealing a novel state-dependent mechanism for neural gain control. More broadly, the strength of recurrent inhibition provided an effective circuit mechanism for graded control of gain at both the single-neuron and network levels. To test these model predictions, we developed a novel dynamic clamp approach that connected ex vivo CRHPVN neurons with an in silico circuit model. Ex vivo CRHPVN neurons exhibited in vivo-like state-dependent shifts in both firing patterns and gain. In summary, we identified a novel neuron-to-network mechanism that underlies state-dependent activity dynamics at the effector neurons of hormonal stress response, enabling rapid and graded control of their activity during stress. This mechanism provides new insights into how stress-responsive circuits flexibly regulate neuroendocrine outputs.

P3-E-156 - Colonic butyrate lowers hepatic glucose production via FFAR2-GLP-1-vagal afferent neuronal signaling

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Hyperglycemia is partially due to increased hepatic glucose production (HGP). Our lab has demonstrated HGP is regulated by the gut microbiota which is a known contributor to host glucose homeostasis. The gut microbiota alters ingested dietary components, generating metabolites such as short chain fatty acids (SCFAs), which are produced in the large intestine from bacterial fermentation of dietary fiber. Our lab has found that dietary fiber supplementation improves glucose homeostasis in western diet-fed mice only if the fiber increases the production of the SCFA butyrate. Butyrate binds to free fatty acid receptor 2 (FFAR2) on enteroendocrine cells to induce gut peptide release, and gut peptides regulate HGP via a gut-brain-liver neuronal axis. Therefore, we hypothesized that endogenous butyrate lowers HGP via gut peptide neuronal signaling. First, colonic infusion of saline or 10mM butyrate during a basal insulin euglycemic clamp in rats showed that colonic butyrate reduced HGP and increased portal GLP-1 concentration compared to saline infusion. Co-administration of a GLP-1 receptor antagonist abolished the effects of colonic butyrate on HGP. Additionally, vagal afferent GLP-1R knockdown abolished the HGP-lowering effects of colonic butyrate. Lastly, blocking FFAR2 signaling through co-administration of a FFAR2 antagonist or with colon FFAR2 lentiviral knockdown abolished the HGP-lowering effect of colonic butyrate. Together, these studies are the first to identify how endogenous butyrate signaling impacts HGP via a FFAR2-GLP-1-vagal afferent axis.

P3-E-157 - Role of voltage gated Na+ channel Nav1.7 in regulating electrical activity of rat subfornical organ neurons

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The subfornical organ (SFO) is a sensory circumventricular organ that plays a key role in regulation of homeostasis. Previous studies demonstrate that SFO neurons show heterogeneity in current expression and spiking behavior, leading to two predominant spiking phenotypes: tonic and burst firing. Electrophysiology studies, supported by Hodgkin-Huxley single compartment models support the notion that unique properties of voltage gated Na+ current play a key role in determining electrical phenotype. Interestingly, transcriptomic studies show that SFO neurons express a unique complement of voltage gated Na+ channels, including Nav1.3 and Nav1.7, in addition to the commonly expressed Nav1.1, Nav1.2 and Nav1.6 isoforms. In this study, we used patch clamp electrophysiology to examine the properties of voltage gated Na+ currents in acutely dissociated SFO neurons, and investigate the contribution of Nav1.7 to action potential activity. Blocking Nav1.7 with the selective toxin ProTxII (20nM) caused 16.2+/- 3.1% decrease in peak transient Na+ current and a 5.13+/- 2.01 mV depolarizing shift in voltage dependence of activation, indicating that Nav1.7 has a relatively hyperpolarized voltage dependence of activation. ProToxII also caused a 24.7+/-2.5% reduction in persistent Na+ current, suggesting Nav1.7 contributes proportionally more to persistent current than other isoforms. ProTxII also alters rates and patterns of action potentials in SFO neurons, indicating that Nav1.7 plays a critical role in determining the electrophysiological properties of SFO neurons.

P3-E-158 - Connecting specific central GLP-1 receptors functionally with glucose homeostasis and energy balance

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Background: The central nervous system regulates metabolism, maintaining energy and glucose homeostasis. Glucagon-like peptide 1 (GLP-1), encoded by the proglucagon (Gcg) gene, is a key neuropeptide produced by NTS neurons, modulating appetite and feeding behavior. While GLP-1's systemic roles are known, its central mechanisms, particularly in feeding and glucose control, remain unclear. Arcuate nucleus of the hypothalamus (ARC), a critical hypothalamic region, receives dense Gcg projections, suggesting GLP-1 signaling in the ARC may regulate energy and glucose balance.This study focuses on investigating the specific role of GLP-1 signaling in the ARC, aiming to elucidate its effects on energy and glucose homeostasis.Objectives: Examine the effects of ARC GLP-1R neuronal activation/inhibition on feeding and to determine their role in glucose homeostasis, insulin signaling, and secretion.Methods: Chemogenetic activation/inhibition of ARC GLP-1R neurons was achieved via Cre-dependent DREADDs in GLP-1R-Cre mice. Food intake, glucose tolerance, and insulin secretion were assessed following ligand administration.Results: Activation suppressed food intake, while inhibition increased it, confirming an anorexigenic role. Inhibition also led to glucose intolerance and impaired insulin signaling. Surprisingly, activation enhanced insulin secretion without altering blood glucose.Conclusions: ARC GLP-1R neurons regulate appetite and glucose homeostasis. While acute activation boosts insulin secretion, chronic effects on glucose regulation require further study.

F - COGNITION, EMOTION AND MOTIVATION

P3-F-159 - Developing a novel virtual reality assessment to quantify navigational impairments in aging and early Alzheimer's disease

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Spatial navigation impairments are among the earliest cognitive deficits observed in Alzheimer's disease (AD), preceding symptoms such as memory loss. However, existing spatial navigation tests are often limited in ecological validity and fail to effectively distinguish between egocentric (self-referenced) and allocentric (environment-referenced) navigation. To address these limitations we developed a novel virtual reality (VR) task designed to quantify age- and disease-related changes in navigation abilities and identify potential behavioural biomarkers for cognitive impairment. In this immersive VR task, participants must keep track of their starting position and two distant landmarks as they traverse along paths of increasing complexity in a naturalistic 3D environment. Navigation performance is assessed using pointing tasks with three primary metrics: egocentric error, allocentric error, and allocentric consistency. Data collection is currently ongoing with cognitively healthy young and older adults to establish baseline measures during normative aging. In the next phase, we will recruit individuals with early AD to examine and compare navigational impairments in this population. We hypothesize that older adults will show

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increased allocentric deficits compared to young adults, with additional egocentric impairments in the AD population. The resulting data will be analyzed to estimate patterns indicative of AD-related cognitive impairment. The ultimate goal is the development of a sensitive, navigation-based behavioural biomarker to facilitate early AD detection.

P3-F-160 - Categorization with automated touchscreens (CAT) task: A novel operant paradigm for studying visual category learning in mice

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Visual object categorization is a fundamental cognitive process, but the neural mechanisms underlying category learning remain largely unexplored. Mice, despite having poor visual acuity, can use visual features for complex object-based tasks. To determine if mice are capable of visual object categorization, we developed an operant Categorization with Automated Touchscreens (CAT) task. For the CAT task, 24 young adult male C57BL/6J mice learned to discriminate between images from two object categories (e.g., scissors, locks). Mice were first presented in the centre of the touchscreen with a sample image from one of 6 exemplars of a category. After a nosepoke to the sample, two choice images appeared on either side: one matching the sample's category and one from a different category. Selecting the matching image earned a milkshake reward. Mice reached a performance criterion of 70% accuracy for 3 consecutive days in ~35 days. Critically, mice generalized category learning to novel exemplars; over 12 days of training with new images, accuracy on the task was greater with new images of objects from familiar categories than with those from an unfamiliar category. Further testing on a visual pairwise discrimination task confirmed that mice could distinguish between two images from the same category; mice achieved a criterion of 80% accuracy for 2 consecutive days after ~25 days. The CAT task is a novel tool to explore the neurobiological mechanisms underlying visual category learning and further our understanding of how perceptual systems process complex visual information.

P3-F-161 - Altered neuronal lactate dehydrogenase a expression in mice results in changes to spatial memory and lipid droplet formation depending on sex and age

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The cellular mechanisms that underly learning and memory consolidation in the brain are bioenergetically demanding. With the decline in neuronal glucose utilization that occurs with age, the brain relies on alternate metabolites such as lactate and lipids as fuel sources to meet energy demands. The improper processing of these metabolites may underlie memory deficits typically observed in later life. Lactate production in neurons is primarily governed by lactate dehydrogenase-A (LDHA) activity, the rate-limiting enzyme that catalyzes pyruvate to lactate conversion. In the present study, the effects of neuronal-specific ldha induction and knockout on learning and memory was examined. The Morris water maze and object location task were used to test spatial learning and memory in induction and knockout mice, respectively. Neuronal ldha induction mice exhibited reduced spatial memory in males compared to age-matched controls. In contrast, memory was improved in old ldha knockout mice compared to age-matched controls. Brain sections were collected to assess changes in lipid dynamics using immunofluorescence microscopy. Levels of perilipin2, which plays an important role in

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maintaining droplet integrity to prevent free-fatty acid toxicity, was reduced in certain hippocampal subregions within neurons of induction mice, but increased in knockout mice compared to controls. Overall, the present study suggests that excess neuronal-specific LDHA accumulation is cognitively detrimental in aged mice, and these findings could be attributed to aberrant lipid processing and metabolism.

P3-F-162 - Preference for a familiar in pain: Opioid dependent behaviour

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Pain perception and consoling behaviours toward distressed individuals are profoundly influenced by social interactions. However, the extent to which familiarity with the individual in pain shapes the behavioural responses to a conspecific in pain and the neural mechanisms underlying these behaviours, remains unclear. To investigate this, we used a social affective preference task to assess approach and avoidance behaviour toward another mouse in pain. Female mice exhibited a preference for interacting with a familiar mouse in pain over a familiar mouse not in pain, whereas males showed no such preference. When exposed solely to a mouse in pain, both sexes displayed increased interaction time with a sibling in pain compared to not in pain. This preference for familiar individuals in pain was mediated by the opioid system, as several opioid antagonists blocked the social approach toward pain in female siblings. These findings underscore the importance of familiarity on social responses to pain and suggest a critical role for opioid receptors in modulating these behaviors. Understanding the interplay between social behavior, pain, and relationship dynamics may offer insights into the neural basis of empathy and the social modulation of pain

P3-F-163 - Sex-specific variations in CB1 receptor expression in the prefrontal cortex and ventral tegmental area following adolescent cannabinoid administration in the nucleus accumbens

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Cannabis is one of the most widely abused substances globally, particularly affecting adolescents during critical periods of neural development. Excessive cannabinoid exposure during adolescence may disrupt the maturation and expression of the endocannabinoid system, particularly within brain regions involved with decision-making and reward processing, such as the prefrontal cortex (PFC) and the ventral tegmental area (VTA). This altered receptor expression has been associated with behavioural complications, supporting the hypothesis that disruptions in the endocannabinoid system during critical developmental periods can have profound effects on neurobiological circuits. This study investigates the effects of repeated intracerebral injections of a CB1 receptor agonist (WIN55, 212-2), mimicking the THC component of cannabis, into the nucleus accumbens shell (NAs) on the VTA and PFC of adolescent rats. Thirty-six (N = 18 per sex) adolescent male and female Wistar rats were administered either five microinjections of WIN55, 212-2 (1uL at 5mg/mL in DMSO; i.c.) or the vehicle (1uL of DMSO) into the NAs over a 10-day period. Animals were euthanized three weeks post-injection, and selected brain sections were cut and stained through immunohistochemistry to quantify the prevalence of CB1 receptor (CB1R) expression in the PFC and the VTA. Results demonstrate a significant sex difference in

CB1R density within the VTA, with females exhibiting higher CB1 expression levels than males. However, neither sex nor WIN55,212-2 exposure significantly affected CB1R fluorescence in the VTA or the PFC. These findings highlight crucial considerations regarding sex differences in cannabinoid receptor expression and underline the impact of cannabinoid exposure during critical neurodevelopmental periods.

P3-F-164 - Learning of an integral 2D auditory task requires the orbitofrontal cortex

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This experiment seeks to elucidate the role of the orbitofrontal cortex (OFC) in facilitating integral representation of an auditory space. To test rats' ability to learn the stimulus dimensions as interdependent, we trained 20 rats on a Pavlovian task in which two sets of auditory cues were configured to represent a 2D-space. The stimuli consisted of 12 distinct tone frequencies and 12 distinct click frequencies, which were combined for 144 compound auditory stimuli. Two of the auditory compound cues were paired with a saccharine reward. We sought to determine whether temporary DREADD-induced inactivation of the OFC across the learning phase of the task would inhibit associations between the compound stimuli and saccharine. We injected AAV8- CaMKIIa-hM4Di-mCherry (experimental, n=11) or AAV8-CaMKIIa-mCherry (control, n=9) bilaterally into the OFC 4 weeks before the experiment. To learn the stimulus set, rats were exposed to "random-walk" paths through the auditory space, such that they would listen to a progression of cues with a particular transition structure. Rats underwent 34 days of training, with DREADD Agonist 21 and saline injections occurring through the first 16 days. Injections were counterbalanced across experimental groups and extinction tests. Learning was assessed through reward anticipation and performance on two extinction tests (days 17 and 34). Findings suggested that OFC inactivation may inhibit integral learning, thereby reducing reward prediction accuracy and delaying acquisition in post-inactivation trials.

P3-F-165 - Neural substrates of working memory capacity limitations in the prefrontal cortex of the freely moving Marmoset

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Working memory (WM) is a crucial cognitive function that supports the retention and manipulation of information necessary for goal-directed tasks. This capacity is inherently limited, with constraints varying across species and sensory modalities. We trained two common marmosets on a touchscreen-based delayed non-match-to-position task to investigate these limitations. During each trial, one to four stimuli were successively presented on the screen, and in each iteration, the subject had to select the stimulus at the novel location. Performance declined as stimuli increased, with errors occurring more frequently when the novel stimulus appeared on the same side as a previously presented stimulus, consistent with prior findings on visuospatial attention and WM capacity.To explore the neural basis of this behavior, two marmosets were implanted with multi-shank electrode arrays targeting the lateral prefrontal cortex (area 46). Neural responses were recorded using a wireless system. Prefrontal neurons exhibited significant modulation during three task epochs: pre-touch, post-touch, and post-reward. Neurons tuned to specific quadrants of the screen were responsive across memory loads, with these selective neurons distributed across the array. This suggests that spatial

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information is maintained through distributed prefrontal activity rather than localized retinotopic organization. Moreover, the observed firing rate modulations across memory loads suggest a dynamic updating of spatial representations, potentially contributing to memory load-dependent performance limitations.

P3-F-166 - Neural substrates of 17ß-estradiol-induced potentiation of cocaine-primed reinstatement in ovariectomized female rats

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Background: Female rats are more sensitive to cocaine-primed reinstatement than males and this effect is driven by the action of the steroid hormone 17β-estradiol (E2) in females. Gross-scale study of the substrates of this effect may aid in the development of pharmacotherapies for cocaine use disorder (CUD) in humans. Methods: Female rats (N=14) were ovariectomized, implanted with indwelling jugular catheters, and trained to self-administer cocaine intravenously on a fixed-ratio 1 schedule over 24 daily, 2h-long sessions. Then the reinforcer was replaced with saline and cocaine seeking was extinguished over 22 daily sessions. Following extinction, rats were treated systemically with 10μg/kg E2 (n=7) or vehicle (n=7) 22h and 1h before a 90-min reinstatement session under extinction conditions. Reinstatement was primed by 10mg/kg cocaine. Following reinstatement, rats were perfused and the immediate early gene protein c-Fos was quantified as a marker of neuronal activation in the cortico-striatal circuitry underlying reinstatement using immunohistochemistry. Results: E2 potentiated cocaine-primed reinstatement and increased c-Fos+ cell counts in the striatum and nucleus accumbens core and shell. E2 increased functional connectivity between various pairs of regions, notably including the habenula and nucleus accumbens core. Conclusions: E2 may increase relapse risk by activating mesolimbic circuitry. The efficacy of pharmacotherapies for CUD that target this system should be evaluated across a range of hormonal milieux.

P3-F-167 - Repeated exposure to nicotine and cigarette smoke extract produce age dependent effects on spatial memory and compulsive-like behaviour in female rats

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Introduction: Tobacco product use has become more prevalent in recent years, increasing the risk of neurological impairment. Previous studies have found that smoking during adolescence leads to deficits in cognition that extend into adulthood and mixed results have been reported when use starts after adolescence. Methods: We exposed adolescent and adult female rats to saline, nicotine, cigarette smoke extract (CSE), or DMSO (vehicle) via 28 daily subcutaneous injections. After exposure, rats performed the marble burying task to assess compulsive-like behavior and object-in-place tasks to assess spatial memory. Results: Preliminary data show that repeated exposure to all non-saline substances increases marble burying compared to saline controls in adolescent and adult female rats; adolescents exposed to nicotine or vehicle show enhanced marble burying compared to adults, suggesting that repeated exposure during adolescence may increase compulsivity. Nicotine exposed adults and adolescents exhibit

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improved short-term memory and deficits in long-term memory; CSE exposed adults exhibit improved long-term memory and adolescents exhibit improved short- and long-term memory. Conclusions: This suggests that CSE and nicotine have differing effects on spatial memory that depend on age and retention delay length and indicates that the influence of other constituent compounds of tobacco should be considered when assessing and designing nicotine-focused research.

P3-F-168 - Muscarinic modulation of parvalbumin neurons in attention

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Cognitive impairments, especially attention deficits, are a hallmark of neuropsychiatric disorders. These deficits greatly affect daily life yet remain poorly treated. Understanding the neural mechanisms of attention is crucial for developing effective therapies. Cognition relies on the balance between excitatory and inhibitory neurotransmission, particularly in the medial prefrontal cortex (mPFC), where parvalbumin (PV)-expressing neurons play a key role. Acetylcholine also modulates cognition via muscarinic acetylcholine receptor subtype 1 (M1-mAChR), which influences PV neuron activity. The goal of this study is to investigate how the modulation of PV neurons by M1-mAChR contributes to attention. We used in vivo fibre photometry with a genetically encoded Ca2+ biosensor to monitor PV neuron activity during a highly translational touchscreen rodent continuous performance task (rCPT). In this task, mice responded to target images for a milkshake reward while withholding responses to non-targets. To examine M1-mAChR effects, we locally injected the antagonist telenzepine into the mPFC before rCPT. Our results revealed that telenzepine impaired attentional performance on the rCPT and disrupted the recruitment of PV neurons necessary for detecting and responding to relevant visual stimuli, suggesting M1-mAChR signalling is essential for the task-dependent activation of PV neurons during attention. Given that attention deficits are poorly treated in neuropsychiatric disorders, these findings offer insights into potential therapeutic strategies.

P3-F-169 - Chronic stress-induced alterations to the activation of new neurons during negative cognitive bias

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Major depressive disorder (MDD) is a debilitating illness that affects 2x more females than males. MDD is associated with decreased neurogenesis and aberrant neural activity in the dorsal and ventral hippocampus (dHPC&vHPC). Negative cognitive bias (NCB), or the interpretation of ambiguous stimuli as negative, is a treatment-resistant cognitive symptom of MDD. Chronic unpredictable stress (CUS) induces depressive-like endophenotypes in rodents of both sexes. The aim of this study is to investigate sex-specific, CUS-induced alterations to neurogenesis and the activation of new neurons during negative cognitive bias.We exposed young adult male and female rats to 21d of CUS or no-CUS then administered the NCB task developed in the lab. To model cognitive bias, we train rats to discriminate between a shocked and non-shocked context. We then place them in an ambiguous context with a mix of cues from the shocked and non-

shocked contexts where high freezing indicates NCB. We stained HPC sections for BrdU (injected 4 weeks before sacrifice) as a 4-week-old cell marker, doublecortin (DCX, expressed in neurons up to 2 weeks old) as a 2-week-old cell marker, and cFos (immediate early gene expressed after activation) as a marker of neural activity. We found that CUS increased NCB in both sexes and reduced the activation of 2-week-old neurons (DCX/cFos co-expression) in the vHPC, but not dHPC, of CUS-exposed males and females. These findings suggest that stress disrupts the integration of new neurons into the circuitry used to interpret the ambiguous context.

P3-F-170 - Sexually dimorphic neural activation and ultrasonic vocalizations in pair bonded prairie voles

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Prairie voles are a monogamous rodent model for studying social bonding, relying on sensory information, such as ultrasonic vocalizations (USVs) to maintain these bonds. However, the neural networks involved in processing vocal cues during social bonding are poorly understood. To explore regions of interest (ROIs) involved in pair bonding and vocal communication, we employed a whole brain imaging pipeline, iDISCO, across varied partner preference conditions (e.g. partner and/or stranger). Neural activity was assessed in over 800 ROIs using the immediate early active gene c-Fos. Twenty-five ROIs had significant changes in activation, encompassing regions involved in bonding (e.g. ventral basolateral amygdala) and vocal processing (e.g. subparafascicular nucleus). Sixteen ROIs correlated c-Fos activation with USV rates (e.g. dorsal nucleus raphe). Partner conditions correlated positively, however, the ROIs varied by sex, indicating a sex-specific neural circuit for affiliative behaviour. In stranger only conditions, male and female neural activation were inversely correlated in varied stress-related ROIs. Auditory parameters, such as duration, correlated to six ROIs (e.g. bed nuclei of the stria terminalis, dorsal nucleus). Male USV duration was negatively correlated to five ROIs spread across partner and partner/stranger conditions. Two ROIs were positively correlated in females, suggesting neural activation of auditory parameters is sex dependent. These findings suggest male and female prairie voles utilize vocalizations to drive distinct neural processes in social bonding.

P3-F-171 - Cerebellar neurodynamics shaped by the habenula during motor planning

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Motor planning enables animals to prepare and take desirable actions to maximize survival. While executing 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 sensory stimuli and internal brain states for motor planning. To investigate this, we have combined calcium imaging using light-field and two-photon microscopies, optogenetic manipulation, and an operant-conditioning task in larval zebrafish. We find the strongly correlated, directed pre-motor signals in the cerebellum and habenula. These pre-motor signals are evoked by sensory stimuli and gained through learning. Disrupting the pre-motor signal in the habenula affects both decision behaviors and signals in the cerebellum. In particular, optogenetic stimulation leads to rotation in the neural manifold in

the channelrhodopsin (CoChR) animals, while in control siblings the same 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 circuit. Moreover, the cerebellum can encode 'irrelevant' information orthogonally, highlighting a cognitive cerebellum.

P3-F-172 - Dissecting spatial and non-spatial memory encoding and retrieval in the lateral entorhinal cortex

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The lateral entorhinal cortex (LEC) is an essential intermediary hub within the hippocampal-neocortical memory network and contains cells that drastically change their activity patterns depending on the familiarity and structure of experiences (Pilkiw et al., 2017; Tsao et al., 2018). However, how these unique coding properties are related to the role of the LEC in memory encoding and retrieval remains unknown. We thus developed a memory task that allows for tracking LEC activity during memory encoding and retrieval within a day. Water-restricted mice underwent two daily epochs (E1, E2), during which they collected rewards in a square-shaped maze. In the cue learning session, a pair of identical visual cues indicated reward locations, and two other visual cues acted as distractors. In the spatial learning session, rewards were delivered at two of four fixed positions. The reward cues and the fixed positions remained the same across two epochs within a day but changed from day to day. In the cue learning sessions, the mice improved the task performance across days faster in E2 than E1. With the spatial learning sessions, the mice's task performance in E1 improved across days, and the performance remained high in E2. The across-epoch memory retention indicates that the mice became capable of switching between learning new information in E1 and retrieving that information in E2. We are currently recording the activity of LEC cells projecting to the dentate gyrus of the dorsal hippocampus during this task and will discuss the findings during the poster presentation.

P3-F-173 - Social isolation impairs learning by limiting the capacity for synaptic plasticity

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Recently, while conducting experiments on how social isolation causes learning impairments in Drosophila, we stumbled across an unexpected finding concerning the nature of impaired memory encoding. Isolated flies show behavioral impairments in learning ability on the aversive olfactory conditioning paradigm. We then used the under-the-microscope learning and in vivo imaging preparation in Drosophila to directly observe their aversive memory encoding in both MBON-g1pedc and MBON-g2a'1 compared with socially experienced controls. Based on the cellular theory for learning and memory, we expected to find an incomplete synaptic depression upon association of the conditioned stimulus and unconditioned stimulus in isolated flies, explaining their behavioural learning impairments, consistent with known learning and memory theory. Much to our surprise, this was not what we observed. The learning-induced synaptic depression was indistinguishable between control and socially isolated flies. Where they did differ, however, was in their pre-learning odour responses. The MBNs of socially experienced flies responded more strongly to odours than did isolated fly MBNs. This meant that the magnitude of

change created by learning-induced synaptic plasticity was higher in grouped flies compared to isolated flies. Consistent with the literature in long-term potentiation, we propose that it is this difference in the magnitude of synaptic plasticity that leads to better versus poorer memory encoding. However, unlike the current theories on memory encoding, our data suggests that differences in the magnitude of change can originate from differences in the initial responses of neurons to the conditioned stimulus, and not the strength of the encoding itself.

P3-F-174 - Lysergic acid diethylamide modulates hippocampal and cortical local field potential rhythms in male mice

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Lysergic acid diethylamide (LSD) is a promising therapeutic for psychiatric disorders, with clinical use dating back to the 1950s. The physiological effects of LSD on the nervous system remain elusive. In vivo electrophysiological data from male C57BL/6J mice suggests LSD modulates thalamic gamma-amino butyric acid (GABA) neuron networks. Clinical neurophysiology experiments have focused on spectral signatures of LSD using electroencephalography (EEG) and magnetoencephalography (MEG), indicating the clinical relevance of these signals. To date, no study has examined the spectral signatures using either method in freely moving mice. As well, no study has examined the physiological effects of LSD on the hippocampus.Here we present the first in vivo electrophysiological investigation of LSD's cortico-hippocampal physiological effects in freely-moving male C57BL/6J mice using intracranial EEG (iEEG) recordings. Following intraperitoneal (IP) administration of 30µg/kg LSD, there was a global decrease in power spectral density (PSD) signal power in both broadband and discrete narrow band oscillatory rhythms of the ventral hippocampus CA1 and CA3 regions. Similar but less robust effects were observed in the somatosensory and medial prefrontal cortices. These data confer with the existing clinical neurophysiology data. Lastly, LSD increased within-cohort PSD signal power variance, suggesting individual-specific effects, and lending further credibility to the entropic brain theory of psychedelic drug actions.

P3-F-175 - Dopamine overdrive and endocannabinoid tone: Dissecting sex-dependent mechanisms in fear adaptation

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Elevated dopamine (DA) signaling and altered endocannabinoid (eCB) tone are key factors in stress-related disorders. We examined DAT-heterozygous (DAT-HET) mice—which display a two-fold increase in extracellular DA versus wild type (WT) and mild regional alterations in anandamide tone—to investigate these interactions. Although DAT-HET mice are often viewed as vulnerable due to neurochemical dysregulation, our findings reveal a nuanced, sex-dependent response in fear adaptation. In a contextual fear conditioning paradigm, compared to DAT-HET males and WT males and females, only DAT-HET females showed reduced freezing at 24-hour recall. Further, a contextual fear generalization test revealed that, when first exposed to the aversive context, these females displayed superior context discrimination, suggesting adaptive resilience rather than cognitive deficits. To probe eCB signaling, we measured locomotor responses after administering a selective MAGL inhibitor that elevates 2-arachidonoylglycerol (2-AG) levels. Compared to WT, DAT-HET mice exhibited increased

locomotion, confirming a sensitivity to 2-AG modulation and suggesting downstream influence on DA activity. These results highlight the complex interplay between DA and eCB tone and underscore the role of sex in fear adaptation. Our findings challenge the notion of uniform vulnerability in high-DA models and point to the therapeutic potential of targeting eCB pathways to foster resilience in stress-related disorders. Future studies will delimit the molecular and circuit mechanisms underlying these sex-dependent effects.

P3-F-176 - Screening for sociability genes using tools from Drosophila neurogenetics

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Sociability, defined as individuals' tendencies to affiliate with conspecifics, is prevalent among animal species. Despite the importance of sociability for many animals including humans, we still have limited information of its natural genetic and neurobiological architecture. To address this knowledge gap, we artificially selected for sociability in fruit flies (Drosophila melanogaster) by running groups of 8 flies through a sociability assay with 8 connected chambers in which they could choose to join others or be alone. Using this assay, we selected either the most or least sociable flies to produce future generations of sociable and non-sociable lineages respectively. After 25 generations, we extracted DNA and RNA from the heads of sociable and non-sociable flies. Using a multi-omics approach, combining genome scans, genome-wide differential gene expression, and differential transcript usage analyses, we have identified over 300 candidate sociability genes. Next, we tested whether 35 of these candidate genes were causally implicated in sociability using RNA interference. We found that knocking down Sec5 had the greatest effect on sociability, reducing it by half (47±8%, P<0.001, n=144 replicates). Sec5 and its mammalian ortholog EXOC2 are critical for neurite growth and neuronal function. To determine whether Sec5/EXOC2 promotes sociability across species, we have generated Exoc2 knockout mice and are currently testing their sociability using established behavioural paradigms as well as a novel assay inspired by our fruit fly assay.

P3-F-177 - Abnormal awake replay events during planning disrupt spatial episodic-like memory

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Spatial navigation depends on hippocampal synchrony for encoding and recalling salient environmental information. In the rodent hippocampus, during awake immobility, location-specific "place cells" reactivate in "replay" sequences that may represent upcoming trajectories and support navigational planning. These synchronous reactivations are detectable via calcium imaging of large-scale neuronal populations as Synchronous Calcium Events (SCEs). Notably, replay has primarily been observed in highly familiar tasks, while its role during episodic-like learning remains underexplored.In our study, we examined the spatiotemporal properties and spatial information content of SCEs under conditions of successful versus impaired spatial learning. We employed a spatial reference memory task combined with freely behaving calcium imaging of CA1 principal neurons using a Miniscope. Mice were trained to navigate toward a rewarded target arm, and to disrupt learning, we manipulated their ability to localize the starting point of each trial.Interestingly, SCEs were most prominent during the initial learning phase. Unexpectedly, successful learning was associated not with replay but with broad activation of

the cognitive map, whereas impaired learning correlated with the replay of immediately preceding experiences. These findings suggest that CA1 reactivation of prior experiences during the planning phase of episodic-like memory may hinder the retrieval of task-relevant information necessary for optimal behavior. Overall, our results challenge the conventional view that hippocampal replay of specific experiences guides behavior in episodic-like conditions.

P3-F-178 - Pulvinar electric microstimulation enhances target detection and reshapes thalamo-cortical coupling during attention

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[1] Princeton University

Lesion studies show that pulvinar, the largest nucleus in the primate thalamus, is critical in attention function. Functional macaque studies have shown that pulvinar regulates information transfer between cortical areas. Other recent work has suggested that pulvino-cortical interactions are modulated by intrinsic theta rhythms: e.g., coupling between pulvinar and parietal cortex shifts rhythmically such that signaling emerges from pulvinar to cortex and vice versa. It remains unclear whether pulvino-originating signals are simply affected by brainwide oscillations or if pulvinar may be causally linked to their propagation. In this study, we simultaneously stimulated and recorded from local populations in the macaque pulvinar. To investigate the propagation of pulvinar-initiated signals and their putative effect on attention behavior, we recorded spiking and local field potential data from the lateral intraparietal area (LIP) and the dorsal aspect of inferotemporal cortex (PITd), two cortical areas that are implicated in attention control and are anatomically connected to pulvinar. We stimulated pulvinar using 50 µA biphasic cathode-leading 16 pulse trains (200 Hz) at varying delays prior to target presentation in a covert spatial attention task. Results show that pre-target pulvinar stimulation enhanced target detection and triggered a thalamo-cortical cascade, modulating thalamo-cortical and cortico-cortical spike-field coupling. These findings establish a causal role for the pulvinar in attention dynamics and suggest translational potential for attention disorders.

P3-F-179 - Neural mechanisms for multi-race face ensemble perception

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Recent work has shed light on the neural mechanisms underlying the perception of face ensembles (i.e., crowds or groups of faces). Yet, little is known about how multi-race ensembles are processed despite their theoretical significance (i.e., both ensemble perception and other-race perception involve a 'regression to the mean') and practical importance (e.g., the prevalence of multi-race groups at this conference). Accordingly, we used electroencephalography (EEG) in East Asian and White adults to assess neural responses elicited by: (i) single-race ensembles (groups of faces depicting only East Asian or only White females); (ii) multi-race ensembles (containing equal numbers from both races) and (iii) outlier ensembles (containing exactly one face of a different race). Neural decoding was conducted across EEG signals between 50-650ms after stimulus onset via temporally cumulative classification.Our analysis revealed that other-race (OR) ensembles (i.e., distinct groups of East Asian faces viewed by White participants and vice versa) can be decoded from each other even in the presence of an OR effect involving poorer recognition for OR than own-race faces. Second, multi-race ensemble perception was dominated by OR faces as revealed by lower decoding

accuracy relative to single-race OR ensembles. In contrast, outlier ensemble perception was dominated by the majority race, regardless of whether it matched the participant's race.Thus, the present findings shed light on the neural mechanisms for ensemble processing, other-race face perception and their interaction.

P3-F-180 - Chronic stress affects regional grey matter volume and cerebral blood flow in older adults with HIV

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[1] McGill University

Background: Despite effective antiretroviral treatment, brain changes persist in chronic HIV, with unclear contributing factors. Research has focused on biological mechanisms like inflammation, but psychosocial factors, such as chronic stress, remain understudied. Evidence from healthy older adults suggests stress impacts the brain, and those with HIV may be particularly vulnerable. Here, we tested whether chronic stress is linked to brain structure (grey matter volume) and cerebrovascular health (global cerebral blood flow) in older people with HIV.Methods: Fifty-eight HIV+ participants (3 women) from the Brain Health Now cohort underwent multi-modal MRI. Grey matter volume in three regions of interest was assessed via voxel-based morphometry, and global cerebral blood flow via arterial spin labeling. A chronic stress index was calculated based on a weighted sum of eight stress-related items where the weights were derived from each item's impact on anxiety, which is a response to stress.Results: Higher chronic stress was associated with reduced grey matter volume in the left hippocampus ($r = -0.27$, $p = 0.03$, 95% CI [-0.49, -0.01]) and lower global cerebral blood flow ($r = -0.31$, $p = 0.01$, 95% CI [-0.47, -0.01]).Conclusion: Our findings suggest chronic stress, a modifiable psychosocial factor, may contribute to brain changes in older people with HIV. This aligns with evidence in healthy aging and highlights a potential avenue for improving brain health in HIV.

P3-F-181 - Laminar architecture of error responses in medial frontal cortex

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Hubel and Wiesel's seminal work in cat primary visual cortex established the concept of columnar organization, forming the foundation of the canonical cortical microcircuit. However, cortical cytoarchitecture is not uniform—the presence of a clearly defined granular layer 4 varies across regions, and laminar profiles identified in sensory cortices do not consistently apply to agranular frontal areas responsible for cognitive functions such as error monitoring. This challenges the notion of a universal canonical microcircuit. We address this gap by using laminar probes to record spikes from individual neurons and local field potentials (LFPs) across layers in the medial frontal cortex (MFC), including the supplementary eye field and the dorsal and ventral banks of anterior cingulate cortex of four macaque monkeys performing a stop-signal task. Although interconnected, these regions are functionally, cytoarchitecturally, and anatomically distinct. Combining converging evidence from 1) single-unit data characterized by spike waveform shape, spike timing patterns, and cross-correlations between distant and neighboring neurons and 2) LFP measured as current source density and spectrolaminar profile, we localized the laminar origins of error-related signals with high confidence. Our findings refine the canonical

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framework in MFC and present a biologically plausible microcircuitry model that monitors and refines behavior.

P3-F-182 - Repeated within-session intra- and extra-dimensional learning in marmosets

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[1] York University

Cognitive flexibility is the brain's ability to suppress current strategies in favor of better alternatives when the context changes. Reductions in cognitive flexibility are a transdiagnostic deficit in several neuropsychiatric disorders. How the primate brain supports this process is not well understood. We trained 4 marmosets on a touchscreen-based Wisconsin Card Sorting Task (WCST) involving 2 dimensions: 3 shapes (star, square, heart) and 3 colors (yellow, red, blue). Marmosets underwent shape training, choosing the correct black shape from pairs to obtain a reward, followed by training on the remaining shape pairings. Similarly, they were trained on colors presented as circular patches. Once learned, they progressed to the full marmoset WCST (mWCST), in which they identify and select the compound stimulus with the target feature ignoring the irrelevant dimension. When they choose 8 correctly in a 10-trial block, the target feature shifted either within dimensions (e.g. red to blue) or across dimensions (e.g. red to heart). All marmosets quickly learned to perform multiple intra- and extra dimensional switches within a daily session. Afterward, animals were introduced to 6 new features, 3 shapes and 3 colours. Marmosets applied their learned skills to make multiple switches within their first two sessions. Next, we will implement a feature reinforcement learning model to simulate rule switching behaviour during the mWCST, providing insights into how animals adapt to intra- and extra-dimensional shifts.

P3-F-183 - Genetic and behavioural implications of Cell Adhesion Molecule 2 (Cadm2) knockout on reward sensitivity in mice

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Psychiatric disorders and substance use disorders (SUDs) often co-occur, sharing genetic and behavioural risk factors such as impulsivity–a heritable trait linked to sensation seeking and poor premeditation. Genome-wide association studies (GWAS) implicate variants in the Cell Adhesion Molecule 2 (CADM2) gene, encoding a synaptic adhesion molecule, in impulsivity-related phenotypes, including attentional deficits and substance use initiation. This study examines Cadm2's role in psychiatric and SUD risk using RNA sequencing and a Pavlovian conditioned approach task in a transgenic mouse model. Bulk RNA sequencing of whole brain, frontal cortex, and striatal tissues from male and female Cadm2-/- (KO) and Cadm2+/+ (WT) mice (n = 3/sex/genotype) revealed widespread gene expression changes. Differential expression analysis identified upregulated genes linked to addiction-related ontologies and dopaminergic neurogenesis ($p < 0.05$, log2FC ≥ |1|), suggesting CADM2 may modulate SUD vulnerability via altered drug metabolism and dopaminergic signaling. Behaviourally, KO mice (n=6/sex/genotype) exhibited deficits during a touchscreen Pavlovian conditioned approach task, indicating impaired set-shifting. KO mice showed impaired cue-reward association reversal ($p < 0.036$) and faster approach latencies to reward-predictive cues ($p < 0.007$), suggesting behavioural inflexibility and heightened reward anticipation. Ongoing studies will validate neural

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circuits and molecular interactions underlying underlying maladaptive reward processing influenced by Cadm2 deletion.

P3-F-184 - Longitudinal assessment of behavioural variability, repeatability, and anxiety responses in adult zebrafish (Danio rerio)

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Zebrafish are widely used in behavioural neuroscience as a model for studying anxiety and stress-related behaviours. However, substantial variability exists within and among individuals, influenced by factors such as sex, age, and environmental conditions, making the interpretation of anxiety-related behaviours challenging. This study aimed to characterize longitudinal patterns of stability and variability in anxiety-like behaviours across individual zebrafish and to assess whether distinct behavioural profiles emerge over time. Using a modified novel tank dive test, we tracked anxiety-related behaviours in adult zebrafish across three time points (90, 120, and 150 days post-fertilization). Behavioural metrics, including time spent in different tank zones, swimming velocity, and immobility, were analyzed for age- and sex-related effects, repeatability, and within-individual variation. Results indicated significant changes in anxiety-like behaviours with age, with fish spending more time in the upper zone and displaying increased swimming velocity over time. While no significant sex differences were observed in general anxiety measures, males exhibited greater within-individual variability in locomotor activity, while females demonstrated higher repeatability in time spent in the lower zone. Furthermore, zebrafish were classified into high, medium, and low-anxiety groups based on cumulative behavioural scores, revealing stable individual differences in anxiety-like behaviours over time.

P3-F-185 - EEG-based decoding of face imagery

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Despite recent progress in unraveling the neural mechanisms for face perception, their counterparts, that subserve visual memory and imagery, are yet to be elucidated. Accordingly, here, we used electroencephalography (EEG) in healthy adults (n=17) to assess neural responses elicited by face images, as participants viewed them or recalled their appearance as prompted by an auditory cue. Specifically, we appealed to pattern classification and multivariate feature selection to decode facial identity using a homogeneous set of images depicting front views of familiar (i.e., famous) and unfamiliar White females. Regarding the processing dynamics, we found, consistent with prior work, that perceptual information can be robustly extracted between ~150ms -1s after stimulus onset. In contrast, imagery information was decoded between ~500ms-3s after the cue. Second, regarding the spatial profile, imagery and perceptual decoding of familiar faces relied on a common set of central and frontal channels. However, unfamiliar face decoding from perception recruited a complementary set of occipitotemporal and frontal channels. Further, representational similarity analysis revealed relatively good correspondence between perceptual and imagery. Last, a combination of visual and semantic information, as captured by convolutional neural networks and transformer models, accounted for the similarity

structure of our data. These findings shed light on the neural representations underlying visual imagery, on their spatiotemporal dynamics and on their relation with perception.

P3-F-186 - MEG resting state functional connectivity predicts metacognition in self-control

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The ability to regulate one's actions is crucial for success in many aspects of life. A key component of developing and maintaining self-control is metacognition or thinking about one's capacity for self-regulation. We investigated the neural correlates of metacognition in self-control in a group of 60 (28 female) individuals using magnetoencephalography (MEG) resting state functional connectivity. Participants completed the Metacognition in Self-Control Scale (MISCS) (Bürgler et al., 2022) and 5 minutes of resting state in MEG. MEG data was pre-processed following best practices (Gross et al., 2013) and source localized using Brainstorm (Tadel et al., 2013). We computed connectivity using the weighted phase lag index (wPLI) for each of the canonical frequency bands (δ, θ, α, β, γ) within each voxel and derived clustering coefficients (Brain Connectivity Toolbox; Rubinov & Sporns, 2010) for each parcel of the Desikan-Killiany atlas. Using a Leave One Out paradigm, we trained a model to predict MISCS scores for each participant. Using this method, the model achieved a Spearman correlation of 0.339 (p = 0.0129) and a Pearson correlation of 0.328 (p = 0.0144). These findings show that resting-state MEG functional connectivity predicts metacognition in self-control scores.

P3-F-187 - The effect of apolipoprotein E4 on cognitive impairment in Parkinson's disease: A structural MRI study using the PPMI cohort

Angenelle Eve Rosal [1], Edgardo Torres-Carmona [2], Sarah Martin [2], Isabelle Boileau [2], Ariel Graff-Guerrero [2], Antonio Strafella [3]

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Apolipoprotein E4 (APOE4) has been linked to cognitive impairment in Parkinson's Disease (PD), though findings are inconsistent. Both cognitively impaired PD subjects and APOE4 carriers show reduced grey matter volume (GMV) and cortical thickness (CT). However, no study has examined APOE4 status, GMV and CT measures, and cognitive function collectively in a PD cohort.This study aims to explore APOE4's role in PD-related cognitive impairment by analyzing its effect on GMV and CT. T1-weighted MRI images from 52 PD APOE4 carriers (58.9 ± 9.2 years; 16 females, 36 males) and 123 non-carriers (63.1 ± 9.1 years; 75 males, 48 females) from the Parkinson's Progression Markers Initiative (PPMI) were processed in FreeSurfer 7.1. Group differences in CT and GMV for Desikan-Killiany atlas regions were assessed using independent t-tests and ANCOVAs (controlling for age, sex, and disease duration). Pearson correlations examined relationships between significantly different regions and 9 cognitive tests. Preliminary analyses showed significant GMV differences between the groups in the right lateral occipital and left inferior parietal cortices, correlating with Hopkins Verbal Learning Retention Test scores, and in the left superior parietal cortex, correlating with Montreal Cognitive Assessment scores. No CT differences were found. These findings suggest that APOE4 may impact GMV of regions linked to cognitive performance in PD, but not CT, highlighting its potential as a biomarker for PD-related cognitive impairment. Further analysis will be conducted to validate these relationships.

P3-F-188 - Investigating GABA-A receptor mechanisms in the antidepressant action of nitrous oxide

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Depression is a leading cause of morbidity, and current therapies are wanting. Recent trial data support nitrous oxide (N2O) as a rapid, effective antidepressant, but its antidepressant mechanisms are poorly understood. N2O transiently upregulates γ-aminobutyric acid type A (GABAA) receptor function, and we previously reported that other GABAA-targeting anesthetics trigger a sustained increase in α5 subunit-containing GABAA receptors (α5GABAARs). Converging lines of evidence implicate α5GABAARs in depression. Thus, we hypothesize that N2O triggers a sustained upregulation of α5GABAAR function, driving its antidepressant effects. Primary hippocampal neuron and cortical astrocyte cultures were prepared from murine embryos (E18) and exposed to either air or 70% N2O/30% O2 for 1 h. After 24 h, currents evoked by low (0.5 µM or 1 µM) or saturating (1 mM) concentrations of GABA were recorded using whole-cell patch clamp techniques. Treated hippocampal neurons also underwent immunofluorescent staining to assess α5 subunit cell-surface expression. The densities of GABA-evoked current recorded in primary neurons and in astrocyte/neuron co-cultures treated with air or N2O were not significantly different, nor were levels of α5 subunit cell-surface expression. Thus, a 1-h treatment with 70% N2O does not alter GABAAR-mediated currents or α5GABAAR surface expression in hippocampal neurons, 24 h after treatment. Ongoing studies are exploring N2O's actions on GABAARs ex vivo, and whether N2O alters inhibitory synaptic GABAergic neurotransmission or behaviors in mouse models of depression.

P3-F-189 - Medial frontal neuronal activities during repeated rule switches in rats

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To study the neural mechanisms underlying cognitive flexibility, we developed a novel rule-switching task for rats. Under the Stay rule, the correct side—left or right, remained the same for every trial in the block. Under the Shift rule, rats alternated their choices from trial to trial to get reward. Rats learned the Shift rule within 3 days. We conducted single-unit recordings from the medial frontal cortex (MFC) while they learned to switch from the Shift to the Stay rule, which they did on the first or second training day. Over 16 days of rule switch training, they progressed through Stages, Beginner to Expert. To identify factors that contributed to performance, we evaluated 16 multilinear regression models using Akaike Information Criterion. Response accuracy was best predicted by the model with Training Stage, Rule, post-switch Trial Position, and Rule-Side interaction. Reaction time was best predicted by Training Stage as the sole factor. Expert animals committed much less perseverative errors and relatively more regressive errors. Using linear regression models, we determined that the most influential factor was Side of choice, explaining activities of 35% of MFC neurons. The second-biggest factor was Rule, which optimally explained activity of 29% of MFC neurons. Neuronal activity during the delay period can be used to predict whether the animal was about to make an error. Our novel task is well suited for the analysis of the single-neuron, population and network-level mechanisms that support this training-related improvement in cognitive flexibility.

P3-F-190 - The dual role of corticotropin-releasing hormone in the prefrontal cortex in stress-induced working memory impairment and active coping behaviour

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Stress impairs cognition. It is widely believed that stress suppresses the activity of the prefrontal cortex (PFC) while increasing the activities of subcortical areas in order to promote active stress coping behaviours, such as fight-or-flight responses. However, recent evidence shows that the PFC remains active under stress and is indeed crucial for active coping behaviour: This indicates that the PFC may play a role in shifting behaviour from cognition toward active stress coping. We focus on a subgroup of PFC GABAergic interneurons expressing corticotropin-releasing hormone (CRH). In the PFC, CRH acts as a neuromodulator and signals via CRH receptor type 1 (CRHR1), which is primarily expressed on pyramidal neurons. Using the trial-unique non-match-to-location (TUNL) task, our lab previously demonstrated that CRH neuron activity and CRHR1 signaling mediate restraint stress-induced working memory impairment. On the other hand, a study from another group showed that CRH neuron activity in the PFC promotes active stress coping behaviours. Therefore, we hypothesized that CRH neurons mediate a behavioural shift from cognition to active stress coping. To test this, we quantified struggling behaviour (i.e., active coping) during restraint stress and found that the frequency of struggling correlated with post-restraint working memory impairment in male but not female mice, indicating a sex-dependent shift between cognitive function and active coping. We plan to further examine the roles of CRH neurons in the behavioural shift using chemogenetic manipulation of CRH neurons as well as gene knockdown of CRH and GABA in CRH neurons. These findings will help elucidate how the PFC reallocates resources under stress.

P3-F-191 - Retrosplenial Cortex encodes peer direction and coordinates social approach behavior in mice

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Tracking and coordinating approach to conspecifics is central to social behaviors in mice and other social animals. The retrosplenial cortex encodes allocentric direction, and in addition encodes directional information to salient environmental features. We asked whether the retrosplenial cortex dynamically tracks the direction of a peer mouse during freely moving social interaction. Utilizing head-mounted miniaturized microscopy of calcium transients in ensembles of neurons, we found 5-10% of cells in the retrosplenial consistently track the dynamic position of a peer mouse relative to the subject mouse across 5-minute social exploration epochs. These egocentric peer direction cells have similar encoding properties to traditional head direction cells, but are not uniformly distributed in all directions, with peer mouse positions immediately in front and behind the subject mouse underrepresented in the ensemble. Chemogenetic inactivation of the retrosplenial cortex reduced the elevated social investigation of a stressed peer mouse, suggesting that retrosplenial cortex coordinates social approach behaviors. Results indicate a central role for the retrosplenial cortex in guiding social approach behavior and demostrate a novel encoding scheme for social information.

P3-F-192 - Decoding of neuronal activity patterns in the Lateral Amygdala during fear memory formation in mice

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The Lateral Amygdala (LA) is a key structure for Pavlovian fear memory learning and recall. Within this structure, fear memories are believed to be stored in groups of neurons referred to as engrams. Although much progress has been made in understanding how individual neurons are allocated to the engram, the activity patterns of engram neurons during encoding have not yet been well characterized.We asked how the activity pattern in LA mediates memory encoding during initial memory formation. We performed longitudinal imaging of the mouse LA using an implantable miniature endoscope and calcium imaging in freely behaving animals. We then tracked the activity of the same neuronal population across several days including fear learning.Neuronal network activity patterns were analyzed using population vectors, machine learning, and active neurons across sessions. We observed that the population activity patterns were dramatically changed after tone-shock pairing and similarities were identified in activity during learning and during recall of the fear memory. The change in activity was particularly noticeable in a subset of putatively identified engram neurons. Therefore, we conclude that changes in the activity of putative engram neurons enable the formation of a similar representation during encoding and recall, as a possible mechanism for storing information in the brain. Our findings offer new insights specifically into the dynamics governing initial engram formation, revealing how putative engram neurons change their activity to encode new information.

P3-F-193 - Behavioural and neural dynamics of category learning across the menstrual cycle

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A growing body of literature demonstrates widespread effects of ovarian hormones on the brain. However, resulting impacts on human cognition remain to be fully elucidated. Here we provide a multimodal account of cognition across the menstrual cycle using category learning – a core cognitive process that requires careful coordination of learning, memory and attention – as a tool for capturing complex cognition. Using a newly developed method, we find that category learning varies across the menstrual cycle in a non-linear fashion that parallels the typical rise and fall of ovarian hormone estradiol across the cycle (N=171): accuracy increases steadily across the early follicular phase, peaks in the late follicular phase, and decreases again over the mid-late luteal phase. We replicate this behavioural effect in a follow-up MRI study (N = 42) and confirm that activation in brain regions supporting concept formation similarly varies across the menstrual cycle. Finally, we take the analysis a step further by examining hormone-gene interactions in participants tested at two points in the menstrual cycle using the same cognitive task (N=64). Results demonstrate that BDNF genotype, which affects neuroplasticity, modulates participants' sensitivity to estradiol fluctuations across the menstrual cycle as reflected in cognitive performance. Our results combine behavioural, imaging and genetic data to provide a comprehensive neurobiological account of learning and memory across the menstrual cycle.

P3-F-194 - Impact of attention on visual short-term memory performance during experimental pain

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Selective attention is crucial for cognitive processing, enabling us focus on the most relevant sensory information. However, pain can disrupt attention, impairing cognitive domains such as memory (Moore et al., 2012). While working memory deficits during experimental pain have been documented, its impact on the visual short-term memory (VSTM), a system dedicated to storing visual information over a few seconds, remains unclear. This study aimed to examine whether brief thermal pain affects VSTM performance and whether this effect depends on the timing of pain delivery relative to attention engagement. Participants (21 females, 18 males, 1 non-binary, mean age 23.28) completed a VSTM task, with some trials involving moderate pain either before or after a visual cue primed attention. While Bayesian evidence indicated that the null model best described the data (BFnull=8.121, P(null|data)=0.299), accumulating evidence suggests a potential timing effect (BFcue=7.557, P(cue|data)=0.285). Notably, when participants were divided into low- and high-performers (based on median performance in control trials), low-performers improved during pain trials improved compared to control, while high-performers declined. These findings suggest individual differences in pain's impact on VSTM, potentially linked to the A-type (attention dominates) and P-type (pain dominates) phenotypic dichotomy (Seminowicz et al., 2004). This highlights how distinct pain coping strategies uniquely influence cognitive performance and the importance of considering individual differences in pain research.

P3-F-195 - Modulation of learning by post-training heroin: A novel investigation in the Barnes maze

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There is significant evidence that opiates can enhance memory consolidation, but this evidence in rats is primarily generated in tasks requiring a single training session. In fact, this laboratory has found evidence for possible impairments by post-training heroin in avoidance conditioning tasks requiring multiple pairings. To further understand the relationship between task requirements and post-training effects of heroin, 60 male Sprague-Dawley rats underwent 10 days of training in the Barnes maze, a task that relies on multiple exposures to aversive stimuli while locating an escape location. Immediately following each session, animals received 0, 1, or 2 mg/kg heroin. Following acquisition, a probe trial occurred, where the escape location was moved 180°. Primary group differences were revealed on the probe trial. Compared to day 10, post-training 1 mg/kg heroin resulted in a significant increase in latency to escape, training hole investigation, locomotion in the training hole quadrant, and a general increase in arena exploration. Conversely, animals treated with 2 mg/kg heroin did not display an increase in latency to escape and training target investigation decreased. These findings demonstrate that heroin's effects changed drastically due to dose, where post-training 1 mg/kg heroin produced behaviour that appeared persistent, while 2 mg/kg heroin post-training generated the opposite. This evidence may be consistent with the modulation of stimulus-response learning by heroin.

P3-F-196 - The role of the dorsomedial striatum in regulating flexible decision making

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Flexible decision making is critical for adapting to a changing environment. Drug addiction is associated with maladaptive patterns of decision making such as overreliance on habitual behavior. While progress has been made in identifying neural correlates of decision processes, there is still uncertainty about the distinct contributions of corticostriatal and thalamostriatal projections. [JPB1] Using transient optogenetic and chemogenetic manipulations of cortical and thalamic inputs to the dorsomedial striatum (DMS), I am testing the hypothesis that these pathways differently regulate flexible choice behavior in mice. Head-fixed mice can successfully identify which of two available spouts has a higher probability of water reward and quickly adapt to perform above chance levels with probability reversal. Confirming the involvement of the DMS in choice behaviour, I find that unilateral optogenetic stimulation of indirect pathway DMS neurons at the time of a decision increases selection of the ipsilateral spout. In testing chemogenetic manipulations, I have found that inhibition of corticostriatal inputs reduces value updating, making choice behavior seem more habitual. In ongoing work, I hypothesize that inhibition of thalamostriatal inputs will increase choice variability and augment value updating. Since endocannabinoid signaling regulates the excitability of these pathways as well as animals' reliance on flexible versus habitual modes of decision making, I also plan to test how chronic Δ9-THC consumption influences animals' behaviour on this task. [JPB2] This work is critical for understanding the role of the striatal circuits and the impact of cannabis exposure on decision-making processes.

P3-F-197 - Temporal dynamics of neuronal excitability in the lateral amygdala mediates allocation to an engram supporting conditioned fear memory

Annelies Hoorn [1], Andrew Mocle [2], Alessandro Luchetti [1], Alexander Jacob [3], Sungmo Park [3], Bozhi Wu [1], Sofiya Zbaranska [4], Asim Rashid [3], Paul Frankland [3], Sheena Josselyn [3]

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Memories are encoded by ensembles of neurons (engrams) that are active during learning. Within a given brain region, eligible neurons compete for allocation to an engram and neurons with increased excitability at the time of training are likely to be allocated to the engram. Previous findings show that neurons with increased excitability during training, also have increased excitability for ~6h. Here, we examined the temporal dynamics of neuronal excitability important for memory allocation. We focused on the lateral amygdala (LA) and cued fear memory. To examine the functional role of excitability on memory allocation, we artificially increased excitability in a small subset of LA neurons before fear conditioning, biasing engram allocation up to 9 hours before training. Next, we examined endogenous excitability, using calcium imaging in freely moving mice, allowing to image the activity of neurons over time. We found that the most excitable neurons during learning where also significantly more excitable up to ~6 hours prior to learning, when compared to the less excitable neurons. To understand the functional role of these endogenously active neurons and memory allocation, we used a calcium based activity dependent-tagging system (scFLARE). We found that during the test, silencing the neurons that were more excitable 1 hour, but not 24 hours prior to learning, leads to attenuated memory expression. Together, these findings highlight the temporal specificity of excitability in the LA and its pivotal role in selecting which neurons become part of a memory engram.

P3-F-198 - Response conflict neurons: Re-evaluation in medial frontal cortex of non-human primates

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A major theory of cognitive control is based on the quantity "conflict", which is the co-activation of incompatible response processes. In humans performing tasks that create response competition, neural evidence supporting the theory was identified with activation in medial frontal cortex, particularly ACC, observed with fMRI and single unit recordings in humans and inferred from event-related EEG. In non-human primates performing a saccade countermanding task, neurons identified with response conflict have been described in SEF but not in ACC. We re-examined the incidence of neurons signaling response conflict in supplementary eye field (SEF) and the dorsal and ventral banks of cingulate cortex (d/vCC) with more reliable sampling offered by 32-channel linear electrode arrays spanning cortical layers in two male Macaca mulatta performing saccade countermanding. We analyzed single neuron modulation of 2410 isolated units (813 DMFC, 976 dMCC, 621 vMCC) sampled in 164 penetrations at 32 grid locations spanning 26-34 mm anterior to the interaural line. Neurons modulating specifically when response conflict was maximal were found in cingulate cortex—more commonly in dCC than in vCC. Such neurons were more common in SEF. These findings confirm the presence of a response conflict signal in medial frontal cortex of monkeys although in a small fraction of neurons. Further research is investigating other measurement criteria and population-level descriptions to determine whether the weak response conflict signal in cingulate cortex is sufficient or negligible.

P3-F-199 - Development of a novel model to examine state-dependent responses to a dynamic threat: influence of sex and role of the basolateral amygdala

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Physiological state recontextualizes environmental stimuli to prioritize immediate needs and restore homeostasis. Stimuli become differentially influential when energy intake is critical for survival. The basolateral amygdala (BLA) integrates stimuli that guide motivated decisions, such as predator avoidance during foraging. We developed a novel semi-naturalistic model to examine behavioral conflict and BLA function.Adult rats, either sated or food-deprived (FD), are placed in a food-baited open arena with a covered refuge opposite a dynamic predator robot. The robot, triggered by food-area entry, lunges at the animals, requiring them to weigh threat engagement against food procurement.Across sexes, robot presence significantly increases avoidance, characterized by flight to the refuge. Interestingly, while the robot suppresses food intake in FD males, FD females habituate to the robot and rapidly increase intake over repeated exposures. This suggests sex differences in approach/avoidance, with males being more risk-averse.Suppressing BLA-projection neurons via Gi-coupled DREADDs does not significantly alter foraging or robot encounters. Photometry recordings reveal robust BLA-projection neuron responses to both robot attacks and food procurement in both sexes. Ongoing analyses aim to delineate behavioral sequences and corresponding neural activity to clarify the BLA's role in these responses.

P3-F-200 - Chronic Gestational Stress increases maternal anxiety-like behaviours in the presence of offspring without affecting pup-oriented behaviours in an aversive environment

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During the perinatal period, maternal rats undergo physiological changes that optimize pre- and postnatal environments to enhance offspring survival. One adaptation is reduced HPA axis responsiveness to stress, likely mitigating its adverse effects on offspring by preserving maternal care behaviors. This study examines the impact of chronic gestational stress on maternal risk-taking in an offspring retrieval task. Pregnant rats underwent unpredictable restraint and bright light exposure (45 min, three times daily) from gestational days 14–20. Control (CT; n=9) and Gestationally Stressed (GS; n=10) dams were tested in a modified Elevated Plus Maze (EPM) with three open arms. A baseline test was conducted on postnatal day 8 (P8), followed by a retrieval task 24 hours later, where three pups were placed at the edges of the open arms, and dams were given 5 minutes to retrieve them. At baseline, GS dams did not show significant differences in anxiety-like behaviors (time in closed arm: t=-1.49, p=0.160; rearing: t=1.02, p=0.325). During retrieval, they exhibited significantly increased anxiety-like behaviors (time in closed arm: t=-2.21, p=0.046; rearing: t=4.547, p<0.001). However, pup-oriented behaviors did not differ between groups —licking/grooming (t=0.227, p=0.786), pup retrieval latency (first pup: t=-1.796, p=0.253; last pup: t=-0.602, p=0.668), and nest time (t=-1.597, p=0.134). These findings suggest that while gestational stress increases maternal anxiety in aversive environments, maternal care behaviors remain intact, supporting an adaptive mechanism that ensures offspring care despite heightened stress responses.

P3-F-201 - Evaluating proxemic functions of the rat parietal lobe

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Nearly all social behaviour involves dynamic management of interpersonal space. Past work on the neural basis of social proximity ("neuroproxemics") has implicated the amygdala in maintaining appropriate distance from others, likely due to the region's role in assigning and coordinating emotion when personal space is violated. Motor systems of the frontal cortex may similarly adjust movements in response to quickly changing information about the location of others. Surprisingly little is known, however, about how social-spatial information is initially computed, including how it is dynamically updated during movements of self and other. We used a combination of excitotoxic lesions and recently developed, multi-animal movement tracking methods (including DIPLOMAT) to evaluate the role of posterior parietal (PPC) and retrosplenial (RSC) regions of the parietal lobe in managing interpersonal space. Preliminary results show no group differences in levels of social investigation or play, nor changes in simple movement metrics (e.g., exploration and velocity/acceleration in an empty, novel environment). However, detailed analyses suggest subtle changes in how RSC-lesioned rats interact with others, with an increased probability of face-to-face behaviors after anogenital sniffs. Ongoing analyses of approach patterns in these animals seeks to fill critical gaps in our understanding of how individuals manage interpersonal space, and to help establish a new field of neuroproxemics.

P3-F-202 - Structured learning drives progressively multiplexed representations in the hippocampus

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The hippocampus represents a variety of concrete and abstract features in well-trained animals, demonstrated through often tightly restrictive behavioral tasks. This approach reveals the capacity for the hippocampus to reflect whichever task it is engaged in, but masks the internal model of what an animal could be learning over the course of experience. We used one-photon Miniscope calcium imaging in freely behaving mice to track how the hippocampus (dCA1) progressively builds its representation of a task structure over several weeks of learning a difficult multi-trial image-location association touchscreen task. Generalized Linear Models revealed most neurons becoming tuned to a progressively larger set of arbitrary behavioral features, from over 90% tuned to position alone during pretraining to <15% in expert mice. Mice developed highly structured behaviors as they became familiar with the task, and while cell tuning profiles initially spanned these overrepresented behaviors, they generalized to span the entire feature space of behaviors in expert mice. A surprisingly large subset of neurons maintained this multiplexed tuning during free exploration in the inter-trial-interval (ITI). However, spatial decoding from population activity was significantly better—and sometimes predictive of future locations—during task engagement than during ITI. Thus, sufficient task demands can drive the hippocampus to develop detailed behavioral representations with learning, highlighting its general role in forming a flexible and comprehensive internal model of experiences.

P3-F-203 - Investigating the effects of acute thc exposure on motivation and THC exposure and food deprivation on incentive value and reward salience

Savannah Lightfoot [1]

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Natural rewards, like food, and drugs of abuse, like cannabis, act on the mesolimbic dopamine reward pathway and through its interaction with the endocannabinoid (eCB) system, can affect positive valence states, including reward, incentive value, and motivation. Due to the regulatory role the eCB system plays in reward processing and motivation and with the advancements in drug delivery for rodent studies, basic science approaches can now be employed to better establish the impact of cannabis use on motivation, incentive value and goal-directed behaviours in a highly translational manner.In experiment 1: 8 male and 8 female rats were trained on a progressive ratio operant task to earn sucrose, once responding was stabilized, rats were exposed to THC and breakpoint was tested. In experiment 2 and 3: 16 male and 16 female rats were trained on a self paced 2-lever action sequence to earn sucrose. Once responding was stabilized, half the rats were exposed to THC and the other half were food deprived for 20hrs prior to an incentive learning opportunity. THC exposure significantly increased the number of active responses, the number of rewards and breakpoint, regardless of sex. THC exposure had no impact on incentive learning. Twenty hours of food deprivation was sufficient to increase incentive value of sucrose and increase seeking lever responses during a retrieval test 24hrs later. This research indicates that THC is sufficient to increase motivation and effort in rodents but not sufficient to increase the incentive value of a palatable sucrose reward.

P3-F-204 - Sex differences in depression-like behaviors: Insights from chronic stress models in mice

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Depression models, including Chronic Mild Stress (CMS), Chronic Stress (CS), and Chronic Corticosterone Injections, are widely used to study depression-like behaviors. Stressful stimuli activate key brain regions such as the frontal cortex, amygdala, substantia nigra pars reticulata, and basal ganglia. C57BL/6 and Swiss mice differ in emotional responses, with Swiss mice displaying greater stress sensitivity and territorial dominance. This study aimed to characterize a novel depression model using 16 days of Restraint Stress Test (RST) followed by Chronic Social Defeat Stress (CSDS) in male and female C57BL/6 mice. Depressive behaviors were assessed using the Splash Test (ST) and Forced Swim Test (FST).Eight-week-old C57BL/6 mice underwent RST for 2 hours daily without access to food or water, followed by CSDS exposure to aggressive Swiss male mice for 10 days. Behavioral interactions were recorded and analyzed. On day 17, ST revealed that female mice exhibited reduced self-grooming frequency (p<0.001) and increased FST immobility (p<0.0001) compared to controls. Male mice showed anhedonia-like behavior, characterized by reduced self-grooming frequency (p<0.0001) and duration (p<0.001), along with increased FST immobility (p<0.001). Our findings indicate that chronic stress induces depression-like behavior, with female C57BL/6 mice being more stress-sensitive due to estrous cycle influences, whereas males exhibit greater resilience, potentially linked to territorial aggression in the CSDS context.

P3-F-205 - Cue-evoked representations of specific outcomes in the gustatory portion of the insular cortex

Matthew Gardner [1]

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The ability to learn from the environment and anticipate motivationally relevant stimuli is essential for goal-directed behavior and decision-making. Previous research has shown that neurons in the gustatory portion of the insular cortex (IC) develop responses to sensory cues linked to taste availability. These responses occur for both general cues that predict an upcoming taste (Samuelsen et al., 2012) and for specific cues associated with either sucrose or quinine (Vincis et al., 2016; Gardner et al., 2014). Yet, it's not clear whether the specific cue responses encode the identity of the taste predicted or the hedonic value of the taste. To investigate whether IC encodes predictions of distinct taste identities, we designed a Pavlovian trace conditioning task using five auditory cues, each signaling a different outcome—one of four tastes (sucrose, NaCl, citric acid, quinine) or no taste. Our findings showed that rats (n = 5) exhibited specific orofacial movements in response to the different auditory cues, suggesting they learned the tone-taste associations. Further analysis of single-neuron activity (n = 231) recorded in vivo during task performance revealed significant cue-selective responses in IC, indicating that primary taste cortex can be activated by predictions of taste identity.

P3-F-206 - Assessing the long-term effects of microbial intervention on inflammation and clinical response in major depressive disorder: An exploratory pilot study

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The staggering prevalence of Major Depressive Disorder (MDD) and its impact on quality of life necessitates the drive for innovation and effective treatment options. Emerging research suggests the interaction between the gut microbiome and depression may be mediated through the gut-brain axis (GBA), a bi-directional signaling pathway between the gastrointestinal tract and

the brain. In efforts to better understand this dynamic relationship, this study explored the use of a gut-microbiome-targeted intervention, as treatment for depression. Microbial Ecosystem Therapeutic (MET-2), a novel microbial-based intervention, was administered once daily to adults aged 18-45 with MDD. Participants completed a variety of clinical scales and questionnaires to assess clinical response, along with blood collection measures to assess for changes in inflammation. Preliminary data will help determine if changes in inflammatory markers (CRP, TGF- β, IL-6, and IL-10) following microbial treatment can help predict long-term clinical outcomes and response to treatment. Any findings from this study will further help identify how novel pathways such as the GBA, are involved in the pathophysiology and development of psychiatric disorders.

G - NOVEL METHODS AND TECHNOLOGY DEVELOPMENT

P3-G-207 - Focused ultrasound enhances targeted gene delivery of intravenous AAV to the brain

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Background: Recombinant adeno-associated viruses (AAVs) hold promise for brain disorders but often require intracranial injection, limiting therapeutic reach and being invasive. Intravenous (i.v.) AAV delivery is restricted by the blood-brain barrier (BBB), reducing efficiency. Recent AAV variants can cross the BBB, enabling widespread brain transduction. MRI-guided focused ultrasound (FUS) transiently modulates BBB permeability, enhancing gene delivery in small or large brain regions targeted. Hypothesis: We hypothesize that combining IV BBB-penetrating AAVs with FUS will optimize gene delivery, enabling both widespread and region-specific transduction for neurodegenerative diseases. Methods: Three-month-old C57BL/6 mice received i.v. AAV-PHP.V1.CAG.TdTomato (V1) and AAV9.CAG.EYFP, followed by FUS targeting various brain regions. Immunohistochemistry and confocal microscopy were done 3 weeks post-delivery. Results and Conclusion: Our findings demonstrate that FUS enhances targeted gene delivery for AAV9 and V1 while maintaining global expression in the brain for V1, offering a non-invasive strategy for precise (AAV9) or widespread and locally enriched (V1) gene therapy. This approach could improve treatments for neurodegenerative disorders such as Alzheimer's and Parkinson's disease.

P3-G-208 - Seizure onset zone localization guided by neural network explainability tools for intracranial EEG data

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Introduction. Intracranial electroencephalography (iEEG) is the gold-standard tool for Seizure Onset Zone (SOZ) localization among patients undergoing resective surgery, but the time demands of data analysis pose barriers to clinical efficiency. Neural networks (NNs) offer automated iEEG processing, yet understanding their internal workings remains challenging, and SOZ localization is constrained by limited labeled training data. Objective. This study aims to

leverage novel NN explainability tools to 1) identify interpretable motifs associated with the SOZ, and 2) build a pipeline for SOZ prediction that is not reliant on SOZ labels. Methods. A pre-trained seizure detection NN was augmented with an explainer algorithm to assign electrode and input feature importance scores. These importance scores were compared with clinically annotated electrode labels to predict relationships with the SOZ in an open-source dataset (OpenNeuro ds003029, N=13). Results. Our explainability pipeline successfully predicted an electrode belonging to the SOZ for 9 (69.2%) patients. Electrode importance scores were higher for SOZ vs. non-SOZ electrodes ($p<0.001$). A recurring motif involving networks of high importance electrodes and the clinically annotated SOZ was present for all patients. Input feature importance scores revealed associations of the beta and gamma iEEG bands for NN SOZ localization and seizure detection. Conclusion. NN explainability tools for iEEG data help unravel interpretable motifs relating to SOZ localization, offering a promising approach to aid epilepsy surgery planning.

P3-G-209 - Chatting with data: Foundational framework leveraging LLMS for analyzing animal behavior

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The nature of scientific research compels asking questions and seeking answers. In the digital age, this paradigm has shifted toward computational methods aimed at verifying hypotheses. Behavioral neuroscience is a promising field for applying these computational tools. However, as the complexity of these tools increases, the time required to integrate different components of an experiment grows exponentially; from data collection to analysis to results. Here, we propose a retrieval-augmented generation (RAG) framework with a customized large language model (LLM) as its core. This framework aims to streamline the lifecycle of behavioral studies in neuroscience. First, it enables researchers to design experiments and data acquisition by accessing relevant literature, textbooks, and datasets. Once data is acquired, the assistant can help in processing it and extracting behaviorally rich insights. This is achieved by integrating the assistant with animal visual data foundational models. Finally, the assistant facilitates interactive data analysis. Researchers can task the assistant with extracting patterns, identifying regions of interest, visualizing findings, and generating reports. To demonstrate the contributions of this framework in facilitating experimental and analytical workflows, we show its application in measuring common behaviors in data and provided the results alongside the time consumed. The proposed framework empowers researchers from diverse backgrounds to access no-code AI tools and take full control of the analysis involved in the research process.

P3-G-210 - Analysis of behavioral data from rodent stress paradigms via continuous-time Markov models

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Rodent behavioral data is difficult to analyze because of its high dimensionality and large number of data points. Conventional approaches biologists use are normally restricted to comparing time spent in different states and thus fail to appreciate how behavioral states evolve with time. Stochastic approaches like continuous-time Markov modeling allow for the evolution of behavioral states to be modeled interpretably. Despite the utility of Markov modeling, it has not

been frequently applied to behavioral data from rodent stress paradigms. In this study, we use continuous-time Markov models to understand the trajectory of rodent behavior during a stress paradigm with a robotic predator. Our work shows that behavioral steady-state distributions normally derived from transition matrices can be accurately approximated using basic arithmetic. Moreover, our work demonstrates that principal component analysis used in conjunction with machine learning can decode rodent sex and the presence of a robotic predator from the approximated steady-state distributions. This study highlights the utility of Markov modeling in generating informative metrics that can be used for classification tasks. Future work should aim to incorporate time-locked imaging data (e.g., fiber photometry) into subsequent analyses so that links between neuron activity and behavior can be uncovered.

P3-G-211 - Evaluating the impact of dual-task EMG biofeedback on dynamic balance and gait speed in Multiple Sclerosis

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Physical activity has been identified as a beneficial therapy for people with multiple sclerosis (MS), particularly for addressing impaired balance. While gamified balance training systems are rising in popularity, they often lack precise control over muscle engagement. Incorporating EMG biofeedback (EMG-BF) addresses this limitation by ensuring targeted muscle activation, thereby enhancing training effectiveness.The aim of this study is to evaluate the impact of dual-task EMG-BF relative to traditional balance (BAL-EX) exercises.Participants were randomly assigned to receive EMG-BF (n=3), or BAL-EX (n=4). The intervention included a 6-week training period with a total of 18 sessions. Measures of dynamic balance and gait speed were assessed by Mini-BESTest and 25 ft walk test respectively, and were measured at baseline and in the week following the last intervention session. Additionally, perception of difficulty and enjoyability of the were measured following the intervention.Post intervention measures of dynamic balance increased in one participant in the BAL-EX and one in the EMG-BF. Measures of gait speed increased for one participant in both groups. Finally, while difficulty increased 25% in the EMG-BF compared to BAL-EX, enjoyability was 8% higher in this group, demonstrating that participants were still satisfied despite higher difficulty. Overall, the biofeedback intervention appears to be improving balance and functionality, showing promise as a novel intervention tool. Further data collection is required to compare the effectiveness of these two interventions.

P3-G-212 - The development of fully automated transitive inference task for mice

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Transitive inference (TI) allows us to deduce indirect relations based on previously acquired information. In TI tasks, subjects are first trained to discriminate overlapping premise pairs (A>B, B>C, C>D, D>E). Then, they are probed with a novel transitive pair (B vs D) and a novel non-transitive pair (A vs E) intermixed with trained premise pairs. The standard TI tasks for rodents use olfactory cues as items and involve their manual presentations in a specific sequence. This procedure is time-consuming, labor-intensive, and inconsistent, increasing the chance of

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human error. Thus, we developed a fully automated TI task for mice, in which items are five identical visual cues arranged horizontally on a wall. To prevent mice from mapping item relationships onto a linear spatial representation, the hierarchy among items was decoupled from an explicit spatial continuum (i.e., the left side being consistently better than the right side). We observed that 58% of mice chose correctly in trained premise, novel transitive, and non-transitive pairs, while the rest chose correctly in trained premise and non-transitive pairs but not in transitive pairs. Notably, when the hierarchy was defined along the spatial continuum, all mice chose correctly in all three pair types. Thus, similarly to humans (Smith and Squire, 2005), mice exhibit individual differences in the transitive expression of item relationships learned without explicit spatial linearity. This opens opportunities to investigate the neuronal basis of individual differences in intellectual capability.